

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**INVESTIGATIONS INTO THE INFLUENCE OF FERTILISER
HISTORY AND CLIMATE REGIME ON THE SOIL FERTILITY,
SOIL QUALITY AND PASTURE PRODUCTION OF
WAIRARAPA HILL SOILS**

**A thesis submitted in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy in Soil Science
Massey University**

James Laing Moir

2000

27/09/00

TO WHOM IT MAY CONCERN

This is to state the research carried out for my PhD thesis entitled "Investigations into the influence of fertiliser history and climate regime on the soil fertility, soil quality and pasture production of Wairarapa hill soils" in the Institute of Natural Resources, Massey University, Turitea Campus, New Zealand is all my own work.

This is also to certify that the thesis material has not been used for any other degree.

Candidate:

J. Mair

Date:

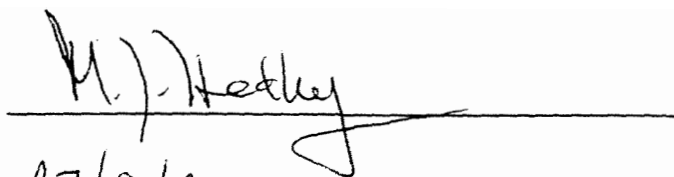
27.9.00

27/09/00

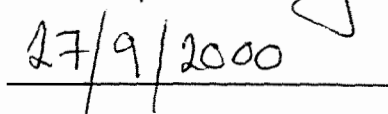
TO WHOM IT MAY CONCERN

This is to state the research carried out for the PhD thesis entitled "Investigations into the influence of fertiliser history and climate regime on the soil fertility, soil quality and pasture production of Wairarapa hill soils" was done by James Laing Moir in the Institute of Natural Resources, Massey University, Turitea Campus, New Zealand. The thesis material has not been used for any other degree.

Supervisor:



Date:



ABSTRACT

The effects of long-term application of single superphosphate (SSP) on soil plant-available nutrient supply and indicators of soil biological quality was investigated on Wairarapa hill soils ranging widely in previous fertiliser history (from 0 to 250 kg SSP ha⁻¹ yr⁻¹) and climate regime (annual and seasonal rainfall distribution). At 12 field sites spring pasture response to strategic N fertiliser application was measured, while the plant-available nutrient (P, N and S) supplying capacity of the soils was assessed in glasshouse studies. Based on the pasture growth patterns in field and glasshouse studies, a new climate-driven, soil fertility dependent pasture growth model was developed and tested. In addition, the suitability of the BiologTM GN microtitre plating system was assessed as an indicator of soil 'quality', using these Wairarapa hill country soils.

Results of soil analyses indicated that small increases in mineralisable N, in the order of 280 kg mineralisable N ha⁻¹, with increased rates of fertiliser (P and S) may represent inefficient use of P and S fertiliser. Soil mineralisable N increased by approximately 8.6 kg mineralisable N ha⁻¹ for every 1 unit increase in Olsen P. The ratio of accumulated plant-available N:P:S of these soils, resulting from long-term SSP applications, is approximately 17:2:1. Olsen P status was shown to be strongly correlated with measures of plant-available N and S.

Pasture growth response in the field to strategic N fertiliser (30 kg N ha⁻¹) applied in spring was highly variable across sites, and within the range of 0:1 to 31:1 kg DM kg N⁻¹. Simple single factors representing soil fertility indices, or climatic regime, could not explain the variation in site-to-site pasture growth response to applied N. Factors constraining N response are discussed.

In glasshouse studies, on samples of the same soils, ryegrass and white clover showed large yield differences (clover, 0.27-2.29 g DM pot⁻¹; ryegrass, 0.22-2.25 g DM pot⁻¹) on low P status and high P status soils respectively. Glasshouse DM yields did not correlate with those measured in the field, confirming that at field sites yield responses to nutrient availability are strongly modified by (site-specific) climate. The relationship between Olsen P and clover yield in the glasshouse (curvilinear, $R^2 = 0.80$) was similar to that

previously seen in (spring) field conditions. The S:P and N:P ratios of clover in the glasshouse trials confirm that P availability in these soils is the major growth-limiting factor, probably followed by S or N, which becomes limiting when P availability is adequate to high.

A modified Stanford and DeMent bioassay technique was used to estimate the amount of plant available N, P and S in each soil. Using an exhaustive cropping regime, these soils exhibited a large variation (range) in ryegrass yields when soils were the sole source of P and N. Yields for each soil were strongly correlated with various soil tests for N, S and P availability. S availability to plants was less variable across soils, but the smaller variation in S limited yield was still strongly correlated with the variation in a newly developed soil hydrogen peroxide-extractable S test. Results from both glasshouse experiments provide strong evidence that the Olsen P soil test is a valuable soil fertility indicator of plant-available P, N and S on legume-based pasture soils with a history of superphosphate use. The amount of dry matter production, when considered with the quantity of soil used for each treatment (-N, 100g; -P, 50g; -S, 25g), suggest that these soils have large pools of plant-available or mineralisable P and S, and, relative to plant demand, small pools of soil mineralisable N. A four-fold increase in field DM production resulted from a 3-fold increase in soil mineralisable N at these sites. This suggests that the rate of N cycling probably also increases with yield increase, and that the size of the soil mineralisable N pool is not directly related to pasture N supply.

A new climate-driven, soil fertility dependent pasture production model has been developed and tested using actual DM yields from the field trial sites. The model assumes that pasture growth is proportional to evapotranspiration, and that the proportionality constant (k) depends on soil fertility*. Soil-limited evapotranspiration is calculated from a simple daily soil water balance model. Values for k varied from 11 to 19 kg DM ha⁻¹ mm⁻¹ of evaporation. With the exception of growth after severe drought conditions, the model shows potential to closely predict actual pasture yield. It is hoped that discrepancies between the modelled and measured production may lead to useful

* Pasture growth per mm of evapotranspiration was strongly related to soil available P status at these sites. From results of the glasshouse study, it was concluded that Olsen P was a strong indicator of 'general' (plant-available P, N and S) across these sites, and therefore suitable for use as the soil fertility proportionality constant in the pasture production model.

speculation and further research on the interacting effects of weather and fertility on pasture growth.

The BiologTM GN microtitre plate system, for comparing substrate use patterns of 95 single C compounds was assessed as an indicator of soil microbial functional diversity across the 12 test hill soils. Preliminary studies showed that saline extracts of different fertility status pasture soils used for BiologTM microtitre plate assay inoculation contain significant amounts of readily available C. It was concluded that in order to interpret the substrate use patterns correctly, this effect must be corrected for.

The BiologTM microtitre plate system, for use as an indicator of soil quality and health, was shown to have limited application to this range of pasture soils with differing pasture histories. Adaptive factors, such as constitutive and inducible enzyme activities, were shown to complicate the interpretation of microbial growth on the C substrates. Substrate use patterns also changed when soils were rewetted and incubated. Possible 'indicator' substrates were identified, but it was concluded that these were low-energy decomposition products, and as such, are not useful as indicators of microbial functional diversity across these soils. Further research would be required to establish how stable the substrate use patterns are, or the relevance of these indicators to field soil processes. However, as a research tool, the BiologTM assay showed potential to separate these soils on the basis of microbial functional diversity. The direction of future research, and limitations of current techniques used in this field are discussed.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to the following people.

First and foremost, I thank my chief supervisor Mike Hedley, who gave inspiration, enthusiasm and support throughout the course of this project.

Dave Scotter, who gave major contribution and momentum to the pasture production model in this thesis.

Charles Russell, for collaborative efforts, and comment on the soil micro biology components of this thesis.

Alex Mackay and Russ Tillman, for constructive comment.

Bob Toes, for valuable assistance with field work, including many early morning starts. I also thank all academic and technical staff, and postgraduate students of the Soil Science Department, for their contributions. Special thanks to Shivaraj Gurung.

Thanks is given to the farmers involved in this study, Messers; A. Day, D. Blackwood, B. Christensen, D. Dunlop, N. Kilmister, D. Kinnell, K. O'Riley and D. Williams.

I also acknowledge the 'Riverside' Trust; FLRC; MURF; Royal Society of New Zealand; Commonwealth Science Council; and numerous postgraduate scholarship providers for financial contributions to this project.

To Vicki, and to my parents, for their continuing support.

To Jane and Shona, for love and support throughout.

TABLE OF CONTENTS

Abstract ii

Acknowledgements v

Table of Contents vi

List of Tables xiv

List of Figures xvi

List of Plates xxii

CHAPTER 1

INTRODUCTION

Introduction 1

References 4

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction 7

2.2 New Zealand Hill Soils 7

2.3 Nutrient Cycling in Grazed Pastures 9

2.4 Soil Fertility and the Efficiency of Fertiliser Use 11

 2.4.1 Phosphorus and Sulphur 11

 2.4.2 Nitrogen 14

2.5 Improved Diagnostics with Herbage Analysis 15

2.6 Sustainable Land Management 16

2.7 Conclusions 17

2.8 References 18

CHAPTER 3

DESCRIPTION OF FIELD SITES AND CHARACTERISATION OF SOIL FERTILITY STATUS

3.1	Introduction	25
3.2	Site Description	25
3.3	Soil Analyses.....	30
3.3.1	Methods and Materials.....	30
	<i>Soil Sampling and Preparation</i>	30
	<i>Total N, P and S Content</i>	30
	<i>Plant-available Nutrients, pH and Bulk Density</i>	30
3.3.2	Results and Discussion.....	31
3.3.2.1	Total P, N and S.....	31
3.3.2.2	Olsen P, Sulphate S, Resin-Extractable P, pH, Bulk Density, P Retention and Mineralisable N.....	34
3.3.2.3	H ₂ O ₂ Extractable Sulphate, Ammonium and Nitrate.....	35
3.3.3	General Discussion	36
3.4	Conclusions	39
3.5	References	39

CHAPTER 4

FIELD STUDIES - ANNUAL PASTURE YIELD AND GROWTH RESPONSE TO STRATEGIC APPLICATION OF NITROGEN FERTILISER

4.1	Introduction	42
4.1.1	Review of Literature	43
4.1.2	Objectives.....	45
4.2	Methods and Materials.....	46
4.3	Results and Discussion	49
4.3.1	Climate	49
4.3.1.1	Rainfall and Evapotranspiration	49
4.3.1.2	Drainage.....	51
4.3.2	Soil Moisture	53

	viii
4.3.3 Annual Pasture Yield	55
4.3.4 Annual Yield and Nitrogen Response	59
4.3.4.1 Statistical Analysis of Annual Pasture Yields.....	60
4.3.5 Spring Nitrogen Response	60
4.3.5.1 Control Yield	60
4.3.5.2 Response to Applied N	63
4.3.5.3 Statistical Analysis of Spring Pasture Yields	63
4.3.5.4 Discussion	64
4.3.5.5 N Response Relative to Economic Returns	69
4.4 General Discussion.....	70
4.5 Conclusions	71
4.6 References	72

CHAPTER 5

GLASSHOUSE STUDIES TO DETERMINE PLANT-AVAILABLE NUTRIENT RESOURCES OF SELECTED WAIRARAPA HILL SOILS

5.1 Introduction	76
<i>Experiment 1</i>	77
<i>Objective (Experiment 1)</i>	77
<i>Experiment 2</i>	77
<i>Objective (Experiment 2)</i>	77
5.2 Methods and Materials - Experiment 1	78
5.2.1 Soil Sampling and Preparation	78
5.2.2 Soil Chemical Analyses	78
<i>Plant-Available Phosphorus</i>	78
<i>Phosphate-Extractable Sulphate</i>	78
<i>Mineralisable Nitrogen</i>	78
<i>Exchangeable Cations and CEC</i>	79
<i>pH (H₂O)</i>	79
<i>pH (0.1M CaCl₂)</i>	79
<i>H₂O₂ Extractable S and N</i>	79
5.2.3 Experimental Design.....	80
5.2.3.1 Soil Moisture and Glasshouse Conditions	80

5.2.3.2	Plant Establishment	80
5.2.3.3	Yield Measurement	81
5.2.3.4	Nitrogen Fixation	81
5.2.3.5	Plant Tissue Analysis	82
	<i>Nitrogen and Phosphorus</i>	82
	<i>ICP-AES Analysis</i>	82
5.2.3.6	Post-Experiment Soil Analyses	82
5.3	Methods and Materials - Experiment 2	83
5.3.1	Stanford and DeMent - Experimental Design	83
5.3.1.1	Pot Design	83
5.3.1.2	Soil Moisture.....	84
5.3.1.3	Plant Establishment	84
5.3.1.4	Basal Nutrient Application.....	85
5.3.1.5	Yield Measurement	86
5.4	Results and Discussion - Experiment 1	87
5.4.1	Soil Analyses	87
	<i>Soil pH</i>	87
	<i>Extractable Phosphate</i>	88
	<i>Mineralisable Nitrogen and Extractable Sulphate</i>	89
5.4.2	Herbage Yield and Nutrient Concentrations	90
	<i>Herbage Yield</i>	90
	<i>Nitrogen Fixation</i>	94
	<i>Relationship Between Yields, Nutrient Uptake and Soil Tests</i>	95
	<i>Nitrogen, Phosphorus, Potassium and Sulphur in Herbage</i>	96
5.5	Results and Discussion - Experiment 2	101
	<i>Effect of Fertiliser History and Climate on N and S Pools</i>	108
5.6	Summary and Conclusions	111
	<i>Experiment 1</i>	111
	<i>Experiment 2</i>	111
5.7	References	112

CHAPTER 6

A CLIMATE-DRIVEN, SOIL FERTILITY DEPENDENT, PASTURE PRODUCTION MODEL

6.1	Abstract	118
6.2	Keywords	119
6.3	Introduction	119
6.4	Model Development.....	123
	6.4.1 Calculation of E.....	123
6.5	Data for Model Development and Validation.....	126
6.6	Results and Discussion	127
	6.6.1 Parametisation of k	127
	6.6.2 Relationship Between Olsen P and Growth Per Unit E	129
	6.6.3 Model Validation	130
	6.6.4 Application of Model.....	133
6.7	Conclusions	134
6.8	Acknowledgements	135
6.9	References	135

CHAPTER 7

USING BIOLOG™ MICROTITRE PLATES AS A METHOD FOR STUDYING CHANGES IN THE FUNCTION AND CARBON SUBSTRATE USE CHARACTERISTICS OF MICROBIAL POPULATIONS IN PASTURE SOILS: I. INFLUENCE OF SOIL EXTRACT ADDITION ON MICROBIAL GROWTH

7.1	Introduction	140
7.2	Methods and Materials	143
	7.2.1 Soil Sampling.....	143
	7.2.2 Soil Chemical and Physical Characterisation.....	144
	<i>Soil Fertility and Physical Condition</i>	<i>145</i>
	7.2.3 Biolog™ Substrate Assays.....	146
	7.2.3.1 Field Dry Soils.....	146
	7.2.3.2 Glasshouse Moistened Soils.....	147

7.2.4	Plating Studies to Determine Numbers of Colony Forming Units.....	148
7.2.5	Carbon in Soil Solution.....	149
7.2.6	Estimation of Biologically Respirable C Contained in Saline Soil Extract.....	150
7.2.7	Carbon Content of Biolog™ Microtitre Plate Substrates	150
7.3	Results and Discussion.....	151
7.3.1	Colour Development in Control Well Inoculated with Soil Extract ...	151
7.3.2	Factors Likely to Effect Absorbance in the Control Well Inoculated with Soil Extract	154
7.3.2.1	Influence of Colour of Soil Extract on Control Well Absorbance	154
7.3.2.2	Influence of Carbon Input from Saline Extracts of Soil	155
7.3.2.2.1	Total Carbon Content of Saline Extracts of Soil	156
7.3.2.2.2	Bioassay to Determine Bio-Available C Content of Saline Soil Extract	160
7.3.2.2.3	Other Factors Related to Available C Concentrations in Soil Extracts.....	164
7.3.2.2.4	Mass Balance of C Input to Biolog™ Wells	165
7.3.3	Changes in Microbial Population.....	167
7.4	Conclusions	172
7.5	References	173

CHAPTER 8

USING BIOLOG™ MICROTITRE PLATES AS A METHOD FOR STUDYING CHANGES IN CARBON SUBSTRATE USE CHARACTERISTICS OF MICROBIAL POPULATIONS IN PASTURE SOILS: II. INFLUENCE OF SOIL LOCATION AND FERTILISER HISTORY

8.1	Introduction.....	178
8.2	Methods and Materials.....	182
8.2.1	General Methods	182
8.2.2	Data Processing.....	182
	<i>Numbers of Substrates Used</i>	183
	<i>Discriminate and Cluster Analysis</i>	183

8.3	Results and Discussion	183
8.3.1	Number of Substrates Used by Each Soil	183
8.3.2	Numbers of Soils Using Each Substrate	192
8.3.3	Identifying 'Indicator Component' Substrate Use Patterns	195
8.3.4	Correlation of Numbers of Substrates Used with other Soil Characteristics.....	200
8.3.5	'Inducible' Substrate Use.....	205
8.4	Conclusions	205
8.5	References	207

CHAPTER 9

SUMMARY AND IMPLICATIONS FOR FUTURE RESEARCH

Summary	211
Future Research	213

APPENDICES

Chapter 2

2.1	Literature Review	215
-----	-------------------------	-----

Chapter 4

4.1	Economics of Strategic Fertiliser N Application	240
4.2	N Response Trial - Pasture Yield Figures	243

Chapter 5

5.1	Field and Glasshouse Site Yield Rankings.....	249
5.2	Nutrient Uptake.....	251
5.3	Clover P Uptake	253
5.4	Nutrient Uptake and Olsen P Correlations.....	254
5.5	Herbage Nutrient Ratios.....	255

Chapter 7

7.1	Regression Analysis	258
7.2	Soil Extractable C	260
7.3	Soil Analyses.....	262

Chapter 8

8.1	Biolog TM C Substrate Information	263
8.2	C Substrate Use Frequency	266
8.3	Cluster Analysis	267

LIST OF TABLES

Table 3.1	Site aspect, soil group and fertiliser history	27
Table 3.2	Soil chemical analyses and field bulk density for all field soils ($\pm$ std. error).....	35
Table 3.3	Soil hydrogen peroxide extractable S and N content across all field soils.....	36
Table 4.1	Annual pasture yield response (kgDMkg^{-1} N applied) to nitrogen fertiliser application (@30 kgNha^{-1}) at all Wairarapa field sites (calculated as [increase in DM (kg) / kg N applied])	59
Table 4.2	Mean annual pasture yields (kgDMha^{-1}). Treatments in columns with different letters are significantly different at $P < 0.01$. +N yields are different from control yields across rows at $P < 0.1$ when marked with *	60
Table 4.3	Mean spring pasture yields (kgDMha^{-1}). Treatments in columns with different letters are significantly different at $P < 0.01$. +N yields are different from control yields across rows at $P < 0.1$ when marked with *	63
Table 4.4	Constraints on hill pasture growth response to spring applied N (30 kgNha^{-1})	67
Table 5.1	Soil water holding capacity (gravimetric water content) at 40 cm tension (suction potential).....	84
Table 5.2	Summary of total nutrient supplied by basal nutrient solution to all treatments.....	86
Table 5.3	Soil chemical analyses and field bulk density for all glasshouse soils .	88
Table 5.4	Mean ryegrass and clover dry matter yields (gDM pot^{-1}) across all soils. Total mean yields in columns with different letters are significantly different at $P < 0.05$	92
Table 5.5	Nitrogen fixation ($\text{kgNha}^{-1}\text{d}^{-1}$) by clovers on day of harvest. Total mean nitrogen fixation in the final column with different letters are significantly different at $P < 0.05$	95

Table 5.6	Mean dry matter yields (gDM pot ⁻¹) and statistical analysis in Stanford and DeMent experiment. Total mean yields (within treatments) in columns with different letters are significantly different at P < 0.05 ..	101
Table 7.1	Summary of Soil Chemical and Physical Characteristics	144
Table 7.2	Absorbance (590nm) of 0.85% w/v NaCl soil extracts at time of extraction (T ₀). absorbance values are means of four replicates	155
Table 7.3	Total carbon contents of soil saline extracts for Field-Dry and Glasshouse-moistened soils, by methabsorbance of LECO and Total Organic Carbon (TOC) Analyser (µgCg ⁻¹ soil, oven-dry basis)	157
Table 7.4	Carbon contents of selected Biolog TM substrates, total C (µgC/well).....	166
Table 7.5	Nutrient agar plate counts of bacteria and fungi colonies from whole soil inoculum (Field-Dry and Glasshouse-Moistened soils). Values quoted are cells mL ⁻¹ (culturable-colonies) at 10 ⁻³ dilution	168
Table 8.1	Substrates falling into the category of “Suitable Indicators”	197

LIST OF FIGURES

Figure 3.1	Total soil phosphorus A , Sulphur B , and Nitrogen C content of the 0 - 7.5 cm horizon across all Wairarapa field sites. Adapted from Moir et al. (1997).	33
Figure 3.2	The relationship between total soil P content and A Olsen P, and B Mineralisable N content across all field sites.....	37
Figure 3.3	The relationship between Olsen P and A Mineralisable N, and B H ₂ O ₂ extractable S content across all field sites.....	38
Figure 4.1	Monthly cumulative rainfall and evapotranspiration from August 1994 to March 1995 at A Mauriceville, B Gladstone and C Whareama.	50
Figure 4.2	Soil water drainage (mm) for the 0-7.5 cm soil depth from August 1994 to March 1995 at A Mauriceville, B Gladstone and C Whareama.....	52
Figure 4.3	Soil volumetric water contents (0-7.5 cm depth) from late May to December 1994 at A Mauriceville, B Gladstone and C Whareama sites.....	54
Figure 4.4	Cumulative annual pasture yields for control plots at A Mauriceville, B Gladstone and C Whareama sites for the 1994/1995 season	56
Figure 4.5	Cumulative annual pasture yields for nitrogen fertilised plots at A Mauriceville, B Gladstone and C Whareama sites for the 1994/1995 season.....	57
Figure 4.6	The relationship between annual pasture yield and Olsen P status for the 1994/95 season.....	58
Figure 4.7	The relationship between annual pasture yield and soil mineralisable N status for the 1994/95 season.....	59
Figure 4.8	The relationship between spring pasture yield and A Olsen P, B Resin P and C mineralisable N status for the 0-7.5 cm soil depth at all sites ..	62
Figure 4.9	Spring pasture growth response to N above control plot growth across all sites for 1994.....	64
Figure 4.10	The relationship between spring growth above control and A Olsen P and B mineralisable N for the 0-7.5 cm depth at all sites	65
Figure 4.11	The relationship between pasture growth above control, and control yield during the spring of 1994 across all sites	66

Figure 4.12	The relationship between pasture growth above control, and control yield during the spring of 1994 across all sites, including actual (●) yields.....	69
Figure 5.1	The relationship between CaP and H ₂ O ₂ extractable sulphate across all soils.....	90
Figure 5.2	The relationship between Olsen P and A ryegrass, and B white clover dry matter yields in the glasshouse across all soils	91
Figure 5.3	The relationship between glasshouse ryegrass yield and glasshouse clover yield A ; and B , The relationship between Olsen P and N fixation levels of white clover on the day of harvest across all soils.....	93
Figure 5.4	The relationship between A , glasshouse ryegrass yield spring field yield; and B , field /glasshouse residual yields and glasshouse minus field Olsen P values across all soils	94
Figure 5.5	Herbage concentration and plant uptake of A P and B N by glasshouse grown ryegrass across all soils	97
Figure 5.6	Herbage concentration and plant uptake of A N, B P, C S and D K by glasshouse grown white clover across all soils.....	98
Figure 5.7	Relationship between Olsen P and A ryegrass % P, B ryegrass P uptake, C clover % P, D clover P uptake, E clover N uptake and F ryegrass N uptake	100
Figure 5.8	Mean dry matter yields across all treatments	102
Figure 5.9	The relationship between Olsen P and A -P treatment ryegrass yield; B -N treatment ryegrass yield; and C -S treatment ryegrass yield across all soils.....	106
Figure 5.10	The relationship between A , soil mineralisable N and -N treatment ryegrass yield; B , Peroxide extractable N and -N treatment ryegrass yield ; and C , Peroxide extractable S and -S treatment ryegrass yield.....	107
Figure 5.11	The relationship between soil total P content and A , -N treatment yield, and B , -S treatment yield across all soils.....	109
Figure 5.12	Predicted (from relationships in Figure 5.11) minus actual yields (gDM pot ⁻¹) for A , -N treatment yield, and B , -S treatment yield across all soils; and also the relationship between total soil P and C , -N , and D , -S residual yields	110

Figure 6.1	Cumulative pasture yield (solid lines) and 0-75 mm depth soil volumetric water content (θ , broken line) at high fertility (HF) and low fertility (LF) sites from August 1993 to April 1994 at A , Mauriceville and B , Whareama	121
Figure 6.2	The relationship between Olsen P and pasture yield for a non-moisture limiting growth period (spring harvests). Data from Moir et al. (1997)	122
Figure 6.3	The relationship between Olsen P and annual pasture yield at Mauriceville (high rainfall) (●, ---), and Whareama (low rainfall)(■, —) sites.	122
Figure 6.4	Relationships between cumulative evapotranspiration (E) and cumulative pasture yield (G) from August 1993 to April 1994 at high (●, —) and low (■, ---) fertility sites at A , Whareama; B , Gladstone; and C , Mauriceville.....	128
Figure 6.5	The relationship between Olsen P and growth per unit E across all Wairarapa field-trial sites (Gladstone sites = ○, Outliers = ■).....	129
Figure 6.6	Actual (data points) and modelled (solid line) cumulative pasture yields at A , Gladstone (1994/5); and Whareama in B , 1993/4 and C , 1994/95. LF, Low fertility (□ or Δ); HF, high fertility (○); UM, unlimited moisture	132
Figure 6.7	The cumulative probability distribution of generated pasture production from 30 November to 30 April at Gladstone, for k values of 11 (low fertility) and 19 (high fertility) kgDMha ⁻¹ mm ⁻¹	134
Figure 7.1A	The pattern of background absorbance (590nm) over time of Biolog TM microtitre plate inoculated assays with extracts from field-dry soils 1-8. Absorbance values are mean values of three replicate extractions	152
Figure 7.1B	The pattern of background absorbance (590nm) over time of Biolog TM microtitre plate inoculated assays with extracts from field-dry soils 9-14. Absorbance values are mean values of three replicate extractions	152
Figure 7.2A	The pattern of background absorbance (590nm) over time of Biolog TM microtitre plate inoculated assays with extracts from glasshouse-moistened soils 1-8. Absorbance values are mean values of three replicate extractions	153

Figure 7.2B	The pattern of background absorbance (590nm) over time of Biolog™ microtitre plate inoculated assays with extracts from glasshouse-moistened soils 9-14. Absorbance values are mean values of three replicate extractions.....	153
Figure 7.3	TOC contents of all soils.....	158
Figure 7.4	The relationship between initial absorbance (Abs) and C concentration of saline soil extracts from A field-dry, and B glasshouse-moistened soils; and the relationship between Abs _{max} and C concentration of saline soil extracts from C field-dry, and D , glasshouse-moistened soils.....	159
Figure 7.5A	Bioassay Standard Curve - Maximum absorbance with Increasing Concentration, single species (WT) inoculum.....	161
Figure 7.5B	Bioassay Standard Curve - Maximum absorbance with Increasing C Concentration, using a (whole community) saline extract inoculum from soil number 8.....	161
Figure 7.6	Estimated bio-available C in saline soil extracts from field-dry and glasshouse moistened soils.....	162
Figure 7.7	The relationship between estimated bio-available C in saline soil extract, and soil-extracted CFUs, for field-dry soils.....	164
Figure 7.8	The relationship between fungal colony counts and A Olsen P, and B soil mineralisable N; and the relationship between total colony counts and C Olsen P, and D Mineralisable N, across all field-dry soils.....	170
Figure 7.9	The relationship between fungal colony counts and A Olsen P, and B soil mineralisable N; and the relationship between total colony counts and C Olsen P, and D Mineralisable N, across all glasshouse moistened soils.....	171
Figure 8.1	Number of Biolog™ C substrates used across all field dry soils after 2, 4 and 6 days of incubation.....	184
Figure 8.2	Graphical representation of “significant” (black cell) or “not-significant” (empty cell) substrate use responses across all field dry soils and all substrates after 2 (A), 4 (B) and 6 (C) days of incubation.....	186

Figure 8.3 Graphical representation of “significant” (black cell) or “not-significant” (empty cell) substrate use responses across all glasshouse moistened and incubated soils and all substrates after 2 (**A**), 4 (**B**) and 6 (**C**) days of incubation. 187

Figure 8.4 Number of Biolog™ C substrates used across all moistened and glasshouse incubated soils after 2, 4 and 6 days of incubation..... 188

Figure 8.5 Numbers of ‘inducible’ (day 2 minus day 6) Biolog™ C substrates across all **A** field dry, and **B** moistened glasshouse incubated soils 189

Figure 8.6 OD (microbial growth) over time across all field dry soils on Biolog™ C substrate cyclodextrin (substrate 2) 190

Figure 8.7 OD (microbial growth) over time across all field dry soils on Biolog™ C substrates **A**, glucuronimide (substrate 63), and **B**, phenylethylamine (substrate 89)..... 191

Figure 8.8 OD (microbial growth) over time across all field dry soils on Biolog™ C substrate acetic acid (substrate 37) 192

Figure 8.9 Number of field dry soils significantly using individual Biolog™ C substrates after **A** 2, **B** 4 and **C** 6 days of incubation 193

Figure 8.10 Number of moistened glasshouse incubated soils significantly using individual Biolog™ C substrates after **A** 2, **B** 4 and **C** 6 days of incubation..... 194

Figure 8.11 Soil inocula OD (growth) over time for control wells, average well colour development (AWCD), Biolog™ C substrate 63 (glucuronimide), and Biolog™ C substrate 80 (D-serine) for **A** field dry soil 13 and **B** moistened glasshouse incubated soil 8 199

Figure 8.12 The relationship between Olsen P and number of Biolog™ C substrates utilised after 4 days incubation across all moistened glasshouse incubated soils 201

Figure 8.13 The relationship between annual pasture yield and **A** spring pasture yield, and **B** number of Biolog™ C substrates utilised after 4 and 6 days incubation, respectively, across all moistened glasshouse incubated soils 202

Figure 8.14 The relationship between soil moisture content at time of sampling and number of BiologTM C substrates utilised after 6 days incubation across all field-dry soils 202

Figure 8.15 The relationship between Soil Mineralisable N and number of BiologTM C substrates utilised after **A** 2, **B** 4 and **C** 6 days of incubation across all moistened glasshouse incubated soils 203

Figure 8.16 The relationship between CFUs and number of BiologTM C substrates utilised after **A** 2, **B** 4 and **C** 6 days of incubation across all moistened glasshouse incubated soils..... 204

LIST OF PLATES

Plate 3.1	Map of the Wairarapa region, including location of field trial sites, and local New Zealand Meteorological Service Climate Stations A , Mangamutu; B , Castle Point and C , Taratahi.	28
Plate 3.2	View of coastal summer-dry Wairarapa hill country, taken at Whareama	29
Plate 3.3	View of Wairarapa hill country, taken from Gladstone, facing North-West towards the Wairarapa plains, and Masterton	29
Plate 4.1	Pasture being harvested from plots at a trial site, using a trim and remove technique	47
Plate 5.1	Design of pots used in Experiment 2, showing the thick rhizosphere mat which forms in the small volume of soil at the bottom of the pot, after plant roots have grown through the upper (sand) portion	85
Plate 5.2	Soil effect on plant growth; from left to right HF soil 1, MF soil 5 and LF soil 4. From top to bottom, treatments are -N, -S and -P.	103
Plate 5.3	Treatment effect across a single soil; HF soil 1 (top), and LF soil 4 (bottom). From left to right, treatments are -N, -P and -S.	104
Plate 5.4	Some root exposures of the Stanford and DeMent bio-assay pot.	105
Plate 7.1	Biolog TM microtitre plates, showing colour development (extent of C substrate oxidation) of varying degrees across the 96 single C substrates (wells). These microtitre plates have been inoculated with soil microbes and incubated for several days.	142

CHAPTER 1

INTRODUCTION

In temperate grasslands, limited nitrogen (N), phosphorus (P) and sulphur (S) availability to plants are often key factors limiting pasture production (Moir et al., 1997; Scholefield and Oenema, 1997). In New Zealand the traditional methods to overcome these key constraints has been to topdress pastures with single superphosphate (SSP), containing P and S, to provide adequate fertility for the legume component (mainly white clover) of the sward to be the sole provider of nitrogen N through biological N fixation (Ball and Tillman, 1994). Pasture renovation with improved cool climate pasture species and regular SSP topdressing have been common in Wairarapa hill country pastures since the advent of aerial topdressing (for the last 30-50 years).

These fertiliser and land management practices are profitable but the efficiency of P and S fertiliser use, or the environmental sustainability of such practices have not been thoroughly researched (Moir et al., 1997). Few studies (e.g. Walker and Adams, 1958; Walker et al., 1959; Jackman, 1964; Gillingham et al., 1980; Lambert et al., 1988; Mackay et al., 1988; Nguyen et al., 1989; Rowarth et al., 1992; Mackay et al., 1995), provide even multiple element information on the changes in chemical soil fertility and pasture productivity that arise from long-term (> 10 years) fertiliser use

Moir (1994), and Moir et al. (1995, 1997), working on hill country pastures of the Wairarapa, have characterised the fertility status of pasture soils which had histories of long-term (30-40 years) SSP fertiliser application. Field trials were conducted, measuring pasture yield at sites varying widely in fertility status (fertiliser history), and climate (annual rainfall) regime. Results showed that both total and easily-extractable soil P, S and N had accumulated in these hill soils, as a direct result of increased fertiliser inputs (Moir, 1994; Moir et al., 1995). Pasture production was strongly influenced by both fertility status and climate. The extractable P status of the soil (Olsen, 1954) was strongly related to field dry matter yield for periods when soil moisture was non-limiting (spring). A Mitcherlich type curve best explained the spread of data. Soil analysis

indicated that as soil P status increased, the nature of soil organic N changed with an increasing proportion becoming readily mineralisable and hence more available to plants (Moir et al., 1997). The authors concluded that the influence of soil P and S fertility on the rate of N cycling in the sward was not well understood. Large increases in pasture yield occurred with increased fertiliser (SSP) use, but the rate and extent of soil N accumulation with increased SSP use in these hill soils was low. This led Moir (1994) to suggest that, increased pasture production resulted from rapid cycling of newly fixed legume N in the pasture system (i.e. rates of N fixation, legume and grass growth and decomposition of soil OM increased with increasing soil fertility).

Given the seasonal moisture limitations in areas of the Wairarapa region, spring pasture growth is critical, and often constitutes a major proportion of the annual pasture production at these hill country sites. Results of previous studies (Moir et al., 1997) have shown that even at high Olsen P values, spring pasture growth is limited by factors other than soil P supply. Such a result suggests that at high Olsen P values, spring pasture growth could be increased at these sites by increasing plant N supply at this time. In the first experimental Chapter of this thesis (Chapter 4), a field experiment is conducted to examine annual pasture yield and growth response to strategic application of nitrogen fertiliser using the field sites examined by Moir (1994).

Experiments at the sites in question have shown that pasture growth is strongly related to both soil fertility status, and climate. However, using results from field trials, it is difficult to separate the effects of climate and soil fertility status, on soil productivity. It is likely that removing the effect of climate would be advantageous when studying the effect of fertility status on the productivity of these soils. Also, in order to fully understand pasture growth responses to fertilisers on these soils, improved information on the nutrient supplying abilities of these soils is required in conjunction with a description of seasonal soil water availability. Two glasshouse studies were undertaken, and are described in Chapter 5 of this thesis. The objective of these trials is to use plant growth in the absence of climatic constraints, to determine the availability of the major growth-limiting nutrients in the 12 Wairarapa hill soils of this study.

Traditionally, researchers have also focused on pasture growth responses to fertiliser P and S applications, and often attempt to relate relative field pasture yields to indices of

soil fertility status, such as Olsen P status. However, the relationships used to best describe soil fertility and pasture growth are highly variable, often due to climatic variation (Sinclair et al., 1997). Also, there is relatively little published information on the relationship between the Olsen P test and pasture response to P fertiliser under 'farmers' field conditions in New Zealand (Sinclair et al., 1997). Moir et al. (1997) reported a strong correlation between Olsen P status and spring pasture growth, but found little correlation between annual pasture growth and Olsen P, presumably due to the variable effect of climate (soil moisture limitations) across field sites. When attempting to estimate or model pasture growth under field conditions, it would therefore appear logical to incorporate site-specific climate effects. There is a need for better methodologies which incorporate the effect of climate into the estimation of field pasture production. In Chapter 6, a pasture growth model is described, and tested. This model uses site-specific climate data, in combination with a proportionality constant dependent on soil fertility, to model daily pasture growth at the 12 Wairarapa field sites. Relationships between evapotranspiration rates and soil fertility status are discussed in this Chapter.

In addition to measures of soil productivity, researchers are currently seeking indices of soil health and quality. Whilst current soil fertility indicators, such as Olsen P, seem appropriate for adaptation to this purpose, much research is being conducted to develop and evaluate suitable indicators of soil biological health and quality (Bardgett et al., 1999; Sparling and Schipper, 1999). The sheep and beef grazed hill soils represented in the trial sites described in this thesis, differing only in fertiliser history and soil moisture regime, provide a single landuse suite of soils with which to examine the functional diversity of soil microflora in pasture soils. In Chapters 7 and 8 the BiologTM microtitre plating system is used to study the carbon substrate use characteristics of culturable microbial populations isolated from the 12 hill country soils examined in this study. Soil microbial C substrate use patterns are discussed in relation to indices of soil fertility, and the applications of this technique for soil health and quality assessment, and for research purposes.

In summary, this thesis comprises nine Chapters. Following this introduction, a concise literature review is presented in Chapter 2, and site description and soil fertility characterisation presented in Chapter 3. The pasture response of these hill soils to low-

level strategic N fertiliser applied in spring is presented in Chapter 4, including a current economic analysis of N fertilisation on sheep and beef hill country. The results and interpretations of two glasshouse plant growth studies are presented in Chapter 5, which cover the plant-available nutrient-supplying power of the study soils. In Chapter 6, a pasture production model is presented, which incorporates climatic and soil fertility data in the estimation of field pasture growth. Investigations into the microbial functional diversity and carbon substrate use characteristics (using the BiologTM microtitre plating system) across this range of Wairarapa hill soils are presented in Chapters 7 and 8. These microbial studies include discussion on the development and evaluation of methodology for using BiologTM plates with soil extracts as inocula. Finally, the relationships between substrate use patterns, soil fertility indices, and soil moisture regimes are explored for potential use as soil quality and health indicators.

REFERENCES

- Ball, P.R. and Tillman, R.W., 1994. Efficient use of nutrients in the intensive pastoral farming industry in New Zealand. In: L.D. Currie and P. Loganathan (Editors), *The efficient use of fertilisers in a changing environment: Reconciling productivity with sustainability*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 96-117.
- Bardgett, R.D., Lovell, R.D., Hobbs, P.J. and Jarvis, S.C., 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. *Soil Biology and Biochemistry*, 31: 1021-1030.
- Gillingham, A.G., Syers, J.K. and Gregg, P.E.H., 1980. Phosphorus uptake and return in grazed, steep hill pastures. II. Above-ground components of the phosphorus cycle. *New Zealand Journal of Agricultural Research*, 23(3): 323-330.
- Jackman, R.H., 1964. Accumulation of organic matter in some New Zealand soils under permanent pasture 1. Patterns of change in organic carbon, nitrogen, sulphur and phosphorus. *New Zealand Journal of Agricultural Research*, 7: 445-471.

- Lambert, M.G., Clark, D.A. and Costall, D.A., 1988. Phosphorus and sulphur accumulation after long-term superphosphate application to grazed hill pasture. In: R.E. White and L.D. Currie (Editors), *Towards the more efficient use of soil and fertiliser sulphur*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 95-100.
- Mackay, A.D., Lambert, M.G., Clark, D.A., Saggart, S. and Hedley, M.J., 1988. Pasture responses to sulphur and phosphorus application in Southern North Island hill country. In: R.E. White and L.D. Currie (Editors), *Towards the more efficient use of soil and fertiliser sulphur*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 285-291.
- Mackay, A.D., Lambert, M.G., Trollove, S.N. and Costall, D.A., 1995. Effect of long-term fertiliser use and sheep grazing on the accumulation and distribution of nitrogen, phosphorus and sulphur in a hill soil. In: L.D. Currie and P. Loganathan (Editors), *Fertiliser requirements of grazed pasture and field crops: Macro and micro nutrients*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 206-219.
- Moir, J.L., 1994. Influences of climate and fertiliser application history on various measures of soil fertility and productivity in Wairarapa hill country. Master of Agricultural Science Thesis, Massey University, Palmerston North, New Zealand, 145 pp.
- Moir, J.L., Hedley, M.J., Mackay, A.D. and Garland, C.B., 1995. Climate, soil fertility and pasture production in Wairarapa hill country. In: L.D. Currie and P. Loganathan (Editors), *Fertiliser requirements of grazed pasture and field crops: Macro and micro nutrients*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 254-260.
- Moir, J.L., Hedley, M.J., Mackay, A.D. and Tillman, R.W., 1997. The effect of fertiliser history on nutrient accumulation and plant-available nutrient supply in legume-based pasture soils. In: J.G. Buchanan-Smith, L.D. Bailey and P. McCaughey (Editors), *XVIII International Grassland Congress*. International Grassland Association, Winnipeg, Canada, pp. 10, 68-69.
- Nguyen, M.L., Rickard, D.S. and McBride, S.D., 1989. Pasture production and changes in phosphorus and sulphur status in irrigated pastures receiving long-term applications of superphosphate fertiliser. *New Zealand Journal of Agricultural Research*, 32: 245-262.

- Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate, USDA.
- Rowarth, J.S., Tillman, R.W., Gillingham, A.G. and Gregg, P.E.H., 1992. Phosphorus balances in grazed hill country pastures: the effect of slope and fertiliser input. *New Zealand Journal of Agricultural Research*, 35(3): 337-342.
- Scholefield, D. and Oenema, O., 1997. Nutrient cycling within temperate agricultural grasslands. In: J.G. Buchanan-Smith, L.D. Bailey and P. McCaughey (Editors), XVIII International Grassland Congress. International Grassland Association, Winnipeg, Canada, pp. 20, 357-370.
- Sinclair, A.G., Morrison, J.D., Smith, L.C. and Dodds, K.G., 1997. Effects and interactions of phosphorus and sulphur on a mown white clover/ryegrass sward 3. Indices of nutrient adequacy. *New Zealand Journal of Agricultural Research*, 40: 297-307.
- Sparling, G. and Schipper, L., 1999. Soil quality changes under intensive and extensive land use in New Zealand. In: L.D. Currie (Editor), *Best Soil Management Practices for Production*. Fertiliser and Lime Research Centre, Massey University, New Zealand, pp. 53-63.
- Walker, T.W. and Adams, A.F.R., 1958. Studies on soil organic matter: 1. Influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulphur, and organic phosphorus in grassland soils. *Soil Science*, 85: 307-318.
- Walker, T.W., Thapa, B.K. and Adams, A.F.R., 1959. Studies on soil organic matter: 3. Accumulation of carbon, nitrogen, sulphur, organic and total phosphorus in improved grassland soils. *Soil Science*, 87: 135-140.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

A comprehensive review of literature, relating to soils and climate of the Wairarapa, and pasture production and soil fertility on clover-based pastures developed through single superphosphate applications was presented in Moir (1994). This review is presented in Appendix 2.1 of this thesis, and readers are directed to this Appendix for a detailed review of these topics.

This literature review Chapter aims to familiarise the reader with a selected group of recent papers not discussed in Moir (1994), and other papers considered to be key to the research presented in this thesis. However, the coverage of literature in this Chapter is brief. Literature review in this thesis is contained within the introductions of individual Chapters, and relates to the results and discussion presented in each Chapter. The main objective of this Chapter is to set the scene for the experimental Chapters and to portray the more in-depth literature reviews in experimental Chapters in a more holistic setting.

2.2 NEW ZEALAND HILL SOILS

In an attempt to describe the physical resource of hill country soils, some workers have documented the variation in hill soils across landscapes.

Campbell (1975) examined the pattern of variation in hill soils of the Wanganui region. Comparing soils of intermediate slope, Campbell showed a sequence of soil development, in the order of low to high land surfaces. Higher land surfaces were associated with greater profile development, and chemical weathering and leaching. It was concluded that cyclic landform development, combined with the range of soil properties related to slope variants, result in large (complex) variation in steepland soil pattern.

Earlier, Campbell (1973), working in the same field area, reported on the pattern of soil variation in a single hill slope. In this work the author describes 4 distinct hill soil units:

1. Ridge - high profile development, low base saturation.
2. Intermediate steep slope - less developed, less weathered (than 1.).
3. Eroded slope - weakly developed and weathered.
4. Accumulation slope - accretion of up-slope material through down-slope movement.

Other workers (e.g. Hall and Olson, 1991) have used similar hillslope models to give comprehensive descriptions of the pattern of soil variation on a single slope. These authors apply the single slope model to predict the variability of soils in the landscape, and provide excellent diagrams to explain the processes involved in hill soil development (Hall and Olson, 1991).

Erosion contributes not only to soil formation on hill slopes (Trustrum et al., 1983), but has long been identified as a major factor limiting agricultural production on hill country soils (Gibbs, 1962). More recently, Lambert et al. (1984) have studied the effect of soil slip erosion on Wairarapa hill pastures. Working on hill country near Masterton, pasture production (and other variables) was measured on uneroded hill slopes, and compared with that on slip scars of 4 ages. The results of this study suggested that pasture production on hill soils formed under pastoral agriculture was unlikely to return to the production levels supported by soils formed under the original forest vegetation. It was also shown that soil N and C contents increased with time since slipping, but were much lower for soils on all slip scars than soils on uneroded sites (Lambert et al., 1984).

Some hill soil variables, and their effect on winter/spring pasture growth were studied by Ledgard et al. (1982). Working on hill country in the Waikato region, it was shown that aspect and land slope have major influence on winter and spring pasture production. In particular, soil N, C and water holding capacity were shown to decline with increasing slope. Aspect and slope also have marked effects on soil moisture status (Gillingham, 1973), and the development of topsoil fertility (Gillingham and During, 1973)

These factors, along with the spatial variation of soil parent material must be considered when selecting sites for study in hill pastures. Parent material of soils in this study have been discussed previously (Moir, 1994), and are given in Appendix 2. For more detailed information on the geology of the Wairarapa region, readers are directed to Eden and Parfit (1992), and Moore and Speden (1984).

2.3 NUTRIENT CYCLING IN GRAZED PASTURES

In addition to the influences of slope, aspect, soil parent material and extent of topsoil development on hill soil fertility, the use of fertiliser and the behaviour of the grazing animal have profound effects on soil nutrient content and pasture production (Gillingham et al., 1980a). Nutrient cycling within grazed soil/plant/animal systems is complex, and central to the understanding of pasture soils. Variations in land slope influence animal grazing and camping behaviours, leading to increased nutrient depletion on steeper slopes, with transfer of nutrients in excreta to track, and low slope camping areas (Gillingham, 1980; Gillingham et al., 1980b; Rowarth et al., 1988; Saggar et al., 1990; Rowarth et al., 1992a; Rowarth et al., 1992b).

A recent, and comprehensive review of nutrient cycling and soil fertility on grazed pasture soils was presented by Haynes and Williams (1993). These authors focus on the dominant effect of grazing animals on the movement of nutrients through the soil/plant/animal system. In particular, they discuss nutrient loss from animal urine and dung patches, where nutrients are deposited in very high concentrations. Virtually all P is returned in animal dung, while K is returned predominantly in urine. N and S are excreted in significant proportions in both faeces and urine.

In relation to the role of fertiliser, Haynes and Williams (1993) comment that the application of fertilisers and lime is necessary for pasture development, to boost natural soil fertility to a level capable of supporting high fertility, high producing plants. Improving pastures in this manner often results in an increase in soil organic matter. The authors emphasise that organic C, N, P and S do not necessarily accumulate at the same rate at a particular site, and soil organic matter C:N:P:S ratios can vary due to soil properties and climatic factors. A gradual decline in soil C:N ratio during pasture development (Walker et al., 1959; Jackman, 1964) is said to result from soil N

accumulating at a faster rate than soil C. It is further suggested that high soil OM content is linked with high microbial activity in pasture soils.

Haynes and Williams (1993) concluded that improved pasture species, used in high production pastures, are generally adapted to high fertility soil conditions. Also, nutrient transformations in excretal patch areas are of central importance to the fertility and productivity of grazed pastures. These authors suggest that there are two major areas of soil fertility where current knowledge is lacking:

1. The storage and transfer of nutrients to and from soil labile organic pools, and
2. The development of dynamic models of nutrient cycling under grazed pasture, that include information on the rates of recycling of key growth limiting nutrients.

Increased knowledge in both these areas of nutrient cycling, and the influence of different climates and land management practice on nutrient cycling is required if the sustainability or environmental impact of hill pastures is to be judged.

Nutrient cycling in grazed pasture systems has also been examined in the Northern hemisphere by Scholefield and Oenema (1997). These researchers suggest that nutrient cycles within soil/plant/animal systems, but with particular respect to nitrogen, are inefficient. However, the authors suggest that the low-input legume-based systems of the Southern hemisphere use N more efficiently than grass only swards, receiving mineral N fertiliser. Legume based swards are commonly N deficient, and soil mineral N is low (leading to less N leaching losses). The C:N ratio of plant tissue is suggested as a strong contributing factor to rates of N mineralisation, and rates of N turnover in grazed pastures (Wedin and Tilman, 1990). Scholefield and Oenema (1997) suggest that mineralisation of N from plant residues is a major area of importance for future research. The efficiency of N cycling in New Zealand hill pastures is mostly unknown because of the difficulty in quantifying inputs through N fixation, and losses by denitrification and leaching (White, 1991).

An active and diverse population of soil biota are essential to nutrient cycling and the sustainability of pasture soils. The effect of management on soil biota, and microbial

processes in hill soils has been comprehensively researched by Bardgett and co-workers (Bardgett et al., 1993; Lovell et al., 1995; Bardgett et al., 1996). In summary, these studies have examined the effects of long-term fertiliser N use, soil drainage, and also the reduction of intensity of management (cessation of fertiliser, lime and grazing sheep) on various indicators of soil microbial numbers and activities. Results indicated that soil microbial biomass N and C were substantially greater in treatment soils receiving no N fertiliser. However, over 4 times less culturable bacteria were in the no N treatment cf. N fertilised soils (Lovell et al., 1995). Later, it was shown that the soil microbial biomass was significantly reduced following the removal of fertiliser, and grazing sheep. This was attributed in part to a fall in soil pH (to pH 4.7). Unfertilised grazed treatments had higher culturable soil fungal numbers, and a narrower fungal:bacterial ratio. This finding was attributed to the dominance of fungal growth on sheep dung on the low pH soil.

More recently, these workers have concentrated on how hill soil microbial populations change with season, and soil fertility status (Bardgett et al., 1997; Bardgett et al., 1999). In both studies, microbial biomass and activity were greatest in summer and lowest in winter. The long-term removal of sheep grazing resulted in significant reductions in microbial biomass, and activity. The abundance of active soil fungi (and fungal:bacterial ratio) was significantly reduced by the removal of grazing sheep. In the second study (Bardgett et al., 1999), microbial activity was lowest on high fertility (most intensively managed) soils, and highest on low fertility, less intensively managed soils. Overall, the findings of these workers support the theory that fungi play a more significant role in soil biological processes of low input, unfertilised grasslands, than in intensively managed systems (Bardgett et al., 1999). To date, there has been no systematic study of microbial functional diversity in New Zealand pastures that vary in aspects of soil fertility (See Chapters 7 and 8).

2.4 SOIL FERTILITY AND THE EFFICIENCY OF FERTILISER USE

2.4.1 Phosphorus and Sulphur

The effects of long-term application of single superphosphate (SSP) fertiliser application to North Island hill soils has been documented by several workers.

The effects of withholding SSP application have been extensively studied at the Whatawhata hill country research station (Gillingham et al., 1990; Rowarth and Gillingham, 1990). Following a 4 year cessation of P application, pasture production, botanical composition and Olsen P status were little affected, although pasture quality deteriorated (at Olsen P values $< 10 \mu\text{gPg}^{-1}$). Level sites and easy slopes were shown to produce 70% of total pasture DM production. It was suggested that pasture growth on moderate and steep slopes was limited by factors other than P (Rowarth and Gillingham, 1990).

Rowarth et al. (1992a) have investigated soil P fractions, and P balances (Rowarth et al., 1992b) at the Whatawhata hill country research station. Olsen P and total P were shown to increase with increasing SSP addition ($10\text{-}100 \text{ kgPha}^{-1}\text{yr}^{-1}$ for 4 years), and decreased with increasing profile depth and slope. Non-occluded P was the largest inorganic P fraction, and was suggested to be involved in active P cycling and P transfer (Rowarth et al., 1992a). When considering P cycling, P was shown to accumulate on low slopes ($< 10^\circ$), and to decline on all other slopes, as a direct result of faecal P transfer (Rowarth et al., 1992b).

The effect of traditional SSP applications on herbage accumulation and pasture botanical composition has been studied by Lambert et al. (1983, and 1986 respectively) at the AgResearch 'Ballantrae' (moist hill country) facility. Grazed farmlets received low ($125 \text{ kgha}^{-1}\text{yr}^{-1}$ SSP) or high ($630 \text{ kgha}^{-1}\text{yr}^{-1}$ SSP) fertiliser inputs. Results showed that high fertility (HF) sites had higher annual dry matter (DM) production than low fertility (LF) sites, and that sward botanical composition was strongly influenced by fertility status. HF pastures had a higher content of ryegrass and legumes than LF pastures, and a smaller content of low fertility tolerant grasses and weed species.

In recent research conducted at the AgResearch hill country station 'Ballantrae', Lambert et al. (1998, 2000) have investigated the effects of long-term SSP application on the nutrient accumulation and organic matter content of hill soils. These workers showed that Olsen P status increased with increasing P application regime. However, responsiveness of the Olsen P test to increasing P input (13 kgPha^{-1} added to raise Olsen P by 1 unit) was lower ($4\text{-}7 \text{ kgPha}^{-1}$ Olsen unit $^{-1}$) than that expected. Trial sites were

considered to be in a developmental “pasture improvement” phase when trials were conducted, and the authors use this reasoning to explain the lack of Olsen P response to fertiliser P input. In contrast to other workers (Walker et al., 1959; Jackman, 1964; Haynes and Williams, 1993), Lambert et al. (2000) suggest that soil C content has decreased as a result of long-term SSP application.

Conducting field studies at the Massey University “Riverside” property in the Wairarapa, Mackay et al. (1990) examined the effect of SSP and lime application on pasture production. One experiment compared the effect of lime at 4 application rates (0, 1.25, 2.5 and 5 tha^{-1}) and also SSP at 4 application rates (0, 125, 250 and 500 kg ha^{-1}). Soils had an Olsen P of 8 μgPg^{-1} , and pH of 5.4. Results suggested that SSP dressings above 250 $\text{kg ha}^{-1}\text{yr}^{-1}$ produced little extra growth, indicating that this rate was at near maintenance requirements for this site (maximum yield = 9-10 $\text{tDM ha}^{-1}\text{yr}^{-1}$). Large increases in seasonal (spring) pasture production were reported at the 250 $\text{kg ha}^{-1}\text{yr}^{-1}$ SSP rate. Increasing lime applications increased pasture growth, but not to the extent seen for SSP treatments.

In a glasshouse based experiment, Mackay et al. (1995) used a subtractive nutrient technique to study the effect of long-term fertiliser use on nutrient accumulation, and supply of N, P and S in a hill soil. Soils were sampled from 3 slope classes and aspects at the farmlets¹ at ‘Ballantrae’, which had fertiliser histories ranging from no fertiliser inputs (Olsen P < 8 μgPg^{-1}) to sites which had received 375 (HF, Olsen P 27 μgPg^{-1}) $\text{kg SSPha}^{-1}\text{yr}^{-1}$ for 16 years². The key finding of Mackay et al. (1995) was that there were only relatively small differences in the plant-available N pool (24 v. 31 kgN ha^{-1}) between LF and HF farmlets, which were not consistent with the large measured difference in plant growth.

This result suggests that the amount of N in the labile pool is far less important than the quantity, quality and rate at which organic matter passes through the pool (Mackay et al., 1995). The role of nutrient recycling rates in pasture production is an area that requires further investigation on a wider range of soils (Chapter 5).

¹ Pasture response to P and S fertiliser applications at these sites was reported by Mackay et al. (1988). In general, results indicated that pastures were P responsive at all but the high fertility treatment.

² The field trial results on the effect of long-term SSP applications on P and S accumulation at these sites (Lambert et al., 1988) has been discussed in a previous literature review (Moir, 1994; Appendix 2).

From the farmer's point of view, the outcome of research into pasture soil fertility and fertiliser use effectiveness should be better prediction of pasture growth rates and nutrient requirements. In a study of 17 long-term field trials, measuring the effects of rates and forms of P fertiliser, Sinclair et al. (1997a) investigated the relationship between pasture DM yield and soil Olsen P status. Results from this study failed to demonstrate that relative yield (RY) at any site, or in any year could be reliably assessed from a common relationship between RY and the Olsen P test (Sinclair et al., 1997a). Variability in the RY v. Olsen P relationship was large within years at individual sites, and also between years within sites.

In fertiliser recommendation modeling, soil Olsen P status is used as a key indicator of likely pasture production (Metherell, 1995). Therefore lack of robustness of the relationship between pasture yield and soil Olsen P status data from mown plot research trials is of concern. Furthermore, the relationships between dry matter yield and Olsen P status have not been compared to those observed on hill farms where pasture production has been influenced by 40-60 years of fertiliser application and stock management practices. The erratic patterns of pasture response to Olsen P over time (Sinclair et al., 1997a) were suggested to result from (within site) climatic fluctuations, and variation in the botanical composition of swards. The interaction between climatic influences and soil fertility on pasture yield have been neglected by hill pasture agronomists, and this area warrants further study (Chapter 6).

The sites selected for work in this thesis may provide comparison of research trial Olsen P / pasture production response curves with those on farmed soils, and by being situated in differing rainfall regimes, it may be possible to investigate climate effects on pasture growth response to soil fertility status.

2.4.2 Nitrogen

A simplified N cycle presented by Moir (1994) suggested that N accumulation in hill soils is very slow, and that such pastures may benefit from strategic fertiliser N application. A number of trials have been reported for lowland pasture, although few studies have examined pasture responses to N fertiliser applications on hill country.

There is a serious lack of quantitative information on nitrogen relationships in hill farming systems (Ball et al., 1982).

A recent study examined N response on dry hill country in the Southern Hawke's Bay (Gillingham et al., 1998). Treatments were low P (Olsen P $9 \mu\text{gPg}^{-1}$) or high P (Olsen P $29 \mu\text{gPg}^{-1}$), receiving nil or $30 \text{ kgNha}^{-1}\text{yr}^{-1}$ applied in winter as urea. Pasture was very responsive to both N and P application on all areas, but best N responses were obtained on steep north-facing aspects of low P status (Gillingham et al., 1998). From these preliminary results, the authors suggest that the most efficient fertiliser use on North-facing steep slopes of seasonally dry hill country would be strategic N fertilisation, and only low (maintenance) levels of P and S fertiliser.

In an earlier N response study, Ledgard et al. (1983) reported highly variable pasture growth response to N fertiliser. These workers reported that no individual pasture or soil parameter was strongly related to pasture DM response to N addition.

Examining the effects of late autumn N applications at 'Ballantrae', Lambert and Clarke (1986) reported N responses of 28 kgDMkg^{-1} N applied. Similar N responses were reported at low and high P fertility. Low fertility sites had an Olsen P status of $8 \mu\text{gPg}^{-1}$ and a pH of 5.1, while HF sites had an Olsen status of $15 \mu\text{gPg}^{-1}$ and pH of 5.7.

Factors controlling N response in hill pastures are not sufficiently clear that yield increase to N application can be predicted. The opportunity is taken in Chapter 4 to study this aspect further.

2.5 IMPROVED DIAGNOSTICS WITH HERBAGE ANALYSIS

Over the last decade the adequate nutrition of pasture has been extensively researched in New Zealand.

Morton et al. (1998) investigated adequate P and S nutrition in pasture on a LF (Olsen P $5 \mu\text{gPg}^{-1}$) recent alluvial soil in Southland. A factorial P x S design was used, with 5 rates of P (MCP at 0, 10, 20, 40 and 80 kg Pha^{-1}) and 5 rates of S (gypsum at 0, 5, 10, 20 and 40 kg Sha^{-1}). Results indicated that adequate (balanced) nutrition of clovers

occurred at S:P ratios of 0.81-0.93. At 95% RY, adequate nutrition was achieved at a clover P:N (x 100) ratio of 7.10, and an S:N (x 100) ratio of 5.64.

Similar experiments were conducted on another Southland site, with comparable low fertility, and soil type (Sinclair et al., 1996a, 1996b and 1997b). Methods were identical to those described by Morton et al. (1998), except for rates of gypsum S, which were 0, 7.5, 15, 30 and 60 kg Sha^{-1} in this trial. In the first of 3 papers (Sinclair et al., 1996a), clover and ryegrass DM production were measured. Results showed that clover was more responsive to S than P, and ryegrass more responsive to P than S. A two-variable Mitscherlich type response function best described patterns of DM response to combinations of P and S fertiliser (Sinclair et al., 1996a).

The second paper of the series reported clover P, N and S ratios in relation to balanced plant nutrition (Sinclair et al., 1996b). Considering a large and comprehensive data set, the authors concluded that the S:P ratio corresponding to balanced nutrition in clover was in the range of 0.70-0.80 (S:P).

In the third, and final paper, Sinclair et al. (1997b) considered indices of nutrient adequacy in clover herbage. In this study the authors examined the relationships between relative yields of pasture and P and S applications, and herbage P, S and N concentrations. It was shown that the herbage P:N ratio was a more sensitive indicator of P deficiency than S:N was of S deficiency (Sinclair et al., 1997b). From this study these workers concluded that balanced nutrient response curves and corresponding nutrient adequacy indices were more relevant to farming than single nutrient response curves with other nutrients non-limiting (Sinclair et al., 1997b). These ideas are extended in the discussion of herbage analyses in this thesis.

2.6 SUSTAINABLE LAND MANAGEMENT

The long-term sustainability of current hill country management practices is a topical subject currently undergoing discussion in literature. Sustainable management of hill land has been investigated by Mackay et al. (1993). Using the guidelines of the N.Z. Resource Management Act, these workers suggested that current practices do not meet the criteria of the act, especially where soil loss / land degradation is concerned. The

issue of socio-economic sustainability is also questioned. The authors conclude that a greater understanding of the interaction between land resources (including soil and climatic properties) and land use practices is required. Monitoring of on-site biophysical indicators was suggested as key on-going research.

The concept of biophysical indicators of sustainability was reported by Lambert et al. (1996). A selected range of analyses were conducted and assessed as suitable bioindicators, including; vegetation, soil, climate and stocking policies. The most suitable indicators of biophysical resource status were sward botanical composition, and earthworm mass (Lambert et al., 1996). However, the authors concluded that much research is required in order to identify and fully test suitable indicators of biophysical resource.

A number of interesting issues were raised at the International Conference on Sustainable Land Management in 1991. The biophysical resource of hill country was described by Wedderburn (1991) as being a complex mosaic of habitats, ranging in fertility, moisture status, grazing management, soil biota and pests. In these legume-based pasture systems, continual fertiliser additions, to counter inevitable nutrient losses from the grazed pasture systems, are required to maintain current pastoral farming (Goh and Nguyen, 1991; Williams and Haynes, 1991). These workers suggested that in future, it may be possible to use nutrients more efficiently than at present. National nutrient balances were considered by White (1991), who concluded that P and S are applied to N.Z. pastures in adequate quantities, K is being depleted, and that current lime applications are inadequate to combat the rate of soil acidification. The aim of this thesis is to provide data to aid informed comment on the sustainability of hill pastures.

2.7 CONCLUSIONS

The pattern and variation of hill soils is complex, both in the landscape, and within single slopes, and aspects. The complex land topography has a marked influence on soil development and soil fertility and nutrient cycling within grazed pasture systems. A better understanding of nutrient cycling within grazed pasture systems is required to predict the nutrient requirements of soils and judge the long-term sustainability of their land management practices. Particular gaps in knowledge are the comparisons of the

relationship between the diagnostic soil test / pasture growth response curves derived on research plots and the actual relationship observed for these functions (curves) on farmed hill pastures.

The dynamic influence of climate on the rate of pasture growth under differing soil fertility regimes is of real interest to researchers, and producers. Prediction of field pasture yields using single indices of soil fertility status (e.g. Olsen P) is confounded by the effect of climate. Pasture production models need to incorporate the effect of climate on pasture growth.

The long-term sustainability of traditional hill country management practices is of major interest to both researchers, and land governing authorities, both in terms of soil nutrient reserves, and soil quality and health. However, the impact of farm management practices on the quality and health of hill soils is not well understood. Suitable indicators of soil quality and health must be developed in order to provide better assessment of the environmental impact of management practices such as traditional fertiliser policies.

The hill pasture sites studied by Moir (1994) are ideally suited for the conduct of experiments to provide some knowledge to fill these gaps. This thesis reports the conduct and findings of those experiments.

2.8 REFERENCES

- Ball, P.R., Luscombe, P.C. and Grant, D.A., 1982. Nitrogen on hill country. In: P.B. Lynch (Editor), Nitrogen fertilisers in New Zealand agriculture. New Zealand Institute of Agricultural Science, Wellington, New Zealand, pp. 133-147.
- Bardgett, R.D., Frankland, J.C. and Whittaker, J.B., 1993. The effects of agricultural management on the soil biota of some upland grasslands. *Agriculture, Ecosystems and Environment*, 45: 25-45.
- Bardgett, R.D., Hobbs, P.J. and Frostegård, Å., 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biology and Fertility of Soils*, 22: 261-264.

- Bardgett, R.D., Leemans, D.K., Cook, R. and Hobbs, P.J., 1997. Seasonality of the soil biota of grazed and ungrazed hill grasslands. *Soil Biology and Biochemistry*, 29(8): 1285-1294.
- Bardgett, R.D., Lovell, R.D., Hobbs, P.J. and Jarvis, S.C., 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. *Soil Biology and Biochemistry*, 31: 1021-1030.
- Campbell, I.B., 1973. Patterns of variation in steepland soils: Variation on a single slope. *New Zealand Journal of Science*, 16: 413-434.
- Campbell, I.B., 1975. Patterns of variation in steepland soils: Soil differences in complex topography. *New Zealand Journal of Science*, 18: 53-66.
- Eden, D.N. and Parfitt, R.L., 1992. Amounts and sources of phosphate in hill country rocks, South-eastern North Island, New Zealand. *Australian Journal of Soil Research*, 30: 357-370.
- Gibbs, H.S., 1962. Steepland soils and their problems, International Soil Science Conference. New Zealand Soil Bureau, New Zealand, pp. 2-16.
- Gillingham, A.G., 1973. Influence of physical factors on pasture growth on hill country. In: W.G. Thurston (Editor), *Proceedings of the New Zealand Grassland Association*. New Zealand Grassland Association, Te Kuiti, New Zealand, pp. 77-85.
- Gillingham, A.G., 1980. Hill-country topdressing. Application, variability and fertility transfer. *New Zealand Journal of Agriculture*, 141: 39-43.
- Gillingham, A.G. and During, C., 1973. Pasture production and transfer of fertility within a long-established hill pasture. *New Zealand Journal of Experimental Agriculture*, 1(3): 227-232.
- Gillingham, A.G., Gray, M.H. and Smith, D.R., 1998. Pasture responses to phosphorus and nitrogen fertilisers on dry hill country. In: M. Baker (Editor), *Proceedings of the New Zealand Grassland Association*. New Zealand Grassland Association, Nelson, New Zealand, pp. 135-140.
- Gillingham, A.G., Richardson, S., Power, I.L. and Riley, J., 1990. Long term effects of withholding phosphate application on North Island hill country: Whatawhata. In: M.J. Baker (Editor), *Proceedings of the New Zealand Grassland Association*. New Zealand Grassland Association, Wanganui, New Zealand, pp. 11-16.

- Gillingham, A.G., Syers, J.K. and Gregg, P.E.H., 1980a. Phosphorus uptake and return in grazed, steep hill pastures. II. Above-ground components of the phosphorus cycle. *New Zealand Journal of Agricultural Research*, 23(3): 323-330.
- Gillingham, A.G., Tillman, R.W., Gregg, P.E.H. and Syers, J.K., 1980b. Uptake zones for phosphorus in spring by pasture on different strata within a hill paddock. *New Zealand Journal of Agricultural Research*, 23(1): 67-74.
- Goh, K.M. and Nguyen, M.L., 1991. Fertiliser needs for sustainable agriculture in New Zealand. In: P.R. Henniques (Editor), *International Conference on Sustainable Land Management*, Napier, Hawkes Bay, New Zealand, pp. 119-133.
- Hall, G.F. and Olson, C.G., 1991. Predicting variability of soils from landscape models., *Spatial Variabilities of Soils and Landforms*. Special Publication No 28. Soil Science Society of America, Madison, USA, pp. 9-24.
- Haynes, R.J. and Williams, P.H., 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Advances in Agronomy*, 49: 120-199.
- Jackman, R.H., 1964. Accumulation of organic matter in some New Zealand soils under permanent pasture 1. Patterns of change in organic carbon, nitrogen, sulphur and phosphorus. *New Zealand Journal of Agricultural Research*, 7: 445-471.
- Lambert, M.G., Barker, D.J., Mackay, A.D. and Springett, J., 1996. Biophysical indicators of sustainability of North Island hill pasture systems. In: M.J. Baker (Editor), *Proceedings of the New Zealand Grassland Association*. New Zealand Grassland Association, Waitangi, New Zealand, pp. 31-36.
- Lambert, M.G. and Clark, D.A., 1986. Effects of late-autumn nitrogen application on hill country pastures and sheep production. In: J.L. Brock (Editor), *Proceedings of the New Zealand Grassland Association*. New Zealand Grassland Association, Whangarei, New Zealand, pp. 211-215.
- Lambert, M.G., Clark, D.A. and Costall, D.A., 1988. Phosphorus and sulphur accumulation after long-term superphosphate application to grazed hill pasture. In: R.E. White and L.D. Currie (Editors), *Towards the more efficient use of soil and fertiliser sulphur*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 95-100.
- Lambert, M.G., Clark, D.A., Grant, D.A. and Costall, D.A., 1986. Influence of fertiliser and grazing management on North Island moist hill country 2. Pasture botanical composition. *New Zealand Journal of Agricultural Research*, 29(1): 1-10.

- Lambert, M.G., Clark, D.A., Grant, D.A., Costall, D.A. and Fletcher, R.H., 1983. Influence of fertiliser and grazing management on North Island moist hill country 1. Herbage accumulation. *New Zealand Journal of Agricultural Research*, 26: 95-108.
- Lambert, M.G., Trustrum, N.A. and Costall, D.A., 1984. Effect of slip erosion on seasonally dry Wairarapa hill pastures. *New Zealand Journal of Agricultural Research*, 27: 57-64.
- Lambert, M.G., Mackay, A.D. and Costall, D.A., 1998. Long-term fertiliser application and fertility of hill soils. In: M. Baker (Editor), *Proceedings of the New Zealand Grassland Association*. New Zealand Grassland Association, Nelson, New Zealand, pp. 63-67.
- Lambert, M.G., Clark, D.A., Mackay, A.D. and Costall, D.A., 2000. Effects of fertiliser application on nutrient status and organic matter content of hill soils. *New Zealand Journal of Agricultural Research*, 43: 127-138.
- Ledgard, S.F., Brier, G.J., Gillingham, A.G. and Sheath, G.W., 1983. Influence of some soil and pasture components on the growth of hill country pastures II. Response to nitrogen fertiliser. *New Zealand Journal of Experimental Agriculture*, 11: 251-256.
- Ledgard, S.F., Sheath, G.W. and Gillingham, A.G., 1982. Influence of some soil and pasture components on the growth of hill country pastures I. Winter and spring production. *New Zealand Journal of Experimental Agriculture*, 10: 239-244.
- Lovell, R.D., Jarvis, S.C. and Bardgett, R.D., 1995. Soil microbial biomass and activity in long-term grassland: Effects of management changes. *Soil Biology and Biochemistry*, 27(7): 969-975.
- Mackay, A.D., Lambert, M.G., Clark, D.A., Saggar, S. and Hedley, M.J., 1988. Pasture responses to sulphur and phosphorus application in Southern North Island hill country. In: R.E. White and L.D. Currie (Editors), *Towards the more efficient use of soil and fertiliser sulphur*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 285-291.

- Mackay, A.D., Lambert, M.G., Trolove, S.N. and Costall, D.A., 1995. Effect of long-term fertiliser use and sheep grazing on the accumulation and distribution of nitrogen, phosphorus and sulphur in a hill soil. In: L.D. Currie and P. Loganathan (Editors), Fertiliser requirements of grazed pasture and field crops: Macro and micro nutrients. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 206-219.
- Mackay, A.D., Tillman, R.W., Parker, W.J. and Barker, D.J., 1990. Effect of superphosphate, lime, and cocksfoot on summer-dry hill country pasture production. In: M.J. Baker (Editor), Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, Wanganui, New Zealand, pp. 131-134.
- Mackay, A.D., Wedderburn, M.E. and Lambert, M.G., 1993. Sustainable management of hill land. In: M.J. Baker (Editor), Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, Masterton, New Zealand, pp. 170-176.
- Metherell, A.K., 1995. A model for phosphate requirements for pastures - Incorporating dynamics and economics. In: L.D. Currie and P. Loganathan (Editors), The efficient use of fertilisers in a changing environment. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 18-37.
- Moir, J.L., 1994. Influences of climate and fertiliser application history on various measures of soil fertility and productivity in Wairarapa hill country. Master of Agricultural Science Thesis, Massey University, Palmerston North, New Zealand, 145 pp.
- Moore, P.R. and Speden, I.G., 1984. The Early Cretaceous (Albian) sequence of eastern Wairarapa, New Zealand. NZGS Bulletin 97. New Zealand Geological Survey, Wellington, 98 pp.
- Morton, J.D., Sinclair, A.G., Morrison, J.D., Smith, J.C. and Dodds, K.G., 1998. Balanced and adequate nutrition of phosphorus and sulphur in pasture. New Zealand Journal of Agricultural Research, 41: 487-496.
- Rowarth, J.S. and Gillingham, A.G., 1990. Effects of withholding fertiliser on pasture production and phosphate cycling in hill country pasture. In: M.J. Baker (Editor), Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, Wanganui, New Zealand, pp. 17-20.

- Rowarth, J.S., Gillingham, A.G., Tillman, R.W. and Syers, J.K., 1988. Effects of season and fertiliser rate on phosphorus concentrations in pasture and sheep faeces in hill country. *New Zealand Journal of Agricultural Research*, 31(2): 187-193.
- Rowarth, J.S., Gillingham, A.G., Tillman, R.W. and Syers, J.K., 1992a. Effect of phosphate fertiliser addition and land slope on soil phosphate fractions. *New Zealand Journal of Agricultural Research*, 35(3): 321-327.
- Rowarth, J.S., Tillman, R.W., Gillingham, A.G. and Gregg, P.E.H., 1992b. Phosphorus balances in grazed hill country pastures: the effect of slope and fertiliser input. *New Zealand Journal of Agricultural Research*, 35(3): 337-342.
- Saggar, S., Hedley, M.J., Gillingham, A.G., Rowarth, J.S., Richardson, S., Bolan, N.S., and Gregg, P.E.H., 1990. Predicting the fate of fertiliser sulphur in grazed hill country pastures by modelling the transfer and accumulation of soil phosphorus. *New Zealand Journal of Agricultural Research*, 33(1): 129-138.
- Scholefield, D. and Oenema, O., 1997. Nutrient cycling within temperate agricultural grasslands. In: J.G. Buchanan-Smith, L.D. Bailey and P. McCaughey (Editors), XVIII International Grassland Congress. International Grassland Association, Winnipeg, Canada, pp. 20, 357-370.
- Sinclair, A.G., Johnstone, P.D., Smith, L.C., Roberts, A.H.C., O'Connor, M.B., and Morton, J.D., 1997a. Relationship between pasture dry matter yield and soil Olsen P from a series of long-term field trials. *New Zealand Journal of Agricultural Research*, 40: 559-567.
- Sinclair, A.G., Morrison, J.D., Smith, L.C. and Dodds, K.G., 1996a. Effects and interactions of phosphorus and sulphur on a mown white clover/ryegrass sward 2. Concentrations and ratios of phosphorus, sulphur, and nitrogen in clover herbage in relation to balanced plant nutrition. *New Zealand Journal of Agricultural Research*, 39: 435-445.
- Sinclair, A.G., Morrison, J.D., Smith, L.C. and Dodds, K.G., 1997b. Effects and interactions of phosphorus and sulphur on a mown white clover/ryegrass sward 3. Indices of nutrient adequacy. *New Zealand Journal of Agricultural Research*, 40: 297-307.
- Sinclair, A.G., Smith, L.C., Morrison, J.D. and Dodds, K.G., 1996b. Effects and interactions of phosphorus and sulphur on a mown white clover/ryegrass sward 1. Herbage dry matter production and balanced nutrition. *New Zealand Journal of Agricultural Research*, 39: 421-433.

- Trustrum, N.A., Lambert, M.G. and Thomas, V.J., 1983. The impact of soil slip erosion on hill country pasture production in New Zealand, Second International Conference on Soil Erosion and Conservation. Soil Conservation Society of America, Honolulu, Hawaii, pp. 83-89.
- Walker, T.W., Thapa, B.K. and Adams, A.F.R., 1959. Studies on soil organic matter: 3. Accumulation of carbon, nitrogen, sulphur, organic and total phosphorus in improved grassland soils. *Soil Science*, 87: 135-140.
- Wedderburn, M.E., 1991. The biophysical resource of hill country. In: P.R. Henniques (Editor), International Conference on Sustainable Land Management, Napier, Hawkes Bay, New Zealand, pp. 292-296.
- Wedin, D.A. and Tilman, D., 1990. Species effects on nitrogen cycling: a test with perennial grasses. *Oecologia*, 84: 433-441.
- White, R.E., 1991. The soil factor in sustainable agriculture in New Zealand. In: P.R. Henniques (Editor), International Conference on Sustainable Land Management, Napier, Hawkes Bay, New Zealand, pp. 111-118.
- Williams, P.H. and Haynes, R.J., 1991. New Zealand's Pastoral farming: How biologically sustainable is it? In: P.R. Henniques (Editor), International Conference on Sustainable Land Management, Napier, Hawkes Bay, New Zealand, pp. 284-291.

CHAPTER 3

DESCRIPTION OF FIELD SITES AND CHARACTERISATION OF SOIL FERTILITY STATUS³

3.1 INTRODUCTION

In order to evaluate the agronomic effectiveness of long-term and frequent dressings of single superphosphate (SSP) to clover-based hill pastures, Moir (1994) chose to study annual and seasonal pasture production at a range of sites in the Wairarapa that had varying topdressing histories under traditional sheep and beef grazing management. Soil chemical fertility at the field trial sites examined in this study have been comprehensively described in previous studies (Moir, 1994; Moir et al., 1995). Readers are directed to Chapter 7, Table 7.1 for a brief summary of soil fertility at these sites.

Total soil P, N and S are presented in this Chapter, adapted from Moir (1994). Other soil analysis results presented in this Chapter were obtained when the sites were re-sampled in the summer of 1995. Brief site descriptions, adapted from Moir (1994), with plates, are also presented in this Chapter.

3.2 SITE DESCRIPTION

This study was conducted in the Wairarapa region (central and southern east-coast), North Island, New Zealand, and continues from the studies of Moir (1994). After extensive soil sampling in the region, initially fourteen trial sites were selected for study, and established on sheep and beef cattle grazed hill country farms. After the studies of

³ Some data presented in this Chapter contributed to the following conference papers:

(i) "Climate, soil fertility and pasture production in Wairarapa hill country.", presented at the FLRC Workshop, Massey University (Moir et al., 1995).

(ii) "Effects of various fertiliser histories on nutrient accumulation and pasture production in Wairarapa hill country.", presented at the combined NZSSS/ASSS conference, Melbourne, Australia (Moir et al., 1996).

Moir (1994), two sites found to be influenced by previous stock camping behaviour (2 and 7) were abandoned, leaving the 12 field sites studied in this thesis⁴.

Sites were situated in one of three districts, varying widely in climate (annual rainfall and seasonal distribution). Site 13 (Riverside Farm), although near Mauriceville, has rainfall more akin with the Gladstone sites. The three districts were:

1. Mauriceville (high rainfall); mean annual rainfall 1300-1500 mm, with an even annual distribution and no summer dry period;
2. Gladstone (medium rainfall); mean annual rainfall 1000-1200 mm, moderate summer dry period;
3. Whareama (low rainfall); mean annual rainfall 800-900 mm, uneven annual distribution (winter dominant), reliable and extended summer dry conditions.

Within each rainfall district (climate regime), sites ranged widely in fertiliser history (Table 3.1). Application rates ranged from little or no fertiliser applied over the last 20-25 years (low fertility), up to sites which had received 250 kg SSP ha⁻¹yr⁻¹ for 20 years or more. Fertiliser history, soil parent material, and site aspect are summarised in Table 3.1.

Botanical composition of swards also varied widely across sites (Moir, 1994). High rainfall sites 1 and 14 were ryegrass/white clover dominant, while low fertility sites 4, 11 and 12 were dominated by browntop and other low fertility grass species. However, botanical composition varied widely across these hill country sites, and was strongly seasonal. In the previous study, Moir (1994) was unable to explain the complex pattern of sward botanical compositions at these sites using simple measures of soil fertility and/or climate.

⁴ For simplicity, trial site numbering was not changed for this study. Because two sites (sites 2 and 7) were abandoned at the start of this study, "site 2" and "site 7", or "soil 2" and "soil 7" do not appear, and are not used, in this thesis. Sites are however still numbered numerically from 1 to 14, although only 12 sites are studied. This sequence of site numbers applies to all research presented in this thesis.

Sites were selected to avoid stock camp areas, had moderate slope (15-20°), and were N, NW or NE facing (Table 3.1). Soil groups included Central Yellow-Brown Earths (YBE) in the high rainfall district (Mauriceville), and central Yellow-Grey Earths (YGE) or YGE/YBE intergrades at Whareama and Gladstone.

Description of field experiments (including plates) to measure pasture production are given later in Chapter 4, Section 4.2.

Table 3.1 Site aspect, soil group and fertiliser history

Location	Site Number	Aspect	Soil Group, Parent Material	Fertiliser History (SSP ha ⁻¹ yr ⁻¹)
Mauriceville	1	W	Central YBE	200 kg+ (20 years+)
	3	NW	Limestone	120 kg (15-20 years)
	4	W		No Fertiliser (20-25 years)
	13	Flat	Gravels	180 kg (10-15 years)
	14	NE	Greywacke	
Gladstone	10	N	Redzina Mudstone	200 kg (15-20 years)
	11	NW	Central YGE/YBE	No fertiliser (8 years)
	12	W	Intergrade Loess and sand/siltstone	No fertiliser (15-20 years)
Whareama	5	NE	Central YGE/YBE	250 kg (1969-83)
	6	S	Intergrade	10 kgPha ⁻¹ (1983-1999)
	8	NW	Mudstone and	250 kg (20 years+)
	9	N	Argillite	No fertiliser (12 years)



Plate 3.1 Map of the Wairarapa region, including location of field trial sites, and local New Zealand Meteorological Service Climate Stations A, Mangamutu; B, Castle Point and C, Taratahi.



Plate 3.2 View of coastal summer-dry Wairarapa hill country, taken at Whareama.



Plate 3.3 View of Wairarapa hill country, taken from Gladstone, facing North-West towards the Wairarapa plains, and Masterton.

3.3 SOIL ANALYSES

3.3.1 Methods and Materials

Soil Sampling and Preparation (Summer 1995)

Soil was sampled using a 2.5 cm corer to a depth of 7.5 cm at each site (30-40 cores per site) in early 1995. Samples were air dried for 48 hours at 35° C, then 2 mm sieved for analysis.

Total N, P and S Content

Soil sub-samples were dried at 105°C for 24 hours, then ring ground to a very fine particle size. Total N and P were determined by Kjeldahl digest (McKenzie and Wallace, 1954), and total S by dry oxidation (Landers et al., 1983). All digest mixtures were analysed for the respective nutrient content using a Technicon II Auto analyser.

Plant-Available Nutrients, pH and Bulk Density

Plant-available phosphorus (P) in soils was extracted by the method of Olsen et al. (1954), and also by ion-exchange resin membrane extraction (also S; Saggar et al., 1990), followed by P analysis by method of Murphy and Riley (1962).

Soil mineralisable nitrogen (N) content was measured using a soil anaerobic incubation. The incubation technique was a modified version of that used by Keeney and Bremner (1966) and Waring and Bremner (1964). Air-dry soil (5g) sub-samples were suspended with 20 mL of distilled H₂O. This test was conducted in triplicate in 50 mL centrifuge tubes, then incubated at 30°C for 21 days. Samples were mixed once daily using a vortex mixer. After incubation, N was extracted by adding 10 mL of 3M KCl solution (extraction strength = 1M KCl), and shaking on an end-over-end shaker for 20 minutes. Samples were then centrifuged at 5000 rpm for 5 minutes, followed by filtration. Supernatants were analysed for total N content using the colourmetric method on a Technicon II Auto Analyser (see later, this section).

Soil pH in water was measured at a water:soil ratio of 2.5:1 (Blakemore et al., 1987). Soil pH in 0.1M CaCl₂ solution was measured at a solution:soil ratio of 2.5:1 (Blakemore et al., 1987).

Phosphate retention by soil was measured by method of Blakemore et al. (1987). Soils were shaken with a 5:1 phosphate solution (1 mgP mL⁻¹):soil ratio for 16 hours, followed by P measurement by vanadomolybdate method (Saunders, 1965).

Mineralisable organic (carbon-bound) N and S soil contents were also estimated using a dilute hydrogen peroxide treatment (S. Saggar, pers. comm). Air-dry soil samples (2g, in triplicate) were treated with 2.5 mL of an 8% by volume H₂O₂ solution. Samples were mixed on a vortex mixer, then left to sit for 30 minutes to oxidise soil carbon. Soils were extracted by adding 17.5 mL of 0.286 M KCl solution (final strength 0.25 M KCl), and shaking on an end-over-end shaker for 30 minutes, then filtered (Whatman 41). Supernatants were taken to dryness (85°C, 24 hours), then rehydrated with 10 mL of deionised H₂O, mixed by vortex mixer. The HI reducible S content of the H₂O₂ soil extract was determined colorimetrically by method of Johnson and Nishita (1952) modified to work on a Technicon Autoanalyser. The NH₄⁺ and NO₃⁻ concentrations were determined colorimetrically by method of Searle (1975, 1984) and Kamphake et al. (1967) respectively, on a Technicon II Autoanalyser. To determine S, NH₄⁺ and NO₃⁻ recovery, several soils were spiked with solutions containing these ions. Inorganic S, NH₄⁺ and NO₃⁻ contents of soils were estimated by extracting all soils with deionised water only, followed by the standard analysis. Soil bulk density for the 0 -7.5 cm soil horizon was measured by bulk density coring (8 cores per site, in April of 1995).

3.3.2 Results and Discussion

3.3.2.1 Total P, N and S

Total elemental P, N and S content of the soils used in this study have been comprehensively described and discussed by Moir (1994). For this reason, nutrient accumulation in these hill soils is not discussed in any detail in this thesis. A very brief summary of previous findings relating to these soils is given in this section. Readers are directed to Moir (1994) for further detail.

Total phosphorus (P), nitrogen (N) and sulphur (S) contents of the 0 - 7.5 cm soil horizon are presented in Figures 3.1A, 3.1B and 3.1C respectively. Soil phosphorus content ranged widely from 430 μgPg^{-1} at a low fertility site, to 1470 μgPg^{-1} at a high fertility site (Figure 3.1A). The key factor influencing total P levels was fertiliser history. Long-term application of SSP resulted in a large (approximately 1000 μgPg^{-1}) accumulation of P in the 0-7.5cm soil depth. Soil P accumulation was lower at the low rainfall sites, presumably because of lower fertiliser inputs, which reflects farmers recognising the lower productivity potential of these pasture systems given the climatic (rainfall) constraints.

Total soil N and S also accumulated as a result of increased P and S fertiliser application, but to a much lesser extent than soil P. Regression analysis revealed a poor relationship between total soil N and P, and showed only modest increases in soil N with increasing soil P (Moir et al., 1997). The N:P ratio across sites varied from more than 10:1 to < 5:1 indicating large differences in the use of soil and fertiliser P in building the soil organic N pool through legume growth. Total soil S was also poorly related to total soil P, and again showed a far greater accumulation of P than S, due probably to variable and significant S leaching losses (Moir, 1994). Climate also influenced N and S accumulation. Sites with high historical fertiliser inputs and low rainfall (e.g. site 8) had greater (total N and S) accumulation when compared to equivalent sites in high rainfall areas (e.g. site 1). This could in part be attributed to greater N and S leaching losses in areas of high rainfall (Lambert et al., 1988).

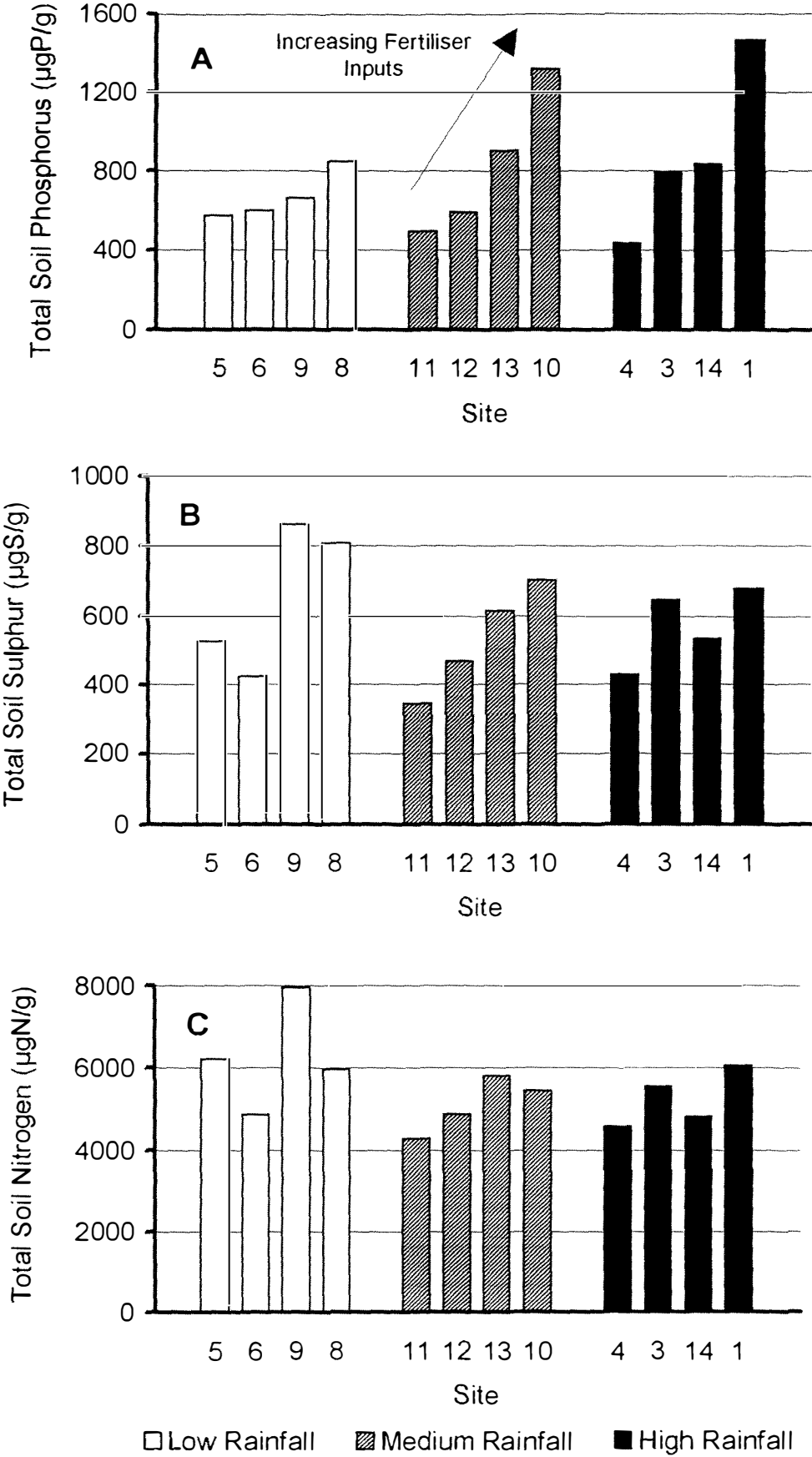


Figure 3.1 Total soil phosphorus **A**, Sulphur **B**, and Nitrogen **C** content of the 0 - 7.5 cm horizon across all Wairarapa field sites. Adapted from Moir et al. (1997).

3.3.2.2 Olsen P, Sulphate S, Resin-Extractable P, pH, bulk density, P Retention, and Mineralisable N

Results of soil chemical analyses, and bulk density measurements are presented in Table 3.2. Soil pH_{H2O} values ranged from 5.3 (Site 4) to 5.8 (Site 8). Soils with pH values in the “optimum” range for pasture growth (approximately 5.8; Edmeades et al., 1984) include sites 1, 8 and 11. pH values of < 5.5 at sites 4 and 9 may impose limitations on pasture growth.

Soil Olsen P values ranged from 11 (site 12) to 39 μgPg^{-1} (site 10; Table 3.2). High Olsen P values were associated with high fertiliser (SSP) inputs, and were strongly related to soil total P values (Figure 3.2A, $R^2 = 0.81$). Values were similar to those reported previously for these sites (Moir, 1994), except for site 8, a high fertility highly fertilised site, that returned an unusually low Olsen P value of 22 μgPg^{-1} in summer, 1995.

Resin P values ranged from 17 (site 4) to 87 $\mu\text{gP/g}$ (site 10). Using the criteria set down by Saggar et al. (1992) on YGE soils, soils 1, 3, 8, 9, 10 and 14 with resin P values > 40 μgPg^{-1} are unlikely to be P responsive (Saggar et al., 1992). Phosphate retention was low at most sites, excluding sites 4 and 13, which had moderate P retention (40-50%; Saunders, 1965).

Mineralisable N ranged widely across sites, from 107 (site 4) to 366 kgNha^{-1} (site 10; Table 3.2). Increasing mineralisable N values were associated with increasing total soil P values and with Olsen P status (Figure 3.3A), indicating that historical (P and S) fertiliser applications have resulted in 2-3 fold increases in the soil plant-available N pool.

Soil bulk density was mostly in the range of 0.9-1.0 gcm^{-3} , which are typical values for silt-loam soils of this type (Gradwell and Birrell, 1968). Soil from site 10 had a moderately high bulk density value of 1.21 at the time of sampling, which may be expected to limit pasture growth. The high bulk density was consistent with the paddock being stocked with heavy beef cattle during the previous wet winter.

Resin-exchangeable sulphate ranged from 9 (sites 6 and 11) to 27 μgSg^{-1} (site 8; Table 3.2). These values are (as expected for this type of extraction) higher than, but proportional to, those reported previously, using the calcium phosphate extraction method (Moir, 1994).

Table 3.2 Soil chemical analyses and field bulk density for all field soils ($\pm$ std. error)

Site	pH (H ₂ O)	Bulk Density (g cm ⁻³)	Olsen P ($\mu\text{g P g}^{-1}$)	P Ret ⁿ (%)	Mineralisable N (kg N ha ⁻¹)	Resin-Exchangeable Anions ($\mu\text{g g}^{-1}$)	
						P	S
1	5.76 $\pm$ 0.02	0.95 $\pm$ 0.03	38 $\pm$ 5.5	25.6 $\pm$ 0	313 $\pm$ 24	86.6 $\pm$ 3.7	21.9
3	5.65 $\pm$ 0.08	0.92 $\pm$ 0.05	28 $\pm$ 0.4	33.2 $\pm$ 0.4	246 $\pm$ 2	36.4 $\pm$ 3.3	20.0
4	5.32 $\pm$ 0.08	0.97 $\pm$ 0.09	13 $\pm$ 0	52.8 $\pm$ 0.3	107 $\pm$ 1	16.9 $\pm$ 6.7	17.5
5	5.57 $\pm$ 0.02	0.85 $\pm$ 0.07	16 $\pm$ 2.6	16.4 $\pm$ 3.0	212 $\pm$ 6	29.2 $\pm$ 0.6	16.3
6	5.59 $\pm$ 0.01	0.88 $\pm$ 0.07	10 $\pm$ 0.8	20.4 $\pm$ 1.3	169 $\pm$ 9	21.4 $\pm$ 3.2	8.8
8	5.80 $\pm$ 0.07	0.99 $\pm$ 0.07	22 $\pm$ 0.8	18.8 $\pm$ 0	259 $\pm$ 9	67.5 $\pm$ 0.9	26.9
9	5.43 $\pm$ 0.05	0.82 $\pm$ 0.05	16 $\pm$ 0	27.3 $\pm$ 0.9	139 $\pm$ 9	38.1 $\pm$ 12	16.3
10	5.68 $\pm$ 0.07	1.21 $\pm$ 0.04	39 $\pm$ 0.4	21.3 $\pm$ 7.8	366 $\pm$ 3	87.3 $\pm$ 1.5	20.6
11	5.71 $\pm$ 0.02	0.97 $\pm$ 0.07	13 $\pm$ 0.3	17.3 $\pm$ 1.4	142 $\pm$ 14	20.8 $\pm$ 1.3	8.8
12	5.53 $\pm$ 0.06	1.14 $\pm$ 0.1	11 $\pm$ 2.1	21.1 $\pm$ 1.5	225 $\pm$ 15	22.7 $\pm$ 4.0	12.5
13	5.61 $\pm$ 0.09	0.85 $\pm$ 0.06	14 $\pm$ 0.9	39.7 $\pm$ 1	266 $\pm$ 4	31.0 $\pm$ 5.2	20.6
14	5.55 $\pm$ 0.03	0.9 $\pm$ 0.02	21 $\pm$ 0.4	26.8 $\pm$ 1.9	242 $\pm$ 7	42.3 $\pm$ 1.1	18.1
Mean	$\pm$ 0.05	$\pm$ 0.06	$\pm$ 1.2	$\pm$ 1.6	$\pm$ 9	$\pm$ 3.6	N/A
Error [†]							

[†]Standard deviation across 4 samples per site

3.3.2.3 H₂O₂ Extractable Sulphate, Ammonium and Nitrate

Hydrogen peroxide extractable SO₄, NH₄ and NO₃ values are presented in Table 3.3. Extractable sulphate S ranged from 19.5 (sites 4 and 6) to 45 μgSg^{-1} (site 8; Table 3.3). Nitrogen extracted by this method ranged from 49.4 (site 14) to 121.9 μgNg^{-1} .

Table 3.3 Soil hydrogen peroxide extractable S and N content across all field soils

Site	Sulphate S (μgSg^{-1})	Nitrogen (μgNg^{-1})		
		NH_4	NO_3	Total N
1	35.0 $\pm$ 1.4	109.4 $\pm$ 2	12.5 $\pm$ 0.7	121.9 $\pm$ 3
3	32.0 $\pm$ 0	88.9 $\pm$ 12	4.8 $\pm$ 0.4	93.7 $\pm$ 12
4	19.5 $\pm$ 2.2	63.9 $\pm$ 1	0.7 $\pm$ 0.9	64.6 $\pm$ 2
5	30.0 $\pm$ 1.4	64.9 $\pm$ 14	4.0 $\pm$ 0	68.9 $\pm$ 14
6	19.5 $\pm$ 0.7	68.9 $\pm$ 1	1.0 $\pm$ 0	69.9 $\pm$ 1
8	45.0 $\pm$ 1.5	29.9 $\pm$ 3	20.5 $\pm$ 0.7	50.4 $\pm$ 4
9	30.0 $\pm$ 0	91.2 $\pm$ 7	0.7 $\pm$ 0.9	91.8 $\pm$ 8
10	32.5 $\pm$ 2.1	67.9 $\pm$ 5	14.5 $\pm$ 0.8	82.4 $\pm$ 6
11	32.3 $\pm$ 0.3	86.4 $\pm$ 0	0.5 $\pm$ 0.7	86.9 $\pm$ 1
12	21.0 $\pm$ 1.4	60.9 $\pm$ 1	1.3 $\pm$ 0	62.2 $\pm$ 1
13	21.8 $\pm$ 1	93.9 $\pm$ 6	0.8 $\pm$ 0	94.7 $\pm$ 6
14	28.5 $\pm$ 0.7	45.4 $\pm$ 3	4.0 $\pm$ 0	49.4 $\pm$ 3
Mean Error [†]	$\pm$ 1.1	$\pm$ 4.6	$\pm$ 0.4	$\pm$ 5

3.3.3 General Discussion

The Relationship Between Total Soil P, Olsen P, and Mineralisable N

Soil Olsen P status was strongly correlated with soil total P content (Figure 3.2A, $R^2 = 0.81$). The slope of the linear relationship of Figure 3.2A indicates that Olsen P has increased by 30 units for a total P accumulation of 675 kgPha⁻¹ (bulk density equals 900 kgm⁻³) in these soils (for the range of 500-1500 μgPg^{-1} total soil P). This suggests that in the long-term, 22 kgPha⁻¹ are required to raise Olsen P by one unit on these soils under sheep and beef farming. Mineralisable soil N was also strongly correlated with total soil P (Figure 3.2B, $R^2 = 0.83$). Soil mineralisable N increased by approximately 0.39 kgNha⁻¹ for every 1 kg of accumulated soil total P, or 8.6 kg mineralisable Nha⁻¹ for every 1 unit increase in Olsen P. The ratio of accumulated plant-available N:P:S of these soils is approximately 17:2:1. This small unit of available N increase per unit of applied

P suggests that traditional P and S fertiliser has resulted in only very slow accumulation, perhaps inefficient accumulation of soil mineralisable plant-available N. Total soil N and S were not strongly correlated with measures of plant-available (readily-extractable) soil N and S (data not shown), nor increases in total P.

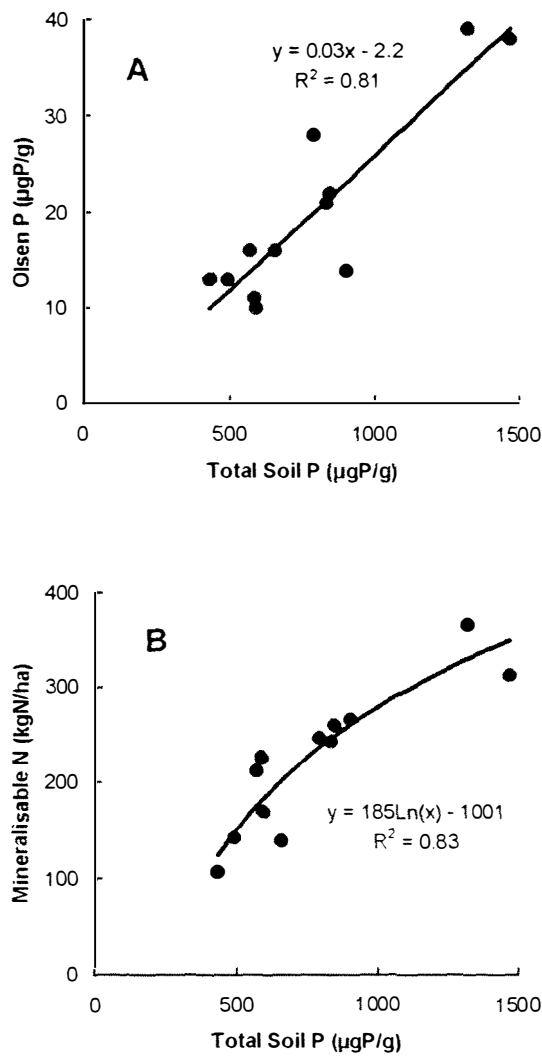


Figure 3.2 The relationship between total soil P content and **A** Olsen P, and **B** Mineralisable N content across all field sites

The Relationship Between Olsen P, Mineralisable N, and H₂O₂ Extractable S

Olsen P status was strongly correlated with mineralisable N status (Figure 3.3A, $R^2 = 0.64$), and to a lesser extent, hydrogen peroxide extractable S status (Figure 3.3B, $R^2 = 0.43$). This result indicates that Olsen P may not only be a strong predictor of soil plant-available P, but in these P and S fertilised legume-pasture soils may also be a reasonably

predictor of the soil mineralisable plant-available N and S status. This relationship will be explored later in this thesis (Chapter 5).

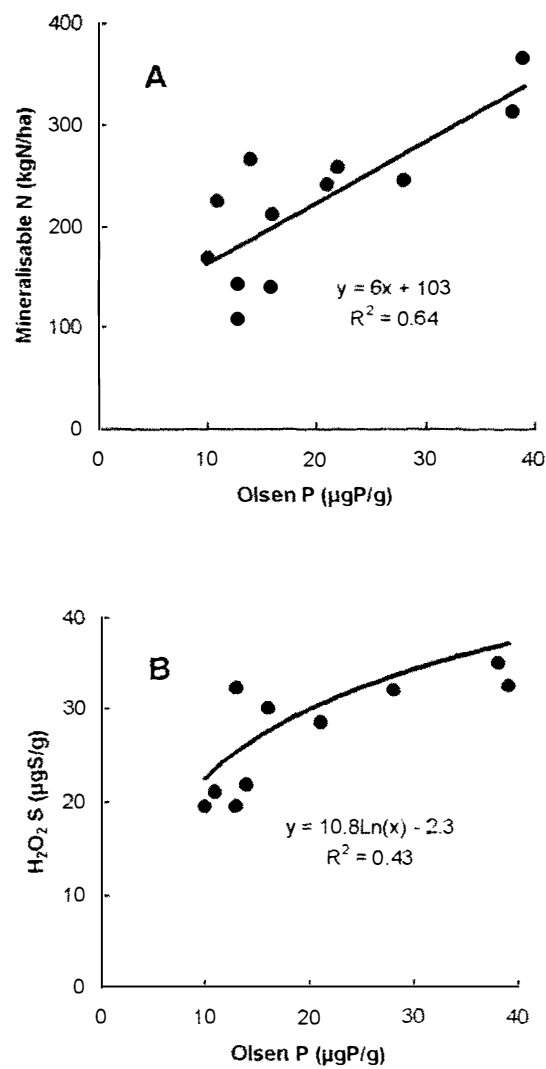


Figure 3.3 The relationship between Olsen P and **A** Mineralisable N, and **B** H₂O₂ extractable S content across all field sites

3.4 CONCLUSIONS

Increasing rate and frequency of superphosphate topdressing on the 12 Wairarapa hill country soils has resulted in increasing soil nutrient status as indicated by standard tests for soil P, S and N status.

Soils were shown to have accumulated large quantities of P, and to a lesser degree, S and N, as a direct result of fertiliser (SSP) application. Total soil P was strongly correlated with Olsen P, and soil mineralisable N status, and indicated that plant available P and S has also increased as a direct result of historical SSP application. Small increases in mineralisable N with increased rates of fertiliser (P and S) may represent inefficient use of P and S fertiliser use, and warrant further investigation (See Chapter 5).

Soil Olsen P status was shown to be strongly correlated with measures of plant-available soil N and S, and it is suggested that Olsen P may be a useful “surrogate” indicator of mineralisable plant-available N and S. Further studies should focus on testing the predictive abilities of the Olsen P test as an indicator of other plant-available macro-nutrients (such as N and S). Such studies could provide valuable information about the suitability of the Olsen P test as an indicator of plant-available P, N and S in soil, and aid the current limited understanding of the relationship between Olsen P status and pasture growth.

The relationships between soil nutrient supply, soil fertility indicators, and plant growth are studied in detail in the Chapters that follow.

3.5 REFERENCES

Blakemore, L.C., Searle, P.L. and Daly, B.K., 1987. Methods for chemical analysis of soils. Scientific Report 80, New Zealand Soil Science Bureau, Lower Hutt, New Zealand.

- Edmeades, D.C., Feyter, C. and O'Connor, M.B., 1984. Lime and phosphorus requirements for hill country yellow-brown earths. In: J.L. Brock (Editor), *Proceedings of the New Zealand Grassland Association*. New Zealand Grassland Association, Gisborne, New Zealand, pp. 98-106.
- Gradwell, M. and Birrell, K.S., 1968. Physical data for modal profiles. In: J. Luke and J. Heine (Editors), *Soils of New Zealand*. N.Z. Soil Bureau Bulletin. New Zealand Soil Bureau, Wellington, New Zealand, pp. 132-146.
- Johnson, C.M. and Nishita, H., 1952. Microestimation of sulfur in plant material, soil, and irrigation waters. *Analytical Chemistry*, 24: 736-742.
- Kamphake, L.J., Hannah, S.A. and Cohen, J.M., 1967. Automated analysis for nitrate by hydrazine reduction. *Water Research*, 1: 205-216.
- Keeney, D.R. and Bremner, J.M., 1966. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. *Agronomy Journal*, 58: 498-503.
- Lambert, M.G., Clark, D.A. and Costall, D.A., 1988. Phosphorus and sulphur accumulation after long-term superphosphate application to grazed hill pasture. In: R.E. White and L.D. Currie (Editors), *Towards the more efficient use of soil and fertiliser sulphur*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 95-100.
- Landers, D.H., David, M.B. and Mitchell, M.J., 1983. Analysis of organic and inorganic sulphur constituents in sediments, soils and water. *International Journal of Environment , Analytical Chemistry*, 14: 245-256.
- McKenzie, H.A. and Wallace, H.S., 1954. The kjeldahl determination of nitrogen: A critical study of digestion conditions-temperature, catalyst and oxidising agent. *Australian Journal of Chemistry*, 7: 55-70.
- Moir, J.L., 1994. Influences of climate and fertiliser application history on various measures of soil fertility and productivity in Wairarapa hill country. Master of Agricultural Science Thesis, Massey University, Palmerston North, New Zealand, 145 pp.
- Moir, J.L., Hedley, M.J., Mackay, A.D. and Garland, C.B., 1995. Climate, soil fertility and pasture production in Wairarapa hill country. In: L.D. Currie and P. Loganathan (Editors), *Fertiliser requirements of grazed pasture and field crops: Macro and micro nutrients*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 254-260.

- Moir, J.L., Hedley, M.J., Mackay, A.D. and Tillman, R.W., 1997. The effect of fertiliser history on nutrient accumulation and plant-available nutrient supply in legume-based pasture soils. In: J.G. Buchanan-Smith, L.D. Bailey and P. McCaughey (Editors), XVIII International Grassland Congress. International Grassland Association, Winnipeg, Canada, pp. 10, 68-69.
- Murphy, J. and Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27: 31-36.
- Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate, USDA.
- Saggar, S., Hedley, M.J. and White, R.E., 1990. A simplified resin membrane technique for extracting phosphorus from soils. *Fertilizer Research*, 24: 173-180.
- Saggar, S., Hedley, M. J., White, R. E., Gregg, P. E. H., Perrott, K. W. and Cornforth, I. S., 1992. Development and evaluation of an improved soil test for phosphorus: 2. Comparison of the Olsen and mixed cation-anion exchange resin tests for predicting the yield of ryegrass grown in pots. *Fertilizer Research*, 33(2): 135-144.
- Saunders, W.M.H., 1965. Phosphate retention by New Zealand soils and its relationship to free sesquioxides, organic matter and other soil properties. *New Zealand Journal of Agricultural Research*, 8: 30-57.
- Searle, P.L., 1975. Automated colorimetric determination of ammonium ions in soil extracts with "Technicon Autoanalyzer II equipment". *New Zealand Journal of Agricultural Research*, 18: 183-187.
- Searle, P.L., 1984. The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen. A review. *Analyst*, 109: 549-568.
- Waring, S.A. and Bremner, J.M., 1964. Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. *Nature*, 201: 951-952.

CHAPTER 4

FIELD STUDIES - ANNUAL PASTURE YIELD AND GROWTH RESPONSE TO STRATEGIC APPLICATION OF NITROGEN FERTILISER⁵

4.1 INTRODUCTION

In temperate New Zealand pastures, inadequate nutrient levels and soil moisture often limit pasture growth rates and subsequent pasture production for grazing livestock. This is especially true of N.I. East-Coast hill country, which often experiences moisture limitations soon after spring (Radcliffe, 1975). Although radiation is at optimum levels for pasture growth at this time of year, lack of adequate moisture can prevent maximum pasture growth-rates being realised.

Given these climatic limitations, producers must maximise stock production, and therefore pasture growth, during climatic intervals when environmental conditions are suitable for plant growth. In very dry districts, such as coastal Wairarapa hill country, this means that pasture growth must be optimised over a very short growth period over early-mid spring, before the onset of near-certain summer moisture limitations. For the hill-country farmer, this season is critical not only because the majority of pasture dry matter growth occurs at this time, but also because in some seasons this critical growth may occur over a short period of only several weeks.

It is clear that management practices at this time of year have the potential to strongly influence annual pasture production, and further, livestock production. For the hill country producer in higher-rainfall districts, that is districts which receive higher rainfall and/or more "reliable" summer rainfall than their coastal dry counterparts, management of spring growth may not be as critical, as pasture growth is more plentiful, and reliably

⁵ Some data presented in this Chapter contributed to the following conference paper: "Effects of various fertiliser histories on nutrient accumulation and pasture production in Wairarapa hill country.", presented at the combined NZSSS/ASSS conference, Melbourne, Australia (Moir et al., 1996).

spread over summer and autumn seasons. With the bulk of East-Coast hill country being summer-dry, it is important to develop strategies to increase pasture growth rates over spring.

In the absence of moisture limitations, soil nutrient status is a major factor influencing pasture growth rates during spring. At this time of year, climatic conditions are often most favourable for growth, and high pasture growth rates create high demand for plant-available soil nutrient resources. In particular, lack of soil phosphorus, nitrogen and sulphur are the major constraints on pasture growth. Moir et al. (1997), studying pasture growth on Wairarapa hill country over spring, found that of many soil nutrient indices, soil (Olsen) P status was the best indicator of pasture growth rates in spring, when soil moisture was not limiting plant growth. The relationship of pasture growth against Olsen P is a diminishing returns type curve. As Olsen P levels rise above 30 μgPg^{-1} there is little increase in pasture yield, suggesting that factors other than Olsen P status were limiting pasture growth at high Olsen P. This result suggests that even at high Olsen P levels, spring pasture growth could be increased at these sites by increasing plant N supply at this time. This could be achieved by the application of N fertiliser.

4.1.1 Review of Literature

Many Wairarapa hill country farmers have incorporated strategic nitrogen (N) fertilisers into fertiliser policies over the past 10-20 years (Morton et al., 1993). Some (Daniel, 1993; Kinnell, 1993), have attributed major management benefits to strategic N applied to summer-dry hill country pastures in the Wairarapa. However, pasture N-response data for this region is scarce.

In a survey of east-coast hill-country farms, Morton et al. (1993) indicated that N fertilisers were being used regularly in addition to phosphorus (P) as maintenance fertiliser, rather than a strategic application to overcome short-term feed shortages. The Wairarapa farms surveyed applied N at a mean rate of 20 kgNha^{-1} , applied over 80% of the farm area. Fertiliser was applied in May or August, mainly as diammonium phosphate (DAP), with 30 % of farms using urea. In general, those farms using N fertiliser also applied higher rates of other fertilisers (especially P) and had higher soil fertility levels and lower browntop and white clover levels in swards, c.f. those farms that

were not applying N fertiliser. Other workers (Birchham, 1977; O'Connor, 1982) have reported and explained changes in sward botanical composition resulting from N fertiliser application.

Working on dry Southern Hawkes-Bay hill country, Gillingham et al. (1998) measured pasture response to 30 kgNha⁻¹ applied as urea in winter. They reported a 24:1 N response on low P sites (Olsen P 9 µgPg⁻¹, 12 kgPha⁻¹yr⁻¹), but no N response at the high P level (Olsen P 28 µgPg⁻¹, 25 kgPha⁻¹). Pasture responses to N were greater from steep (1000 kgDMha⁻¹yr⁻¹) than easy (440 kgDMha⁻¹yr⁻¹) slopes, in winter, and annually. In general, these researchers showed complex response patterns to N and P fertilisers, with low P, steep, North-facing aspects being the most responsive to N fertiliser (43 kg DMkg⁻¹ N). Application of N fertiliser reduced sward clover content by 36% and 47% for low and high P treatments respectively. These workers explain this botanical change in some detail.

Lambert and Clarke (1986) and Clarke and Lambert (1989) investigated the effects of late autumn N application on moist North Island hill country pasture ("Ballantrae"). N was applied as urea at 37 kgNha⁻¹ on low fertility (LF) farmlets (Olsen P 7 µgPg⁻¹, 125 kgpha⁻¹yr⁻¹ SSP), and at 50 kgNha⁻¹ on high fertility (HF) farmlets (Olsen P 15, 375 kgpha⁻¹yr⁻¹ SSP). Responses to N were large, ranging from 30:1 (HF) to 37:1 (LF), and occurred mostly in winter and spring. This represented a major change in the pattern of seasonal pasture production at this site. The authors attributed the large response to N fertiliser to low soil N fertility, a phenomena also observed and discussed by other workers (Ball and Field, 1982; Ball et al., 1982).

Also studying at the AgResearch facility "Ballantrae", Luscombe (1979) applied treatments of 0-160 kgpha⁻¹ as urea in mid-September to a low fertility hill pasture (Olsen P 8 µgPg⁻¹), plus 750 kgpha⁻¹ SSP and 200 kgpha⁻¹ KCL. The largest N responses were measured in early spring, and N was used more efficiently at low application rates (20-40 kgNha⁻¹).

Ball et al. (1976) measured N responses to 58 kgNha⁻¹ applied to low fertility pastures (Olsen P 7-11 µgPg⁻¹) on a Southern Hawke's Bay site in mid-August as urea. A 31:1 response to N fertiliser was measured, representing 36% of total growth on sheep-grazed

plots, and a 14:1 response on mown plots (15% of total growth). Higher responses on grazed plots resulted presumably from additional response to N recycled via urine and dung.

Ledgard et al. (1983) studied pasture N response on Waikato hill country. Urea was applied at 30 and 90 kgNha⁻¹. The 30 kgNha⁻¹ treatment grew less pasture than the higher N rate, but had a higher response efficiency (kgDM per kgN applied). Increased N efficiency at low rates of N application has also been suggested by other researchers (O'Conner, 1982). Ryegrass dominant camp sites, and easy slopes had moderate fertility (Olsen P 17 µgPg⁻¹), while steeper slopes were browntop dominant, and less fertile (Olsen P 11 µgPg⁻¹). N responses were up to 13:1 on easy slopes, and up to 21:1 on stock camp sites. These workers measured many soil and pasture related indices, none of which were correlated to degree of pasture response to N fertiliser.

Some workers (Parker et al., 1989; Parker et al., 1994) have considered strategic N fertiliser applied to dry hill country as a risk management tool. This research combines pasture N responses from Wairarapa trial data with simple probability of climatic scenarios to estimate likely pasture N response, and returns (per kg N applied) for the producer. Such risk management frameworks show potential to provide valuable information to the producer. However, such evaluations rely heavily upon accurate N response data. Trial sites in this study cover a wide range of fertility status and climate regime, and provide an ideal opportunity in which to study hill country pasture response to a single low N application rate, aimed at strategic spring pasture response, for summer-dry risk management.

4.1.2 Objectives

In this study, the objective is to observe the effect of low rates of N fertiliser, applied strategically to Wairarapa hill country in spring. The strategy was to apply a single application of urea one month before maximum pasture growth rates are expected. The pasture growth rate response, if any, to applied N was measured. The results are discussed in relation to the use of strategic N fertilisation of Wairarapa hill country in spring, and the factors which may contribute to the degree of pasture growth response to N fertilisation.

4.2

METHODS AND MATERIALS

Pasture growth was measured at the Wairarapa hill country sites described by Moir (1994) (Chapter 3; Appendix 4.1). Annual pasture production was measured at twelve field sites. Three climate zones were examined; Whareama, low rainfall (<800mm), coastal summer drought; Gladstone, medium rainfall (900-1150mm), inland summer moisture deficient; and Mauriceville, high rainfall (1400mm), inland moist hill country. Within each climate district, sites varied widely in soil fertility status. High fertility (HF) sites had Olsen P values of 35-40 μgPg^{-1} , Medium fertility (MF) sites 18-26 μgPg^{-1} , and low fertility (LF) sites 7-15 μgPg^{-1} . Each trial site was situated on medium slope (approx. 20°) sheep and cattle grazed pastures.

Aspect was mostly NE, E or N facing (sunny) slopes. Sites were originally established in 1993, but were re-established for this trial in July of 1994. Each site, or "plot" was 4m wide by 8m long, enclosed by an electric fence to exclude grazing stock. The plot was split into eight equal sub-plots, each of which contained a 0.5 m² stock exclusion cage, from which pasture was harvested (Plate 4.1).

Plots were randomised, and 4 subplots were designated as being "control" plots, while the remaining four plots received nitrogen fertiliser. At each harvest all pasture in each subplot was cut, and the pasture within the stock exclusion cage was used to calculate pasture growth. Once harvested, the exclusion cage was re-positioned within its subplot.

For this experiment, sites were established in late July 1994. At this time plots were cleared of any rank pasture growth, and exclusion fences were re-erected. Pasture was cut to 2cm above ground level at all sites. On August 11, sites were cut for pasture growth, then nitrogen fertiliser was applied to the designated (50% of the) plots. This was the only application of N fertiliser, and was applied at the rate of 30 kgNha⁻¹, in the form of urea. This low rate was chosen to allow for economic and budgeting constraints of a hill country farmer, where it may only be economic to apply N at low rates, over small areas of the farm. Also, literature (Luscombe, 1979; Ball and Field, 1982; Ball et al., 1982; Lambert and Clarke, 1986) indicated that maximum dry matter responses per kgN could be obtained by applying low rates of N, to N-deficient spring pastures, with high potential growth rates.



Plate 4.1 Pasture being harvested from plots at a trial site, using a trim and remove technique.

From this point onwards, pasture growth was harvested using a domestic hedge trimmer at all sites at 3-4 week intervals, until soil moisture deficits became severe in late November / early December of this year (1994), and pasture growth was severely limited. Sites continued to be harvested until the end of March 1995, at intervals dependent upon adequate growth.

At each harvest, soil moisture content was measured, and regular soil cores were taken for soil nutrient analysis (pH, Olsen P, Mineralisable N, exchangeable cations etc; Reported in Chapter 3, Section 3.3.2). On-site climate measurements were made at daily intervals in each of the three climate zones. Measurements included daily rainfall, maximum and minimum soil temperature at 10cm depth, and daily radiation measurements.

Soil bulk density measurements were also made from soil cores taken to measure soil moisture content.

4.3 RESULTS AND DISCUSSION

4.3.1 Climate

4.3.1.1 Rainfall and Evapotranspiration

Mean monthly rainfall and evapotranspiration data from August 1994 to late March 1995 are presented in Figure 4.1.

At Mauriceville (Figure 4.1A) mean monthly rainfall was 30mm lower over the Dec-January period when compared with 20-year average data. November was very wet, being 180mm above mean values for that month. At Gladstone (Figure 4.1B), monthly rainfall was close to the long-term average, except for the months of August, November and December. At 60mm, Gladstone received less than 50% of mean rainfall for the late spring/early summer period. August and December were very dry at Whareama sites (Figure 4.1C), especially in August when only 10mm of rain fell, compared with the 95mm 20 year average for this month. Low rainfall early in the season, and in November and December therefore caused drought conditions by mid spring in Whareama, and Gladstone, and produced drier than average conditions at Mauriceville in early summer.

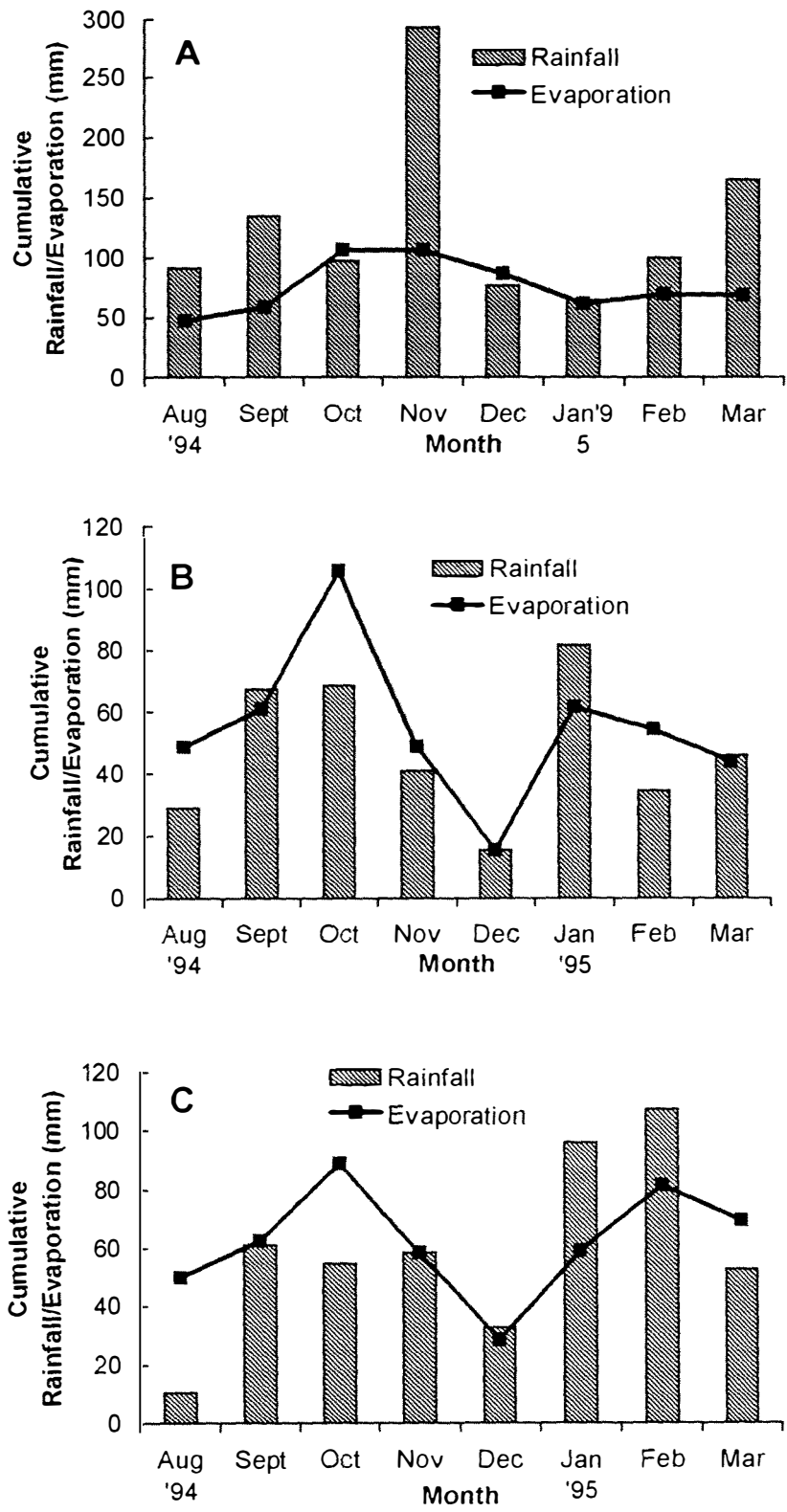


Figure 4.1 Monthly cumulative rainfall and evapotranspiration from August 1994 to March 1995 at **A** Mauriceville, **B** Gladstone and **C** Whareama.

4.3.1.2 Drainage

The soil water balance model described in Chapter 6 was used to predict drainage events from late August to the end of December 1994 (Figure 4.2).

While little or no predicted drainage occurred at Whareama (Figure 4.2C) and Gladstone (Figure 4.2B) sites over the period of this trial, significant drainage did occur at Mauriceville sites (Figure 4.2A). Of particular interest to this study is a drainage event of 50mm on the 15 of August, followed by a further 50mm in the following 10 days. The significance of these drainage events will be discussed later in this section. If we assume a mean soil bulk density of 1 gcm^{-3} , and 50% total porosity, then a 50mm drainage event represents approximately a 10cm displacement of soil water in the surface soil profile.

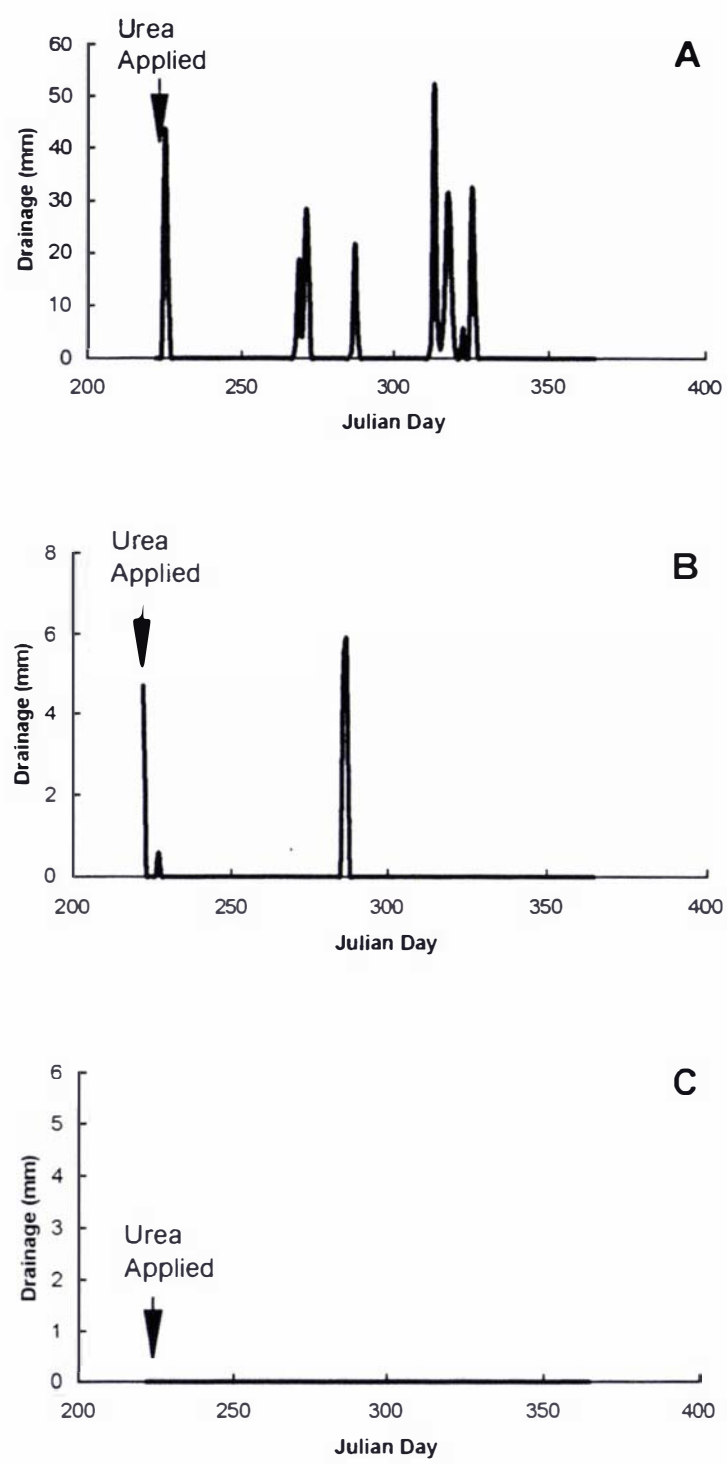


Figure 4.2 Soil water drainage (mm) for the 0-7.5 cm soil depth from August 1994 to March 1995 at **A** Mauriceville, **B** Gladstone and **C** Whareama.

4.3.2 Soil Moisture

Soil moisture contents (volumetric) from late April to late December 1994 are presented in Figure 4.3.

At Mauriceville sites (Figure 4.3A), soil volumetric water content (θ) remained above 0.30 until early November (Julian Day 310), but dried to below 0.20 over the month of December (Julian day 340-365). In comparison, Whareama and Gladstone sites were experiencing severe drought conditions early in the season. At Whareama (Figure 4.3C), sites 5 and 9 soil moisture fell to 0.20 by mid-late August (Julian day 255), and remained this dry for the following three months. Site 6 and 8 were moister, but also dried severely. Gladstone sites (Figure 4.3B) were slightly moister than those at Whareama, but had dried severely ($\theta = 0.20$) by late October. In the previous season, pasture growth ceased when θ equalled 0.20 (Moir et al., 1997).

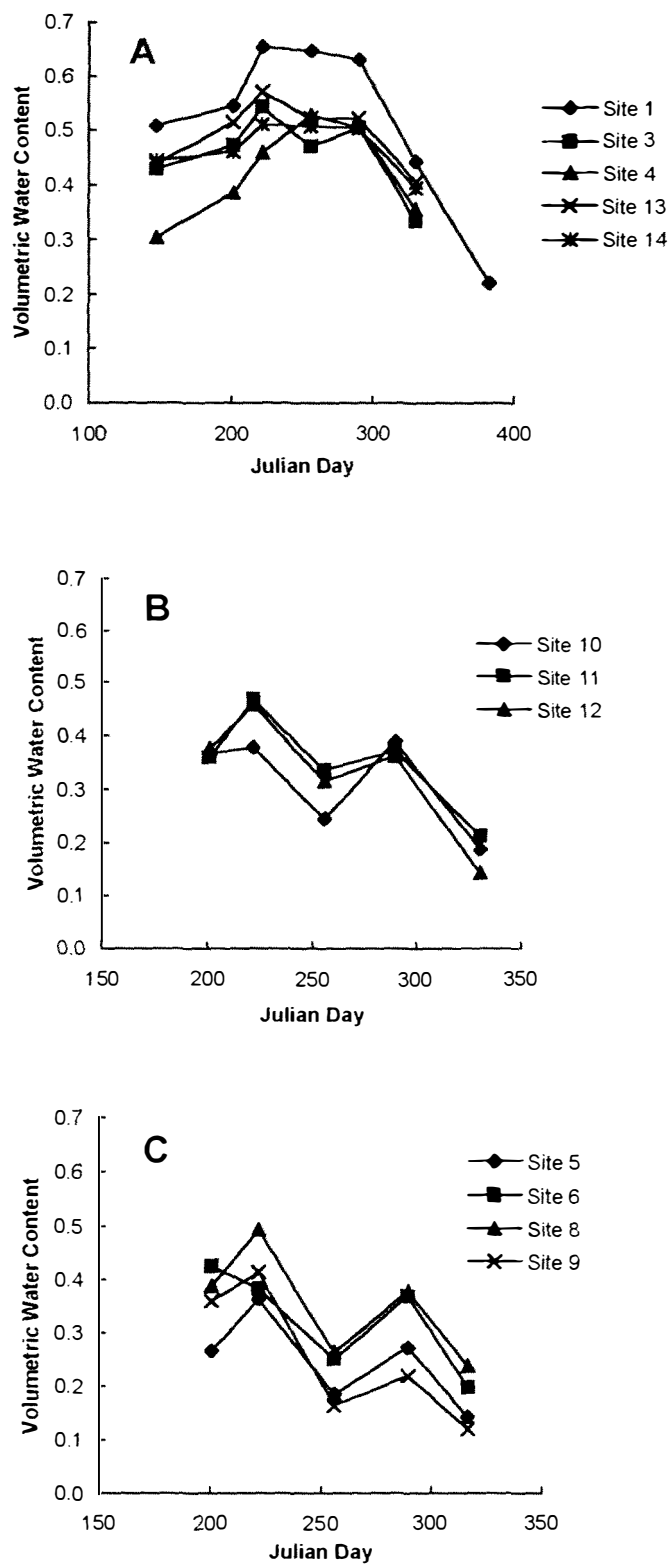


Figure 4.3 Soil volumetric water contents (0-7.5 cm depth) from late May to December 1994 at **A** Mauriceville, **B** Gladstone and **C** Whareama sites.

4.3.3 Annual Pasture Yield

Total herbage yields on control plots from mid August 1994 to late March 1995 are presented in Figure 4.4.

Dry matter production ranged from 1.69 tDMha⁻¹ at Site 6 (Figure 4.4C) to 10.8 tDMha⁻¹ at Site 1 (Figure 4.4A). Most Mauriceville sites yielded above 6 tDMha⁻¹ (excluding Site 4), while most Whareama and Gladstone sites grew less than 4 tDMha⁻¹ during this season. High fertility sites 8 and 10, however, yielded near 6 and 8 tDMha⁻¹ respectively by late March.

Nitrogen fertilised plots produced slightly higher accumulated yields, ranging from 1.69 tDMha⁻¹ (site 5) to 11.7 tDMha⁻¹ (site 1; Figure 4.5).

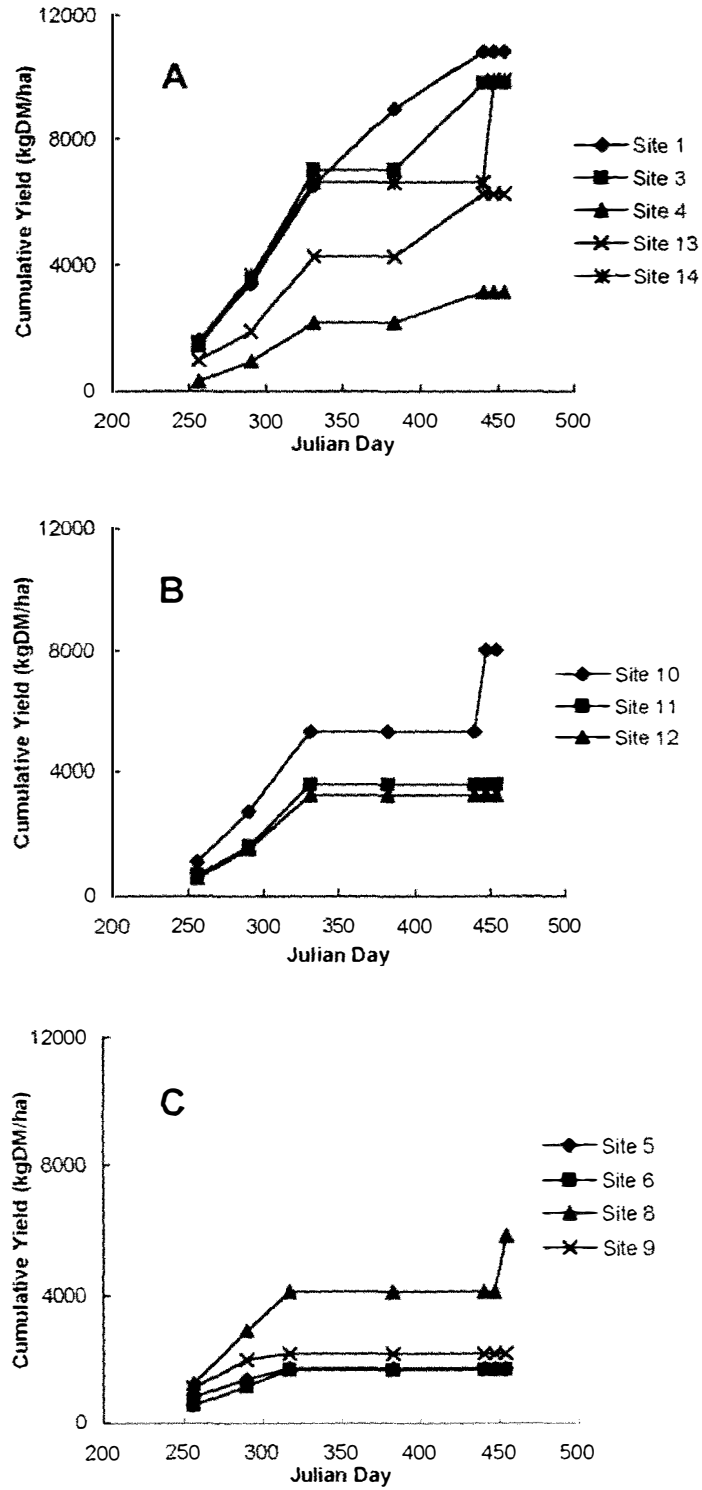


Figure 4.4 Cumulative annual pasture yields for control plots at **A** Mauriceville, **B** Gladstone and **C** Whareama sites for the 1994/1995 season.

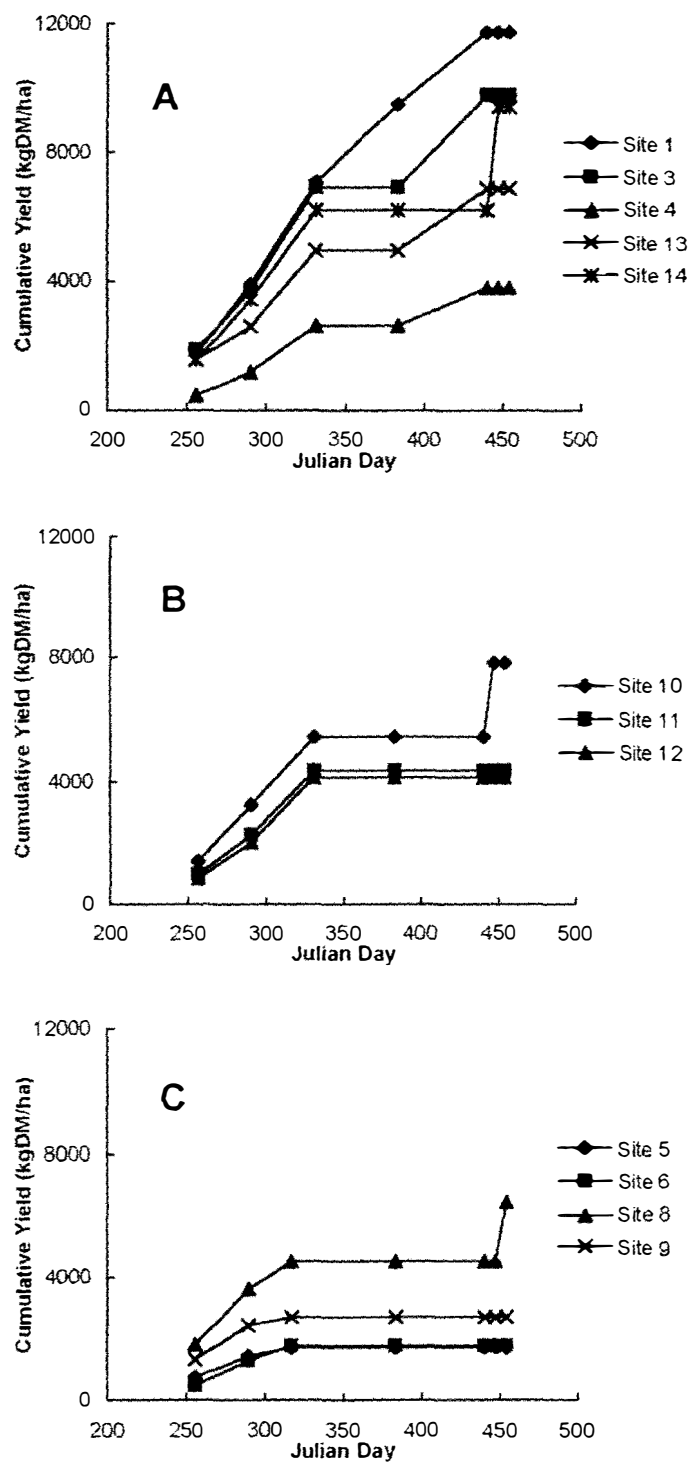


Figure 4.5 Cumulative annual pasture yields for nitrogen fertilised plots at **A** Mauriceville, **B** Gladstone and **C** Whareama sites for the 1994/1995 season.

Of all measured soil fertility indicators, Olsen P status best reflected annual pasture yield at all sites. Nearly 65 % of the variation in annual yield could be explained by Olsen P, using a Sigmoidal line of best fit (Figure 4.6). The shape of this curve suggests a yield asymptote at Olsen P values of 30-35 $\mu\text{gP g}^{-1}$, which is slightly higher than the ranges published by Sinclair et al. (1996a, 1996b, 1997a, 1997b).

In contrast, annual yield was not related to measurements of soil mineralisable nitrogen (Figure 4.7).

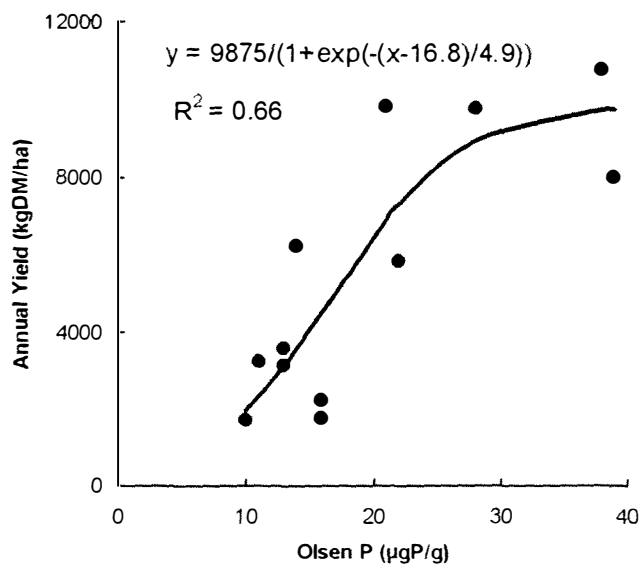


Figure 4.6 The relationship between annual pasture yield and Olsen P status for the 1994/95 season.

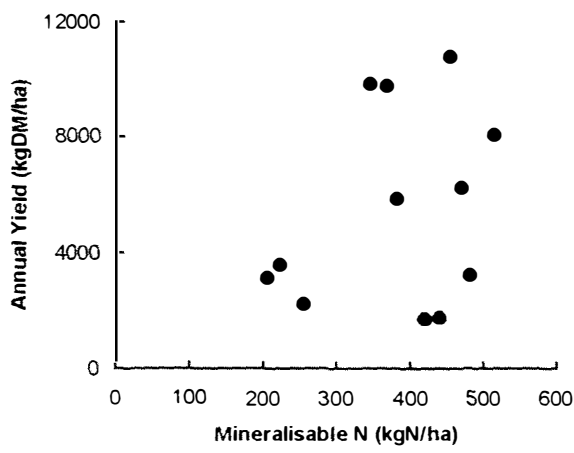


Figure 4.7 The relationship between annual pasture yield and soil mineralisable N status for the 1994/95 season.

4.3.4 Annual Yield and Nitrogen Response

Annual pasture growth responses to the 30 kgNha⁻¹ applied as urea on 11 August 1994 are presented in Table 4.1, and total dry matter yield at all sites in Table 4.2. Responses ranged from 0 at Sites 3, 5 and 14, to responses of 30:1 and 31:1 at Sites 12 and 1 respectively. Without further investigation, the variable pattern of N response at these sites is difficult to explain. For full yield details at all sites, and for all harvests, refer to Figures 4.2.1^A-4.2.13^A, Appendix 4.2.

Table 4.1 Annual pasture yield response (kgDMkg⁻¹ N applied) to nitrogen fertiliser application (@30 kgNha⁻¹) at all Wairarapa field sites (calculated as [increase in DM (kg) / kg N applied]).

Site	1	3	4	5	6	8	9	10	11	12	13	14
N Resp	31:1	0	22:1	0	2:1	13:1	16:1	0	26:1	30:1	21:1	0

4.3.4.1 Statistical analysis of annual pasture yields

Table 4.2 Mean annual pasture yields (kgDMha⁻¹). Treatments in columns with different letters are significantly different at P < 0.01. +N yields are different from control yields across rows at P < 0.1 when marked with *.

Site	Control Yield	+N Yield
1	10790 ^A	11719 ^A
3	9772 ^A	9764 ^B
4	3135 ^{DEF}	3792 ^{DE*}
5	1751 ^F	1687 ^F
6	1690 ^F	1751 ^F
8	4116 ^D	4513 ^{D*}
9	2196 ^{EF}	2676 ^{EF*}
10	8034 ^B	7810 ^C
11	3594 ^{DE}	4358 ^{D*}
12	3234 ^{DEF}	4145 ^{D*}
13	6246 ^C	6868 ^{C*}
14	9848 ^A	9391 ^{B*}
Mean	5367	5706*

4.3.5 Spring Nitrogen Response

4.3.5.1 Control Yield

In this study the nitrogen (N) fertiliser was applied strategically to observe pasture growth responses in spring, when a warming climate, and adequate soil moisture, were likely to be present.

It would follow that most plant growth response to added N would occur within a short period of application, perhaps three to four weeks (Parker et al., 1989; Parker et al., 1994). It may therefore be advantageous to examine this particular growth period in more detail.

In this section results from a period of “spring growth” are presented. This represents pasture growth from the time of fertiliser application (11th August 1994) to October 20 1994.

Pasture growth on control plots over spring was strongly related to Olsen P status ($R^2 = 0.80$; Figure 4.8A), and to a lesser degree, Resin P status ($R^2 = 0.66$; Figure 4.8B). The relationship is curvilinear (Sigmoidal), suggesting that factors other than soil P status were limiting pasture growth above Olsen P values of $30 \mu\text{gP g}^{-1}$. As with annual yield, spring yield was not related to soil mineralisable N concentrations (Figure 4.8C).

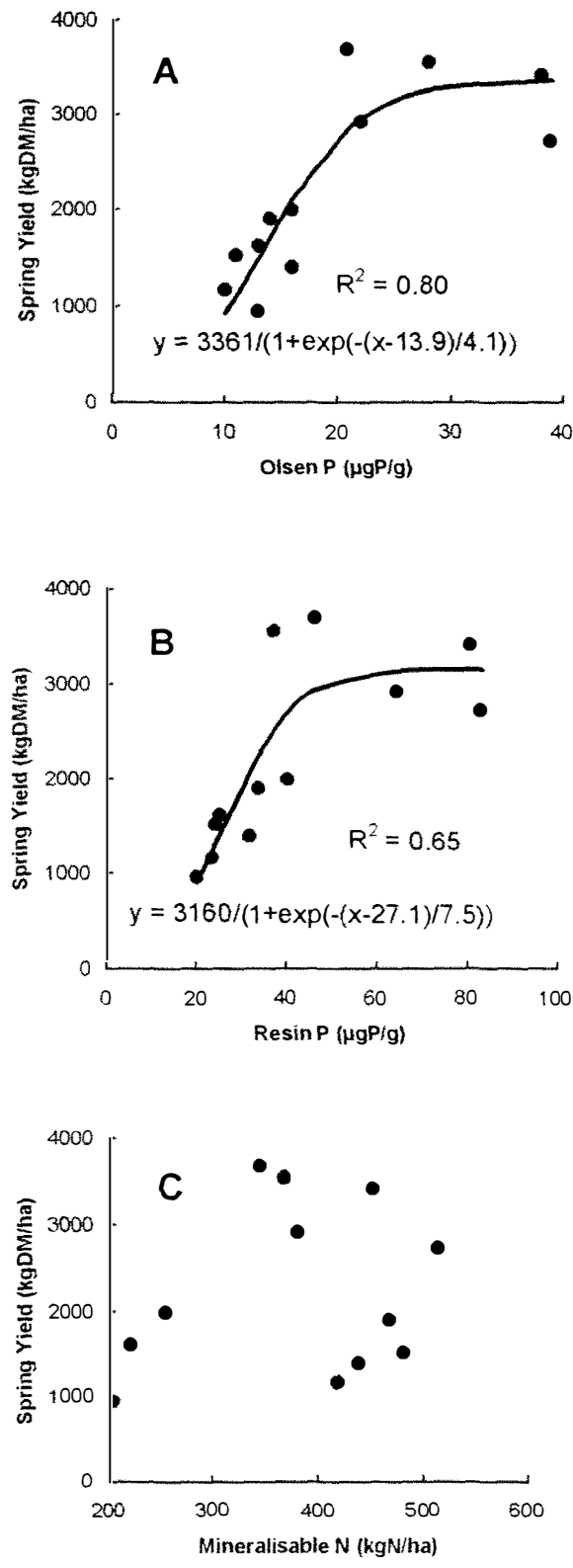


Figure 4.8 The relationship between spring pasture yield and **A** Olsen P, **B** Resin P and **C** mineralisable N status for the 0-7.5 cm soil depth at all sites.

4.3.5.2 Response to applied N (yield above control yield)

Growth above control plots (i.e. +N treatment yield minus control yield) for the outlined growth period are given in Figure 4.9. N fertilised plots tended to out-yield control plots, but only by approximately 500 kgDMha⁻¹ (Table 4.3). This represents approximately only 10-30% of total pasture yield on control plots over this period.

At some sites (Sites 3, 4, 5 and 6), yields were very similar to those on control plots. At site 14, the control plots grew more dry matter than N-fertilised plots.

4.3.5.3 Statistical analysis of spring pasture yields

Table 4.3 Mean spring pasture yields (kgDMha⁻¹). Treatments in columns with different letters are significantly different at P < 0.01. +N yields are different from control yields across rows at P < 0.1 when marked with *.

Site	Control Yield	+N Yield
1	3416 ^A	3905 ^{A*}
3	3547 ^A	3719 ^A
4	958 ^C	1162 ^{F*}
5	1395 ^C	1425 ^{EF}
6	1168 ^C	1272 ^F
8	2919 ^{AB}	3601 ^{A*}
9	1991 ^{BC}	2417 ^{CD*}
10	2728 ^{AB}	3257 ^{ABC*}
11	1618 ^C	2294 ^{DE*}
12	1519 ^C	2043 ^{DEF*}
13	1904 ^{BC}	2578 ^{BCD*}
14	3691 ^A	3415 ^{AB*}
Mean	2238	2591*

4.3.5.4 Discussion

It is important to note that for this time period (spring), DM production at Mauriceville sites represented only 30% of total annual production, while at drier coastal sites, up to 90% of total yield grew in this period. N response during this period at the dry sites can add significantly to the ability of the farmer to feed and fatten high value beef and lamb products before the dry summer conditions set in. Such a result also reflects not only the influence of climate and soil fertility status, but also the important links to, and complexity of, sward composition. It is more likely that swards in drier areas are dependent upon plant species that grow early in the season, and reproduce early before the onset of almost certain soil moisture deficits. Such species may also be slower to re-vegetate upon rewetting, as regrowth is seed and stolon based, rather than vegetative.

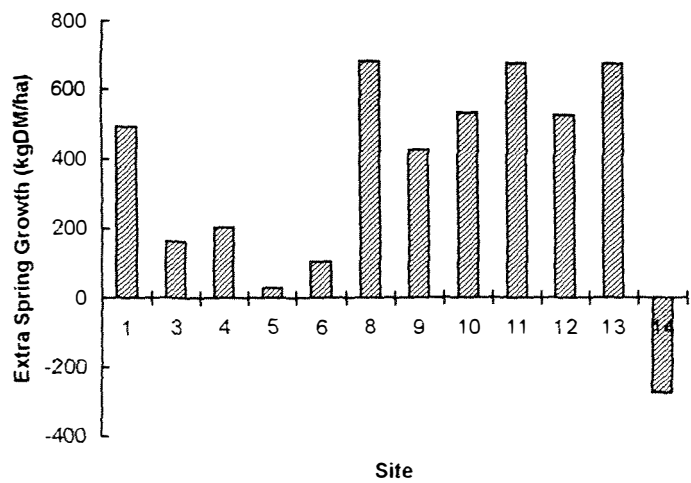


Figure 4.9 Spring pasture growth response to N above control plot growth across all sites for 1994.

Spring yield response to N above control was not related to Olsen P (Figure 4.10A), or soil mineralisable N concentrations (Figure 4.10B). This may be explained, in part, by the fact that the control and +N plots had similar yields, and that the growth above control was often less than 20% of total yield at the same sites. Thus it would appear that the extra N added caused little yield response when a relatively large amount of N already cycled through the sward.

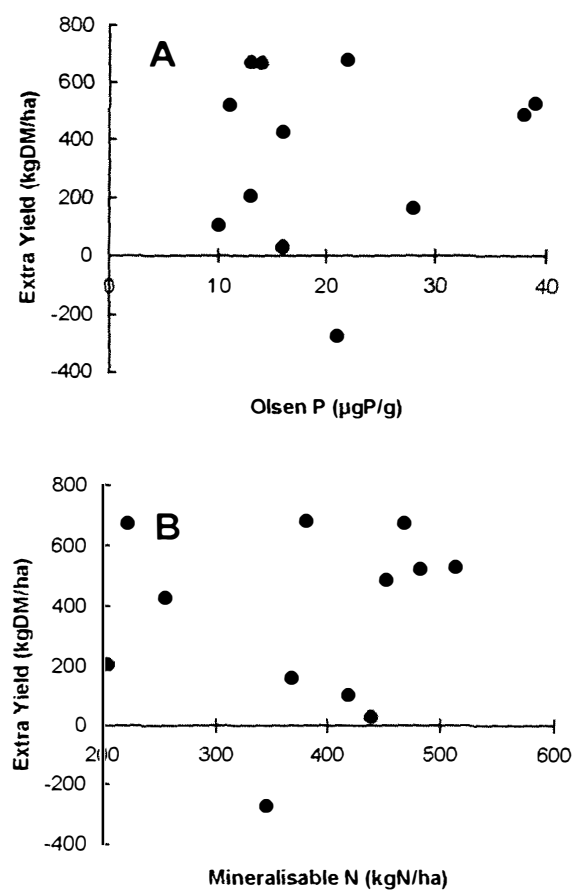


Figure 4.10 The relationship between spring growth above control and **A** Olsen P and **B** mineralisable N for the 0-7.5 cm depth at all sites.

Given such limitations, it may be useful to consider the “yield above control”, or yield response, on a scale which takes the magnitude (i.e. relative scale) of total spring growth into consideration. The relationship between yield on control plots and the yield above control is presented in Figure 4.11.

Approximately 67% of the variation in yield above control could be explained by the level of control yield. The curve which best explained the data was cubic in shape (Figure 4.11). In order to fully understand the significance of this result the pattern of this response curve is investigated further.

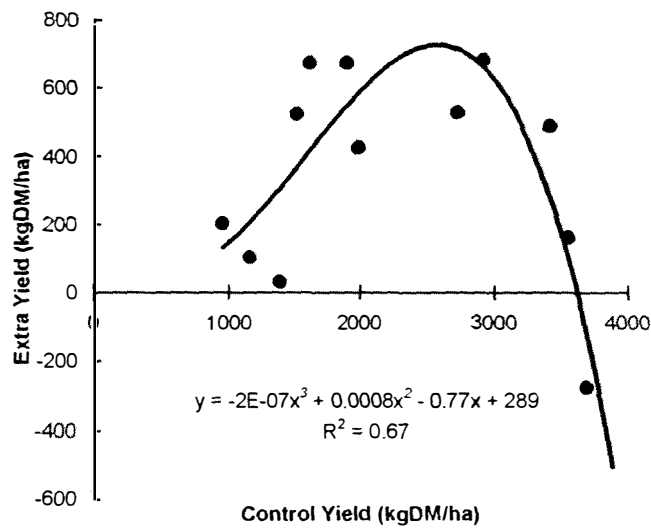


Figure 4.11 The relationship between pasture growth above control, and control yield during the spring of 1994 across all sites.

In Figure 4.12, the response curve of Figure 4.11 has been replicated, and presented with data point labels (site numbers). The shaded bar represents a 25:1 assumed “maximum” response to the 30 kgNha⁻¹ applied.

In order to better understand this response curve, the curve can be split into three distinctly different regions, or segments. Some constraints that may reduce pasture growth response to applied N are listed in Table 4.4. A tick indicates that this constraint operates at one of the numbered sites (1-14).

At yields of 1400 kgDMha⁻¹ and below, response to N fertiliser was low (Figure 4.12). Sites in this category have very low fertility (Sites 4, 5 and 6, Table 4.4). Site 4 had more available moisture (Figure 4.4A-C) than coastal sites 5 and 6, explaining the marginally higher response at that site.

In the central section of the response curve, sites had a high response to applied N, nearing the potential maximum of 25 kgDM grown per kg of N applied as fertiliser. Looking at the first grouping of sites, on the upward slope of the curve (Figure 4.12), is Site 13, a medium-high rainfall site, and Site 11, which is a comparable site, receiving lower rainfall (Table 4.4).

Site 12 has responded less, probably as a result of lower general fertility, and site 9 lower still, again due to lower fertility, and also because of the drier coastal environment. Sites 8 and 10 produced a high response due to high fertility status, and possibly high Et.

Table 4.4 Constraints on hill pasture growth response to spring applied N (30 kgNha⁻¹).

Site	Constraints						Nitrogen Response	
	Leaching loss	Low P Fertility	N Fert <15% N Uptake	High Soil N	Dry Soil	Low Growth Rates	< 15:1	15 to 25:1
1	✓			✓				✓
3	✓		✓				✓	
4	✓	✓				✓	✓	
5		✓			✓	✓	✓	
6		✓			✓	✓	✓	
8								✓
9					✓		✓	
10				✓	✓			✓
11		✓			✓			✓
12		✓		✓	✓			✓
13	✓			✓				✓
14	✓		✓				✓	

Finally, on the downward slope of the response curve, where sites had high control yields, but lower relative response to N fertilisation, are Sites 1, 3 and 14 (Figure 4.12). All three of these sites are within the “high” rainfall zone, and experienced large drainage events within 4-5 days after the fertiliser N was applied (Figure 4.12). Approximately 55mm drained 4 days after application, and a total of 115 mm drained within 14 days of application (Figure 4.2A). This amount of rain would cause most of the fertiliser N to drain below pasture rooting depth at these sites (Sakadevan et al., 1993). Reduced available N may explain the lack of growth response (Table 4.2, 4.3). However, Site 1 still exhibited good growth response to N fertilisation at this rate, which can perhaps be explained by the very high fertility status at this site, and by the high (and perhaps more labile) soil mineralisable N status. Large quantities of N (relative to fertiliser N applied) cycling at this site may also explain the low response to N fertiliser. Sites 3 and 14 have lower fertility status respectively, which supports their respective positions on this response curve. The very low position of Site 14 on this curve may be explained by the very high rainfall at this site, perhaps leading to high levels of N leaching. It is difficult, however, to explain why the control plots yielded higher than N treated plots at Site 14. It is possible that Exchangeable potassium (K) levels may also have been limiting at this site (See low K values: Chapter 5, Table 5.3). Taking soil exchangeable K status, high leaching/rainfall, N fertiliser addition, soil parent materials (Greywacke), lower soil temperatures, and higher altitude into consideration, it may be the case that these conditions have interacted to limit any response to N fertiliser applied at this low rate.

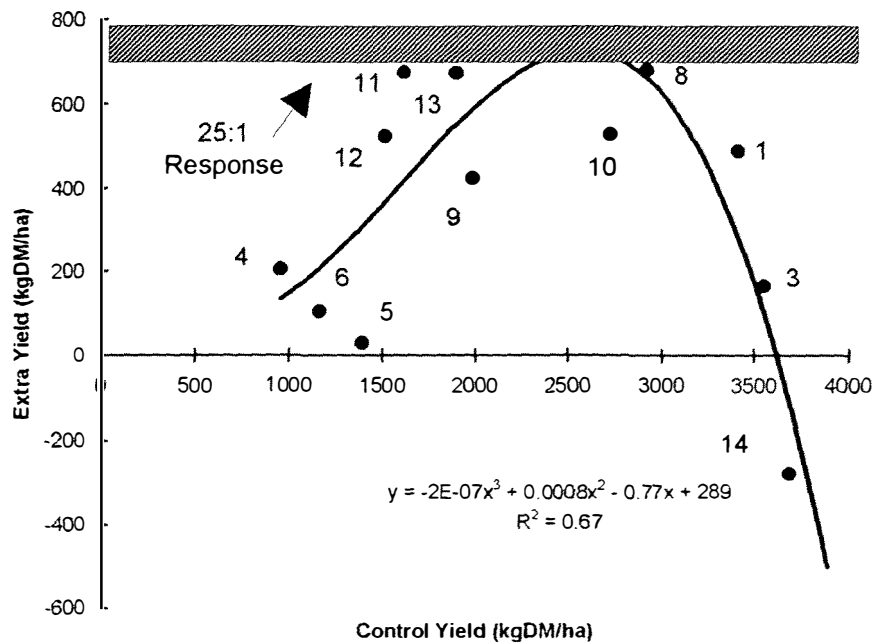


Figure 4.12 The relationship between pasture growth above control, and control yield during the spring of 1994 across all sites (numbered 1-14), including actual (●) yields. Shaded zone = 25:1 growth response to N fertiliser.

4.3.5.5 N Response Relative to Economic Returns

When considering the use of strategic N fertiliser applications to hill country, it may be beneficial to consider the “likely” economic returns to the farmer. Following the methodology of Parker et al. (1994), excluding climatic probabilities, simple costs and returns were calculated (see Appendix 4.1).

This calculation shows that at current lamb and beef prices, the break-even point for pasture response to 30 kgNha^{-1} is around 4.8:1 for 30 kg lambs, and 3.4:1 for 540 kg beef steers, assuming 100% utilisation of extra feed. Under such a scenario, the dollar value of strategic N returns are \$3.16 and \$4.95 per kgN applied for lamb and beef respectively (Appendix 4.1.5.1). These “break-even” N response levels are lower than those measured at most of the Wairarapa trial sites in the spring of 1994. Where the return to farmer from N application varies from \$2.27-\$3.47 / kgN spent on N fertiliser, grazing management/pasture utilisation is good and allows 70% utilisation (Appendix

4.1.5.2). If however extra pasture DM is poorly utilised (50%) then returns are lower (\$1.58-\$2.48 / kgN; Appendix 4.1.5.3). However, even at only 50% utilisation of pasture DM, the break-even response to N fertiliser is only around 10:1 at current market produce prices. These calculations indicate that given current prices for product on overseas markets, N applied at low rates to East Coast hill country pastures is likely to be profitable, dependant upon climatic conditions, and high levels of pasture utilisation.

4.4 GENERAL DISCUSSION

Low rainfall in August and December of 1994 produced drought conditions in Whareama and Gladstone districts, and some moisture limitations to pasture growth in Mauriceville during the 1994/95 season. Pasture production was severely moisture limited by late spring, and resulted in a shorter than average growing season in Whareama and Gladstone. This climate scenario is one where strategic use of N may grow valuable extra pasture during the short growing season.

Pasture dry matter yield ranged from 1.7 to 11.7 tDMha⁻¹ for the 1994/95 season (Table 4.2), and was strongly influenced by climate and soil fertility status. Plots receiving N fertiliser often yielded about 500 kgDMha⁻¹ higher than control plots, with most of the fertiliser response related growth occurring within four weeks of application. Olsen P status was a very good indicator of annual yield at these sites during this season, which contrasts with the previous years data from these sites (Moir, 1994).

Spring pasture yield was strongly related to Olsen P status ($R^2 = 0.80$), confirming the finding of the previous year (Moir, 1995). Similar to studies by Gillingham et al. (1998), the degree of pasture response to application of N fertiliser was extremely variable and could not be explained by soil fertility indices alone. However, Gillingham et al. (1998) did not conduct a comprehensive examination of the relationship between soil fertility status and pasture response to N fertiliser. Approximately 67% of the variation in yield above the control yield could be explained by the amount of control yield (Figure 4.11). Using this curve, the N response at each site could be explained, depending on the region of the curve that the control (-N) yield appeared upon. In general, low fertility and low fertility dry sites had low N responses (fertility limitation), sites with high response had

medium-high fertility, and probably adequate moisture and high Et, and sites with high control yields but lower N responses suffered high N leaching losses during the large drainage events of late August.

The results indicate that many of these pastures may give good responses to low levels (30 kgNha⁻¹) of strategic N fertiliser applied in mid spring, even under moisture limited conditions, or where low rainfall will shorten the growing season. This result is especially important to producers farming summer-dry hill country, who are seeking strategies for managing risk linked with the onset of drought conditions. Hill country in higher rainfall areas may suffer N leaching losses, even in summer months, but high N responses are still possible. There may be more risk associated with applying N fertiliser on high rainfall hill country, but the lower financial risk may warrant application in any case.

4.5 CONCLUSIONS

Over the 1994/95 growing season, at the study sites in the Wairarapa region, pasture growth response to N fertiliser was variable. Simple single factors representing soil fertility indices could not explain the variation in site-to-site pasture growth response to applied N. The variation in pasture growth response to N was likely caused by a number of constraining soil and climate factors. These are discussed but could not be assembled into a diagnostic model to predict site-to-site variations in N response. The efficiency of pasture N use was discussed, and it was concluded that it was not possible to accurately predict the degree of pasture N response at these hill country sites using simple soil or climatic measures.

Taking current produce market prices into account, pasture responses to low rates of N fertiliser were shown to be economic, and likely to be profitable.

In order to better understand both variation in field yields, and responses to N fertilisers, the nutrient supplying abilities of these soils must be determined. Glasshouse studies, which would remove the complicating and variable effect of climate, are proposed. Such studies may provide valuable information on the ability of these soils to provide plant-available nutrient.

Prediction of pasture production in the field is inaccurate, because of the empirical nature of current pasture production models, which do not account for the effect of climate. In a later Chapter (Chapter 6), an attempt will be made to model pasture growth using both climate and simple soil fertility data from these Wairarapa sites.

The data from these N response trials suggest the need for such a model, given that detailed site-specific data (such as presented in this Chapter), will not normally be available to researchers attempting to explain broad-based pasture production in the field.

4.6 REFERENCES

- Ball, P.R. and Field, T.R.O., 1982. Responses to nitrogen as affected by pasture characteristics, season and grazing management. In: P.B. Lynch (Editor), Nitrogen fertilisers in New Zealand agriculture. New Zealand Institute of Agricultural Science, Wellington, New Zealand, pp. 45-64.
- Ball, P.R., Luscombe, P.C. and Grant, D.A., 1982. Nitrogen on hill country. In: P.B. Lynch (Editor), Nitrogen fertilisers in New Zealand agriculture. New Zealand Institute of Agricultural Science, Wellington, New Zealand, pp. 133-147.
- Ball, R., Inglis, J.A.H. and Mauger, J.H., 1976. Tactical application of fertiliser nitrogen to offset a seasonal feed shortage on a heavily stocked sheep farm in Southern Hawke's Bay. In: W.G. Thurston (Editor), Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, Hastings, New Zealand, pp. 166-181.
- Bircham, J.S., 1977. Grazing management for the improvement of browntop pastures in hill country: A programme. In: W.G. Thurston (Editor), Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, Nelson, New Zealand, pp. 87-92.
- Clark, D.A. and Lambert, M.G., 1989. The use of fertiliser nitrogen for increased animal production from New Zealand hill pastures. In: R. Desroches (Editor), XVI International Grassland Congress. French Grassland Society, Nice, France, pp. 1269-1270.

- Daniell, D., 1993. The long-term use of nitrogen fertiliser in intensive hill country farming. In: M.J. Baker (Editor), Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, Masterton, New Zealand, pp. 59-61.
- Fleming, P.H. and Burt, E.S. (Editors), 1991. Farm Technical Manual. Farm Management Department, Lincoln University, Canterbury, New Zealand, A3-A15 pp.
- Gillingham, A.G., Gray, M.H. and Smith, D.R., 1998. Pasture responses to phosphorus and nitrogen fertilisers on dry hill country. In: M. Baker (Editor), Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, Nelson, New Zealand, pp. 135-140.
- Kinnell, D., 1993. Managing for risk on summer dry coastal hill country. In: M.J. Baker (Editor), Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, Masterton, New Zealand, pp. 51-52.
- Lambert, M.G. and Clark, D.A., 1986. Effects of late-autumn nitrogen application on hill country pastures and sheep production. In: J.L. Brock (Editor), Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, Whangarei, New Zealand, pp. 211-215.
- Ledgard, S.F., Brier, G.J., Gillingham, A.G. and Sheath, G.W., 1983. Influence of some soil and pasture components on the growth of hill country pastures II. Response to nitrogen fertiliser. New Zealand Journal of Experimental Agriculture, 11: 251-256.
- Luscombe, P.C., 1979. Nitrogen fertiliser responses on hill country pastures. In: J.L. Brock (Editor), Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, Rotorua, New Zealand, pp. 155-162.
- Moir, J.L., 1994. Influences of climate and fertiliser application history on various measures of soil fertility and productivity in Wairarapa hill country. Master of Agricultural Science Thesis, Massey University, Palmerston North, New Zealand, 145 pp.

- Moir, J.L., Hedley, M.J., Mackay, A.D. and Tillman, R.W., 1997. The effect of fertiliser history on nutrient accumulation and plant-available nutrient supply in legume-based pasture soils. In: J.G. Buchanan-Smith, L.D. Bailey and P. McCaughey (Editors), XVIII International Grassland Congress. International Grassland Association, Winnipeg, Canada, pp. 10, 68-69.
- Morton, J.D., Korte, C.J., Smith, D.R., Watt, B.D. and Smith, R.G., 1993. Nitrogen use and farm performance on Wairarapa sheep and beef farms. In: M.J. Baker (Editor), Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, Masterton, New Zealand, pp. 53-57.
- O'Connor, M.B., 1982. Nitrogen fertilisers for the production of out-of-season grass. In: P.B. Lynch (Editor), Nitrogen fertilisers in New Zealand agriculture. New Zealand Institute of Agricultural Science, Wellington, New Zealand, pp. 65-76.
- Parker, W.J., Dake, C.K.G., Wright, L. and Tillman, R.W., 1994. Risk management for tactical nitrogen fertiliser applications in pastoral farming. In: M.J. Baker (Editor), Proceedings of the New Zealand Grassland Association. New Zealand Grassland Association, Hanmer Springs, New Zealand, pp. 223-227.
- Parker, W.J., Tillman, R.W. and Gray, D.I., 1989. Management considerations in nitrogen fertiliser usage on a Wairarapa sheep farm. In: R.H. White and L.D. Currie (Editors), Proceedings of the Workshop on "Nitrogen in New Zealand agriculture and horticulture". Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 65-78.
- Radcliffe, J.E., 1975. Seasonal distribution of pasture production in New Zealand VII. Masterton (Wairarapa) and Maraekakaho (Hawke's Bay). *New Zealand Journal of Experimental Agriculture*, 3: 259-265.
- Sakadevan, K., Hedley, M.J. and Mackay, A.D., 1993. Mineralisation and fate of soil sulphur and nitrogen in hill pastures. *New Zealand Journal of Agricultural Research*, 36: 271-281.
- Sinclair, A.G., Johnstone, P.D., Smith, L.C., Roberts, A.H.C., O'Connor, M.B., and Morton, J.D., 1997a. Relationship between pasture dry matter yield and soil Olsen P from a series of long-term field trials. *New Zealand Journal of Agricultural Research*, 40: 559-567.

- Sinclair, A.G., Morrison, J.D., Smith, L.C. and Dodds, K.G., 1996a. Effects and interactions of phosphorus and sulphur on a mown white clover/ryegrass sward
2. Concentrations and ratios of phosphorus, sulphur, and nitrogen in clover herbage in relation to balanced plant nutrition. *New Zealand Journal of Agricultural Research*, 39: 435-445.
- Sinclair, A.G., Morrison, J.D., Smith, L.C. and Dodds, K.G., 1997b. Effects and interactions of phosphorus and sulphur on a mown white clover/ryegrass sward
3. Indices of nutrient adequacy. *New Zealand Journal of Agricultural Research*, 40: 297-307.
- Sinclair, A.G., Smith, L.C., Morrison, J.D. and Dodds, K.G., 1996b. Effects and interactions of phosphorus and sulphur on a mown white clover/ryegrass sward
1. Herbage dry matter production and balanced nutrition. *New Zealand Journal of Agricultural Research*, 39: 421-433.

CHAPTER 5

GLASSHOUSE STUDIES TO DETERMINE PLANT-AVAILABLE NUTRIENT RESOURCES OF SELECTED WAIRARAPA HILL SOILS⁶

5.1 INTRODUCTION

Long-term fertiliser (P and S) application has been shown to indirectly increase nitrogen levels in pasture soils (Walker et al., 1959; Jackman, 1964; Moir et al., 1995). Accumulation of soil N over many years of pasture development can reduce the nitrogen constraint to pasture production, raise pasture yields and in turn raise the maintenance P and S fertiliser requirements per hectare (Moir et al., 1997; Lambert et al., 1998, 2000).

In the previous chapter, the pasture response to strategic N fertiliser applied in spring was examined on pasture soils with wide ranging long-term fertiliser histories, and levels of nutrient accumulation. Climate was shown to be the major factor confounding an understanding of soil nutrient supply, and response to fertiliser N. From this study, it became clear that attempts to define the productivity of these soils, linked strongly to soil N and P fertility status, were confounded by different patterns of seasonal soil water deficit and surplus. To fully understand pasture growth responses to fertilisers on these soils, improved information on the nutrient supplying abilities of these soils is required in conjunction with a description of seasonal soil water availability.

⁶ Some data presented in this Chapter contributed to the following conference papers:

(i) "Effects of various fertiliser histories on nutrient accumulation and pasture production in Wairarapa hill country.", presented at the combined NZSSS/ASSS conference, Melbourne, Australia (Moir et al., 1996).
(ii) "The effect of fertiliser history on nutrient accumulation and plant-available nutrient supply in legume-based pasture soils.", presented at the International Grasslands Congress, Winnipeg, Canada (Moir et al., 1997).

In this Chapter the nutrient supplying power of these hill country soils was studied in the climate controlled environment of the glasshouse. The hypothesis was that the controlled environment would remove the confounding effects of soil water deficit and surplus (drainage), and would permit patterns of pasture growth response to the nutrients N, P and S to be more clearly related to indices of soil nutrient status. This study involved two pot experiments. The first was a simple experiment examining plant growth and nutrient uptake in a glasshouse environment, and the second involved an exhaustive cropping regime, focusing on the depletion (soil supplying power) of a single nutrient. The objective of both glasshouse trials was to use plant growth, in the absence of climatic constraints, to determine the availability of the major growth-limiting nutrients in soil samples taken from each of the 12 field sites.

Experiment 1

The first experiment examined the effect of climate on pasture growth in order to evaluate indicators of soil fertility that would allow prediction of the overall nutrient supplying power of each soil.

Objective (Experiment 1)

- (i) To use ryegrass and clover growth to indicate nutrient supplying power of the test soils
- (ii) To use ryegrass and clover growth to evaluate standard soil tests and herbage analysis as indicators of nutrient supplying power

Experiment 2

The second experiment involved the exhaustive cropping of a small quantity of soil, where only one nutrient was limiting. The limiting nutrients were N, P or S, which were supplied only by the soils, and may indicate the ability of each of these soils to supply these nutrients (a possible indicator of the size of the plant-available nutrient pools) under controlled climatic conditions.

Objective (Experiment 2)

To use plant growth (to exhaustive cropping) to assess the size of the N, P and S plant-available pools in each soil.

5.2 METHODS AND MATERIALS - EXPERIMENT 1

5.2.1 Soil Sampling and Preparation

Soil was sampled from the Wairarapa hill country sites described in detail in Chapter 3. Approximately 30 kg of field moist soil was collected from each site in late March 1995, from the 0-7.5 cm soil horizon, using a spade, ruler and trimming knife. Having experienced drought conditions over the 1994/95 season (Refer Chapter 4), most soils were very dry (mean $\theta = 0.09$) at the time of sampling, and some were very hydrophobic (see Section 5.2.3.1). Soils were air-dried (approx. 35°C) soon after sampling, then passed through a 2 mm sieve. Plant stem, and thick root material was removed in the sieving process. These soils were used in both glasshouse experiments described in this chapter.

5.2.2 Soil Chemical Analyses

Plant-available Phosphorus

Plant-available Phosphorus (P) of soils was defined as that extracted by the method of Olsen et al. (1954), and also by ion-exchange resin membrane extraction (Saggar et al., 1990), followed by P analysis by method of Murphy and Riley (1962).

Phosphate extractable sulphate

Extractable soil sulphate was determined by a 0.4 M $\text{Ca}(\text{H}_2\text{PO}_4)_2$ soil extraction (Searle, 1979). Extract S content was determined colorimetrically by method of Johnson and Nishita (1952) modified to work on a Technicon Autoanalyser.

Mineralisable Nitrogen

Soil mineralisable nitrogen (N) content was measured using an anaerobic incubation procedure. The incubation technique was a modified version of that used by Keeney and Bremner (1966) and Waring and Bremner (1964). Air-dry soil (5g) sub-samples were suspended with 20 mL of distilled H_2O . This test was conducted in triplicate in 50 mL centrifuge tubes, then incubated at 30°C for 21 days. Samples were mixed once daily using a vortex mixer. After incubation, N was extracted by adding 10 mL of 3M

KCl solution (extraction strength = 1M KCl), and shaking on an end-over-end shaker for 20 minutes. Samples were then centrifuged at 5000 rpm for 5 minutes, followed by filtration. Supernatants were analysed for total N content using the colourmetric method on a Technicon II Auto Analyser (see later, this section).

Exchangeable Cations and Cation Exchange Capacity (CEC)

Soil exchangeable cations (K^+ , Mg^{2+} , Ca^{2+} , Na^+ , H^+) were measured using the ammonium acetate extraction method of Schollenberger and Simon (1945), followed by atomic adsorption spectroscopy (AAS) analysis. All base cations plus an estimate of the acidity equivalents in the extract, were summed to produce the soil CEC value.

pH_{H2O}

Soil pH in water was measured at a water:soil ratio of 2.5:1 (Blakemore et al., 1987).

pH_{0.1M CaCl}

Soil pH in 0.1M $CaCl_2$ solution was measured at a solution:soil ratio of 2.5:1 (Blakemore et al., 1987).

Phosphate Retention

Phosphate retention by soil was measured by method of Blakemore et al. (1987). Soils were shaken with a phosphate buffer (5:1, phosphate solution (1 mgP mL^{-1}):soil ratio) for 16 hours, followed by P measurement by vanadomolybdate method (Saunders, 1965).

H₂O₂ Extractable S and N

The amount of mineralisable organic (carbon-bound) N and S were also estimated using a dilute hydrogen peroxide treatment (S. Saggat, pers. comm.). Air-dry soil samples (2g, in triplicate) were treated with 2.5 mL of an 8% by volume H_2O_2 solution. Samples were mixed on a vortex mixer, then left to sit for 30 minutes to oxidise soil carbon. Soils were then extracted by adding 17.5 mL of 0.286 M KCl solution (final strength 0.25 M KCl), and shaking on an end-over-end shaker for 30 minutes, then filtered (Whatman 41). Supernatants were taken to dryness (85°C , 24 hours), then rehydrated with 10 mL of deionised H_2O , mixed by vortex. The HI reducible S content of the H_2O_2 soil extract was determined colorimetrically by the method of Johnson and Nishita (1952) modified to

work on a Technicon Autoanalyser. The NH_4^+ and NO_3^- concentrations were determined colorimetrically by method of Searle (1975, 1984) and Kamphake et al. (1967) respectively, on a Technicon II Autoanalyser. To evaluate S, NH_4^+ and NO_3^- recovery, several soils were spiked with solutions containing these ions. Inorganic S, NH_4^+ and NO_3^- contents of soils were estimated by extracting all soils with deionised water only, followed by the standard analysis.

5.2.3 Experimental Design

The trial commenced in mid-July, 1995. Air-dry 2 mm sieved soil (500g pot^{-1}) was weighed into 8 cm height x 9.5 cm diameter pots (570 cm^3 volume). Soil from each site was used, replicated four times for each of the two plant species grown (ryegrass, *Lolium perenne*, and white clover, *Trifolium repens*), totalling 96 pots

5.2.3.1 Soil Moisture and Glasshouse Conditions

The soils (500g) were moistened with 200 mL of distilled water. Some soils, notably from sites 9, 1 and 12 were extremely hydrophobic, and took 4-5 days to fully wet up. Soils were maintained at a gravimetric water content of 0.4 for the duration of the trial, and no nutrients were added. Soils were watered to weight with distilled water, on a daily basis. On warm days, when plants were > 8-10 cm in height, pots were watered twice daily, to prevent extremes of soil dryness and plant water stress. Any flow of water through the soil, to the pot saucer, was later re-applied to the soil surface. All pots were housed in glasshouse conditions, with artificial lighting at night. Maximum (daytime) and minimum (night) temperatures for the period were 25 and 15° C, respectively, with an average temperature of approximately 20° C. All pot positions were randomised daily during watering.

5.2.3.2 Plant Establishment

Once moist, approximately 40 seeds were placed on the soil surface, and a small quantity of dry soil (3-5 g for clover pots, 10-15 g for ryegrass pots; of the total 500 g) was sprinkled on top, and firmed. Seeds germinated 7-14 days later, and were thinned to a final density of 30 plants per pot, 23 days after planting. At this point, all clover pots

received 5 mL of a rhizobium inoculum solution, in order to ensure that all soils and plants in this treatment were inoculated with nitrogen fixing bacteria.

5.2.3.3 Yield Measurement

The ryegrass treatments were harvested twice during the duration of the experiment (38 and 56 days post planting), and the clover treatments harvested once (day 56 post planting). Plants were harvested to 10 mm height, the cuts were timed to allow for high plant growth, while keeping the plants in a vegetative rather than reproductive growth stage. All harvested plant tissue was oven-dried at 70° C to constant weight (approximately 48 hours) and weighed for yield measurement. After drying, plant matter was then finely ground for nutrient analysis purposes.

5.2.3.4 Nitrogen Fixation

In clover root nodules, atmospheric nitrogen gas is reduced to ammonia by the nitrogenase enzyme complex of rhizobium bacteria. The nitrogenase enzyme is not specific in action to nitrogen, but preferentially reduces acetylene gas to ethylene gas if clover roots are placed in an acetylene atmosphere. A modified version of the acetylene-reduction assay of Hardy et al. (1968), and Hoglund and Brock (1978) was used to assess the nitrogen fixing rates of white clover (*Trifolium repens*).

Nitrogen fixation was measured on all pots growing clover on the day of harvest (day 56 post-planting). Seven soil core samples, totalling just under 260 cm³ of soil (25 mm diameter, herbage intact), were taken from each pot, and placed in 600 mL glass preserving jars. The jars were then sealed with lids fitted with a "Vacutainer" rubber stopper. Using a syringe, 30 mLs of air was removed from each jar, and 30 mLs of acetylene gas injected into the sample. All samples were then incubated at room temperature (15° C) for one hour. Control jars (gas blanks) were prepared by injecting 30 mL of acetylene into jars containing no soil.

During the incubation, the clover root nodules converted some of the acetylene to ethylene gas, which was released into the preserving jar. After the one hour incubation period, double-ended needles were used with red stoppered "Vacutainer" tubes to take

10 mL gas samples from the sample jars. These gas samples were then analysed for ethylene content by gas chromatography, at AgResearch Grasslands, Palmerston North.

Acetylene levels in gas blanks were subtracted from jars containing soil, and acetylene reduction rates were calculated. These were then converted to N fixation rates, expressed as $\text{kgNha}^{-1}\text{d}^{-1}$.

5.2.3.5 Plant Tissue Analysis

Nitrogen and Phosphorus

To measure total N and P contents of herbage, 0.1 g of ground dry herbage was first digested by the Kjeldahl method (McKenzie and Wallace, 1954). Digest solutions were then diluted, and N and P contents determined by method of Searle (1975, 1984) and Saunders (1965) respectively, on a Technicon II Autoanalyser.

ICP-AES Analysis

Concentrations of S, K, Ca, N, P, Mg, trace elements and heavy metals in all above ground plant tissue were determined by Inductively-Coupled Plasma Emission Spectrometry (ICP-AES, or ICP) analysis (AgResearch Grasslands, Palmerston North). Herbage samples (0.1 g, dried and ground) were digested in 4 mL of conc. nitric acid (HNO_3) at 160°C for 12 hours. Block temperature was then slowly raised to 200°C , and the digest liquid was evaporated (4-8 hours). Hydrochloric acid (HCl 2M, 5 mL) was then added to dissolve the dry sample, and made to a final volume of 25 mL with deionised water. Samples were then analysed by ICP-AES.

5.2.3.6 Post-Experiment Soil Analyses

At the completion of the trial, all soils were removed from the pots, air-dried and 2 mm sieved. Soil pH in H_2O , and 0.1 M CaCl was then determined on all samples (for methodology, refer to Section 5.2.2).

5.3 METHODS AND MATERIALS - EXPERIMENT 2

5.3.1 Stanford and DeMent - Experimental Design

A modified Stanford and DeMent (1957) bioassay technique was used to estimate the amount of plant available N, P and S in each soil. This double-pot technique quickly produces a root mat rhizosphere, which is then placed in contact with the soils to be studied (Plate 5.1). It is an “exhaustive” technique which aims to deplete a nutrient from a small volume of soil. Soils were prepared in the method explained in Section 5.2.1. The quantity of soil used in each of the three treatments (no N, P or S) was varied, based on an estimation of the total amount of the “treatment” nutrient that would be mineralised over the period of the experiment, and be supplied to the plants for uptake. This estimation was based in part on results from Mackay et al. (1995), and also from our soil analyses. The maximum nutrient uptake by plants was estimated as approximately 200 mg N, 10-15 mg P and 7-10 mg S per pot, based on a maximum yield of 3.5 gDM pot⁻¹. Based on these estimates, the -N treatment pots contained 100 g of air-dry soil, -P treatment 50 g and -S treatment 25 g. It is hoped that these treatments would allow complete mineralisation, and plant uptake, of the three treatment nutrients over the duration of the experiment.

5.3.1.1 Pot Design

Each pot consisted of two identical containers 10 cm diameter x 6 cm deep, placed one inside the other (Plate 5.1). The bottom of the inner container was punctured with many holes, which allowed plant roots to grow through to the second container, where the small quantity of soil was placed (after plant establishment). Fine silica builders sand (400g, saturated hydraulic conductivity 70 to 80 x 10⁻³ ms⁻¹) was used as the plant growing medium, having first been acid washed (0.1 M HCl), and leached with distilled water for several days. Treatments were -N, -P and -S, for all 12 soils, replicated four times (total 144 pots).

5.3.1.2 Soil Moisture

Initially, dry sand (400 g) was moistened with 80 mL of distilled water (17 % H₂O by weight, @ 20 cm tension). Throughout the experiment, pots were watered to weight daily, based on maintaining the soils at a constant moisture content, at 40 cm tension. The gravimetric water content at this tension was measured for all soils (measured by method of Haynes apparatus, Table 5.1), and was used to calculate the daily watering weight for each pot. Where necessary, pots were watered 2-3 times per day to maintain this moisture level, and to prevent any occurrence of extreme dryness.

Table 5.1 Soil water holding capacity (gravimetric water content) at 40 cm tension (suction potential).

Site	1	3	4	5	6	8	9	10	11	12	13	14
WHC	38.3	39.1	35.6	43.7	41.3	42.3	38.5	40.2	46.8	43.4	43.0	44.1

5.3.1.3 Plant Establishment

Pots were moistened (sand only), and 20 ryegrass seeds planted at the surface of each in mid August 1995. At germination (late August), all pots received 15 mL of a complete nutrient solution (0.43 mg N, 0.06 mg P, 0.04 mg S, 0.16 mg K, 0.01 mg Mg, plus trace elements per mL), followed by 7 mL 10 days later. After 22 days growth (post-planting), plants were thinned to 15 plants per pot. Nine days later (31 days post-planting), all pots had a fibrous root mat appearing through the pot base (See Plate 5.1), and were ready for introduction to soil. Soil (100g -N, 50 g -P and 25 g -S treatments), was added to the base of the outer pot, and moistened. The sand pot (containing the plants) was then placed on top, allowing the roots to grow into the small soil volume. All plants were trimmed to 15 mm height at this stage.



Plate 5.1 Design of pots used in Experiment 2, showing the thick rhizosphere mat which forms in the small volume of soil at the bottom of the pot, after plant roots have grown through the upper (sand) portion.

5.3.1.4 Basal Nutrient Application

When the plants were first introduced to the soil medium (day 31, post-planting), each pot received 50 mL of their respective basal nutrient solution (either no N, no P or no S). Over the duration of the experiment, all pots received approximately 30-50 mL of nutrient solution per week, dependent on plant growth stage and cover. This supplied all nutrients required for plant growth, excluding either N, P or S, which was supplied only by the soil. The nutrient solution was supplied in the ratio of 12:2:5:1:0.03 (N:P:K:S:Mg + trace elements). Total nutrient applied is presented in Table 5.2. All pot positions were randomised daily at watering.

A “-P Control” treatment was also applied to 8 additional pots. These treatments received no soil, but similar nutrient solution inputs as the -P treatment. The aim of the -

P control was to see how much plant growth would occur when the only P input to the system was from the 22 mL of complete nutrient solution, applied initially, for seedling establishment. As with other treatments, harvests of plants were taken until all growth stopped (assumed to be the point of zero plant availability of P).

Table 5.2 Summary of total nutrient supplied by basal nutrient solution to all treatments

Treatment	Complete Solution	Treatment Solution	Total Nutrient Applied		
			N	P	S
- N	22 mL	297 mL	9.6 mg* (0.1 mg/d)	18.8 mg (0.23 mg/d)	11.3 mg (0.14 mg/d)
- P	22 mL	522 mL	236 mg (1.8 mg/d)	1.3 mg* (10 µg/d)	19.3 mg (0.15 mg/d)
- S	22 mL	297 mL	139 mg (1.7 mg/d)	18.8 mg (0.23 mg/d)	0.78 mg* (9 µg/d)
- P (Control)	22 mL	360 mL	166 mg (2.2 mg/d)	1.3 mg (17 µg/d)	13.5 mg (0.18 mg/d)

*Small amounts of these nutrients applied during seeding establishment phase are assumed to have been taken up by the plants, or removed when plants were trimmed, before soils (and -N, -P and -S treatments) were introduced to the plants. The effect of these very small quantities of basal N, P and S on plant growth thereafter is assumed to be negligible.

5.3.1.5 Yield Measurement

All pots were harvested at intervals until plant growth had stopped (treatment nutrient source exhausted). Harvest intervals were at 63 and 83 days post planting. At the second harvest, all plants in the -N and -S treatments had stopped growing, and were discontinued. Plants in the minus P treatments continued to grow, and were harvested for a third and final time at 127 days post planting (late December 1995).

5.4 RESULTS AND DISCUSSION - EXPERIMENT 1

5.4.1 Soil Analyses

Soil pH

Soil $\text{pH}_{\text{H}_2\text{O}}$ was mildly acidic in all soils (Table 5.3). Soils from sites 3, 4, 9 and 12, were low enough ($\text{pH} \leq 5.3$) to limit ryegrass growth in mineral soils (Edmeades et al. 1984a, 1984b, 1984c). These soil samples were taken from the trial sites in March 1995 and in general are the same as samples taken previously for the study of Moir (1994). Plant growth in glasshouse pots caused both $\text{pH}_{\text{H}_2\text{O}}$ and $\text{pH}_{\text{CaCl}_2}$ levels to decline. The similar decline in both pH levels indicates a true increase in soil H^+ concentration, rather than pH change caused by variation in soil solution ionic strength. It is expected that mineralisation and nitrification of soil organic N would have caused this pH decrease. The pH values below 5.3 at the end of the trial could be expected to begin limiting root extension and plant growth (Wang et al., 1995; Wang et al., 1999).

Table 5.3 Soil chemical analyses and field bulk density for all soils in the glasshouse trials.

Site Soil Sample	pH				Bulk Density (gcm ⁻³)	Olsen P (µgPg ⁻¹)	P Ret ⁿ (%)	Resin P (µgPg ⁻¹)
	Initial		Post Trial					
	H ₂ O	CaCl	H ₂ O	CaCl				
1	5.7	5.07	5.12	4.73	0.97	47.9	24.3	81.0
3	5.4	4.97	5.30	4.86	1.01	20.5	34.4	37.4
4	5.2	4.39	4.93	4.39	0.91	6.9	50.8	20.2
5	5.5	4.83	5.20	4.64	0.83	27.2	15.6	31.9
6	5.5	4.94	5.36	4.77	0.94	16.7	19.6	23.8
8	5.8	5.32	5.55	5.18	0.95	22.2	17.2	64.8
9	5.3	4.61	4.91	4.40	0.82	26.9	25.7	40.5
10	5.6	5.1	5.43	5.00	1.01	27.3	19.8	83.6
11	5.7	5.19	5.52	5.08	0.91	14.4	15.8	25.5
12	5.3	4.57	5.00	4.56	0.87	18.8	22.4	24.5
13	5.6	4.82	5.28	4.79	0.91	14.5	43.6	33.8
14	5.5	4.97	5.26	4.89	1.01	15.9	28.2	46.7
	Exchangeable Cations (cmole charge ⁺ kg ⁻¹ soil)			Min N (kgNha ⁻¹)	SO ₄ [CaP] (µgSg ⁻¹)	H ₂ O ₂ Extraction (µgg ⁻¹)		
	K	Ca	Mg			S	NH ₄	NO ₃
1	1.45	8.00	1.39	454	20.8	43.0	215.9	53.0
3	0.63	8.06	1.13	369	15.5	31.8	122.9	61.3
4	0.54	5.31	1.78	205	8.3	16.0	60.4	0.0
5	1.29	9.61	5.40	440	8.8	30.5	75.2	40.0
6	0.67	8.51	3.81	419	8.8	23.5	81.4	13.7
8	0.97	15.31	5.44	382	17	35.0	57.7	5.5
9	1.14	4.96	3.56	256	7.3	29.0	158.4	46.0
10	1.05	11.85	1.99	515	8.8	27.5	69.7	8.5
11	0.49	6.42	2.57	222	3.5	16.5	41.9	0.5
12	0.69	12.22	2.24	483	11.5	37.0	103.4	1.7
13	0.43	9.04	0.92	469	5.3	12.8	94.9	2.5
14	0.25	11.13	1.05	346	8.5	22.0	52.2	13.5

Extractable phosphate

Olsen P soil tests (Table 5.3) and the P response threshold ranges (<20 µgPg⁻¹ soil) given by Saggar et al. (1993, 1999), Sinclair et al. (1997a), Cornforth and Sinclair (1984) and Cornforth and Sinclair (1982) indicate that soils from sites 4, 6, 11, 13 and 14 are most likely to show ryegrass growth constrained by P availability. Resin P values ranged from 20-81 µgPg⁻¹. According to glasshouse research by Saggar et al. (1992, 1993) ryegrass growth on YBE soil, with resin P values below 40 µgPg⁻¹ can be expected to respond to soluble and reactive phosphate rock P inputs. The resin P data suggest that ryegrass growth on soil from sites 4, 6, 11, 12 and 13 is likely to be constrained by P availability.

The Olsen P values for each site are similar to previous measurements, except for soils 5 and 9, which had uncharacteristically high Olsen P values compared to samples taken by corer in 1995. It is possible that soil sampling depth was too shallow at these two sites when using the spade rather than the soil corer. The influence of soil sampling depth on soil test P values was noted by Zoysa (1997). Phosphate retention was low ($< 30\%$; Cornforth and Sinclair, 1982) at most sites, excluding sites 4 and 13, which had moderate P retention of approximately 50%. High resin P values were generally associated with high Olsen P values, although interestingly, high fertility sites 10 and 8 gave high resin P values, similar to site 1, but Olsen values remained in the medium to high range. Notably, Sites 8 and 10 had some of the higher exchangeable Ca values (Table 5.3) which may have depressed actual Olsen P values (Perrott et al., 1993).

Mineralisable Nitrogen and Extractable Sulphate

Soil mineralisable N ranged from 205 (Site 4) to 515 kgNha⁻¹ (Site 10). Soil sulphate at sites 9, 11, and 13 were at medium to low values where a pasture S response can be expected (Watkinson and Kear, 1995). The new hydrogen peroxide extractable sulphate test (developed by S. Saggart, pers. comm.) ranged from 12 to 43 µgPg⁻¹ and was strongly correlated ($R^2 = 0.70$, Figure 5.1) with CaP-extractable sulphate. Ammonium (extracted by the same process) ranged from 52 to 216 µgNg⁻¹, and was not correlated with the soil mineralisable N values. Nitrate extracted by this method was generally low, but was significantly higher on soils with high mineralisable N levels (Sites 1, 3, 5 and 9). Mineralisable N values are considered medium to high, with the normal range expected for pasture soils to begin at 150 kgNha⁻¹ (M. J. Hedley, Pers. comm.). Long-term cropped soils such as the Kairanga series studied by Carran (1990) return values of 160 kgNha⁻¹.

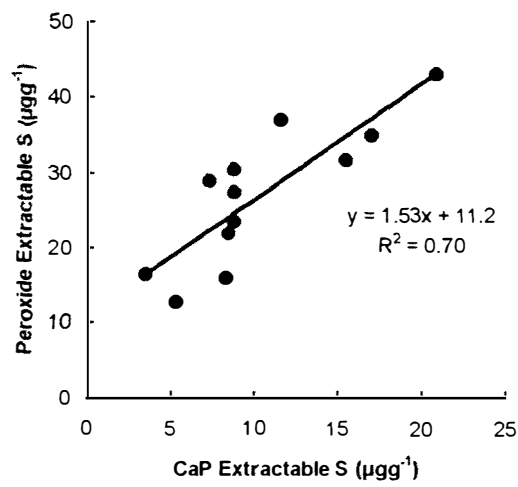


Figure 5.1 The relationship between CaP and H₂O₂ extractable sulphate across all soils.

5.4.2 Herbage Yield and Nutrient Concentrations

Herbage Yield

Total ryegrass yield ranged from 0.22 (Site 4) to 2.05 gDM pot⁻¹ (Site 10; Table 5.4), and was strongly related to soil Olsen P status (Figure 5.2A). Low fertility soils yielded only 30-40% as much ryegrass growth as high fertility soils. Of interest were sites 10 and 5, which showed the potential to out-yield the other high fertility soils, under glasshouse (non-moisture limited) conditions. These two soils had medium to high Olsen P values combined with high mineralisable N values.

Clovers yielded slightly higher than ryegrass, ranging from 0.27 (Site 4) to 2.35 gDM pot⁻¹ (Site 5). Yields were especially high for soils 1, 5 and 9, reflecting the high to medium Olsen P and/or mineralisable N status of these soils.

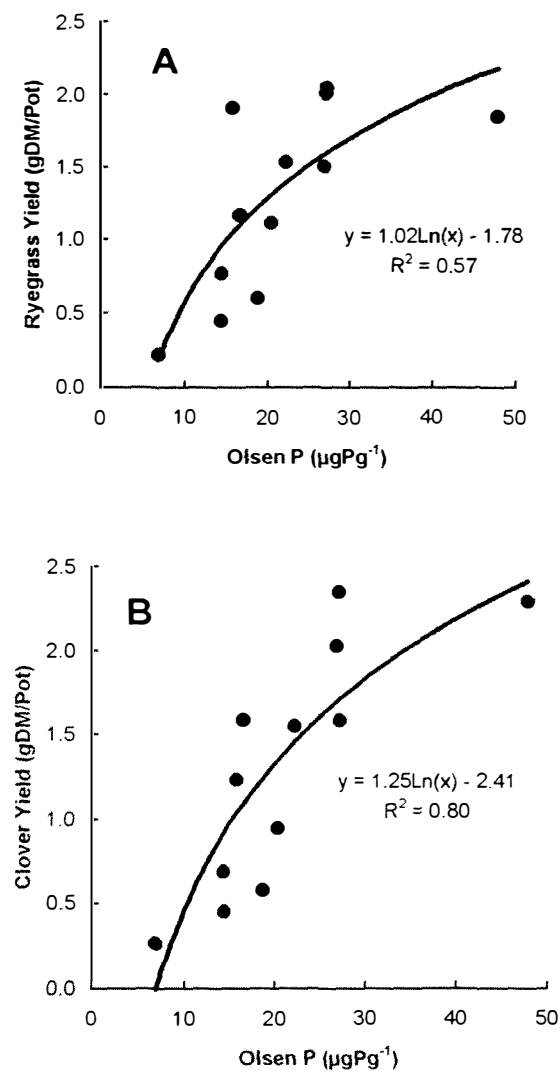


Figure 5.2 The relationship between Olsen P and **A** ryegrass, and **B** white clover dry matter yields in the glasshouse across all soils.

This 10 fold yield difference for both ryegrass and clover (between soils 4 and 1) was much greater than the relative yield difference observed in the field trials in either spring (< 4 fold; 958 versus 3416 kgDMha⁻¹ respectively), or total annual yield (< 4 fold; 3135 versus 10790 kgDMha⁻¹ respectively, Chapter 4, Tables 4.2 and 4.3 respectively). This result supports the theory that higher fertility soils have the potential for rapid nutrient cycling, when soil moisture constraints have been removed. The difference between glasshouse yield and field yield rankings between sites (Table 5.1^A, Appendix 5.1) will be discussed later after the herbage analyses are considered.

Ryegrass and clover yields were relatively similar. There were no notable differences between the herbage mass or relative position of clover and ryegrass yields (Figure 5.3A; Table 5.4) that may have indicated that N fixation by clover was able to overcome a major single factor N limitation to plant growth.

Table 5.4 Mean ryegrass and clover dry matter yields (gDM pot⁻¹) across all soils. Total mean yields in columns with different letters are significantly different at P < 0.05.

Site	Ryegrass			Clover Total
	Cut 1	Cut 2	Total	
1	1.16	0.69	1.85 ^A	2.29 ^{AB}
3	0.57	0.54	1.11 ^C	0.95 ^{DE}
4	0.13	0.09	0.22 ^F	0.27 ^G
5	1.12	0.90	2.01 ^A	2.35 ^A
6	0.70	0.47	1.17 ^C	1.59 ^C
8	0.95	0.59	1.53 ^B	1.55 ^C
9	0.67	0.84	1.50 ^B	2.03 ^B
10	1.04	1.01	2.05 ^A	1.58 ^C
11	0.21	0.24	0.44 ^{EF}	0.69 ^{EF}
12	0.39	0.21	0.59 ^{DE}	0.58 ^F
13	0.40	0.37	0.77 ^D	0.45 ^{GF}
14	0.66	1.25	1.91 ^A	1.23 ^D

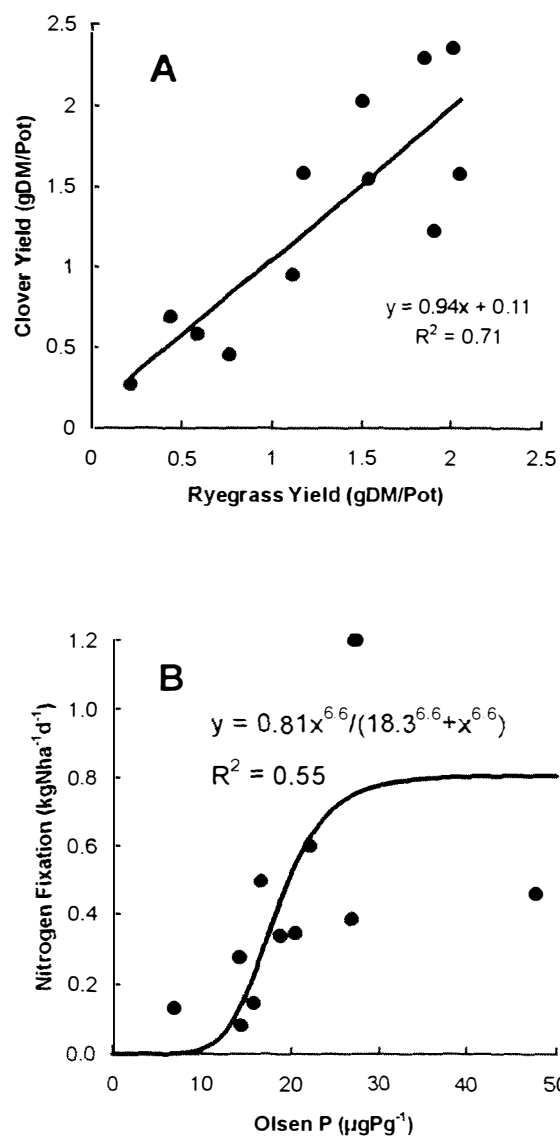


Figure 5.3 The relationship between glasshouse ryegrass yield and glasshouse clover yield **A**; and **B**, The relationship between Olsen P and N fixation levels of white clover on the day of harvest across all soils.

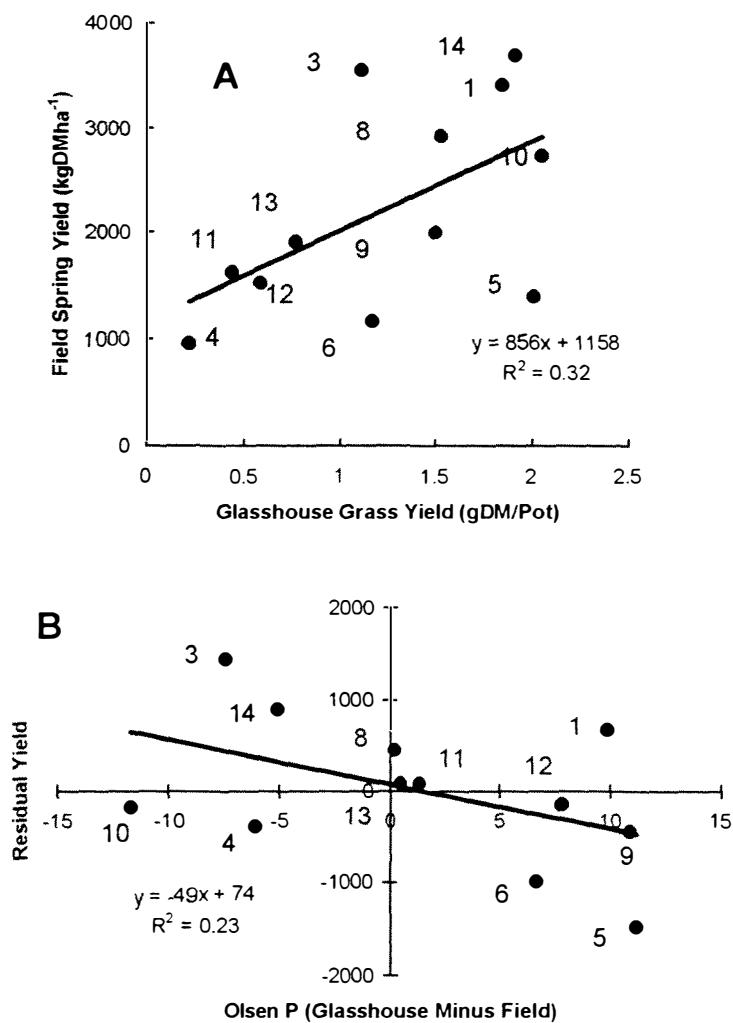


Figure 5.4 The relationship between **A**, glasshouse ryegrass yield spring field yield; and **B**, field /glasshouse residual yields and glasshouse minus field Olsen P values across all soils. Data labels represent site numbers.

Nitrogen Fixation

Nitrogen fixation was highest (1.2 kgNha⁻¹d⁻¹) on soils 5 and 10 (Table 5.5), and lowest (0.13 kgNha⁻¹d⁻¹) on soils 4, 13 and 14. Variation in nitrogen fixation values at harvest could not explain the minor variations in the differences between grass N content and clover N content (data not shown). Of all measured soil properties, variation in N fixation across soils was significantly related to only Olsen P ($R^2 = 0.55$, Figure 5.3B).

Table 5.5 Nitrogen fixation (kgNha⁻¹d⁻¹) by clovers on day of harvest. Total mean nitrogen fixation in the final column with different letters are significantly different at P < 0.05.

Site	Nitrogen Fixation				
	Rep 1	Rep 2	Rep 3	Rep 4	Mean
1	0.32	0.9	0.51	0.12	0.46 ^{BC}
3	0.39	0.35	0.48	0.16	0.35 ^{BC}
4	0.14	0.17	0.11	0.11	0.13 ^C
5	1.34	1.18	1.07	1.22	1.20 ^A
6	0.19	0.22	0.79	0.79	0.50 ^{BC}
8	0.33	0.97	0.69	0.42	0.60 ^B
9	0.38	0.72	0.22	0.22	0.39 ^{BC}
10	1.2	1.39	0.98	1.24	1.20 ^A
11	0.25	0.29	0.24	0.33	0.28 ^{BC}
12	0.38	0.35	0.28	0.34	0.34 ^{BC}
13	0.03	0.11	0.05	0.14	0.08 ^C
14	0.06	0.15	0.21	0.17	0.15 ^C

Relationship between yields, nutrient uptake and soil tests

Ryegrass and clover yields in the glasshouse were strongly related to Olsen soil P test values (Figure 5.2). The pattern of variation in the residuals from this relationship were not related strongly to other measures of either plant nutrient concentration or measures of available soil K, N or S. Field spring yields also showed correlation with field Olsen soil test values (Chapter 4, Figure 4.8A, R² = 0.80).

Even though field spring yields at all sites were taken when pasture growth was not limited by soil moisture content, there was however little correlation between glasshouse ryegrass yields and field spring yield (Figure 5.4A, R² = 0.32). This is partly because sites with the largest positive residual values greater than the line of best fit (sites 3, 1 and 14; Figure 5.4B) represent sites that are in the highest rainfall area and have no soil moisture limitation. In contrast, sites 5 and 6, which have the largest negative residuals, represent sites that moved close to moisture limitation in late spring. In addition, soil Olsen P status varies between the field sites and the glasshouse soils, and this may explain a small amount of the relative yield variation between sites (Figure 5.4B).

Nitrogen, Phosphorus, Potassium and Sulphur in herbage

Nitrogen concentrations in ryegrass (Figure 5.5) ranged from 1.91 % (Site 11) to 3.73 % (Site 14). Total N uptake was also greatest for soil 14 (66 mg), and lowest for soil 4 (4 mg). Clovers (Figure 5.6) had higher N concentrations (2.3-3.8%), and N uptake (8-78 mg) than the ryegrass (Table 5.2A, Appendix 5.2). These plant tissue N levels are below that suggested by other workers (e.g. Sinclair et al., 1996a, 1997b) for balanced nutrition, but lower values are expected when plants are grown rapidly in the glasshouse in restricted volumes of soil. Phosphorus concentrations in ryegrass ranged from 0.168 to 0.38 %, and P uptake 0.41 to 6.45 mg (Table 5.2A, Appendix 5.2; Figure 5.5A). In comparison, clover P concentrations (Figure 5.6A) were lower than in ryegrass ranging from 0.124 to 0.286%. Clover plant P uptake was correspondingly lower, ranging from 0.36 to 6.6 mg P (Table 5.2A, Appendix 5.2). Clover sulphur concentrations varied little across soils, although total S uptake ranged widely, from 0.6 to 5.5 mg S (Table 5.3^A, Appendix 5.2; Figure 5.6).

Clover S uptake was correlated with the new peroxide S test ($y = 0.12x - 0.57$, $R^2 = 0.53$; data not shown). Herbage P and S concentrations measured in this study fall within the range observed by other workers (Sinclair et al., 1997b) as being adequate for balanced clover nutrition. Clover P concentration was also strongly correlated with clover S concentration (Appendix 5.3; Figure 5.1A).

The S:P ratio in clover changes from 1.2 to 0.84 as legume P concentration increases from 0.2 to 0.29. This decline in the clover S:P ratio suggest that S availability may be limiting at the highest P availability. According to Sinclair et al. (1996a, 1996b, 1997b), S:P ratios in the range 0.7 to 0.8 (or lower) indicate S limitation.

In contrast, K:P concentration ratios (data not shown) showed little correlation with P uptake, probably because across all soils the %K in the clover herbage was well above growth limiting values of approximately 2% K (DM basis) (Figure 5.6D). Legume K uptake (mgK pot^{-1}) was strongly correlated to soil exchangeable K values ($\text{K uptake mg/pot} = 71.5 \times \text{meq K } 100\text{g}^{-1} \text{ soil} - 10.7$, $R^2 = 0.71$; data not shown).

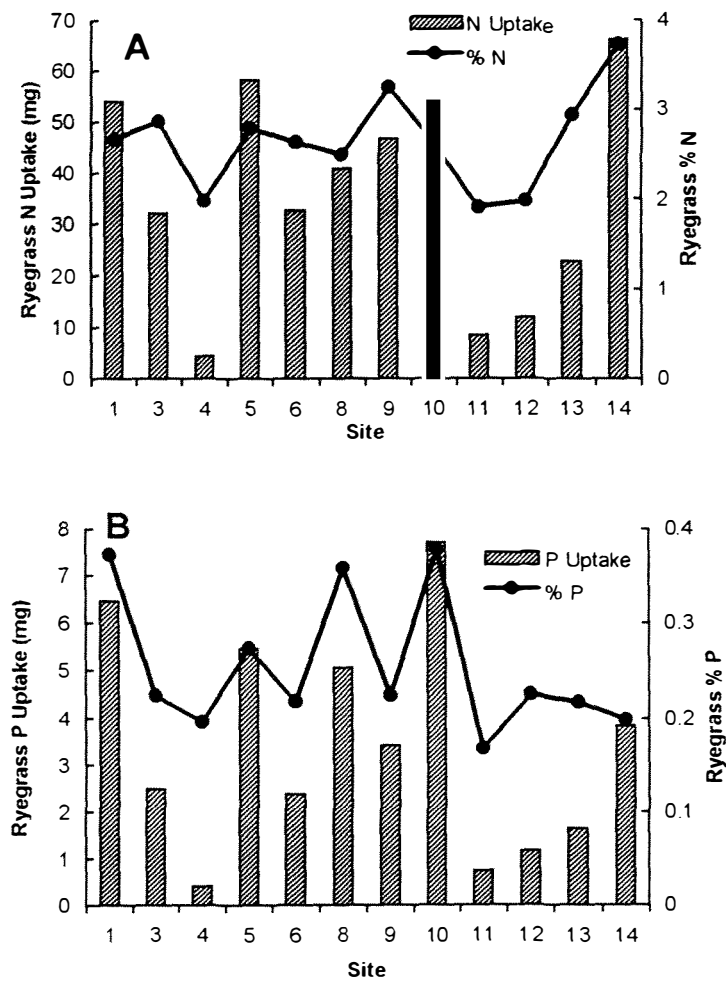


Figure 5.5 Herbage concentration and plant uptake of **A** P and **B** N by glasshouse grown ryegrass across all soils.

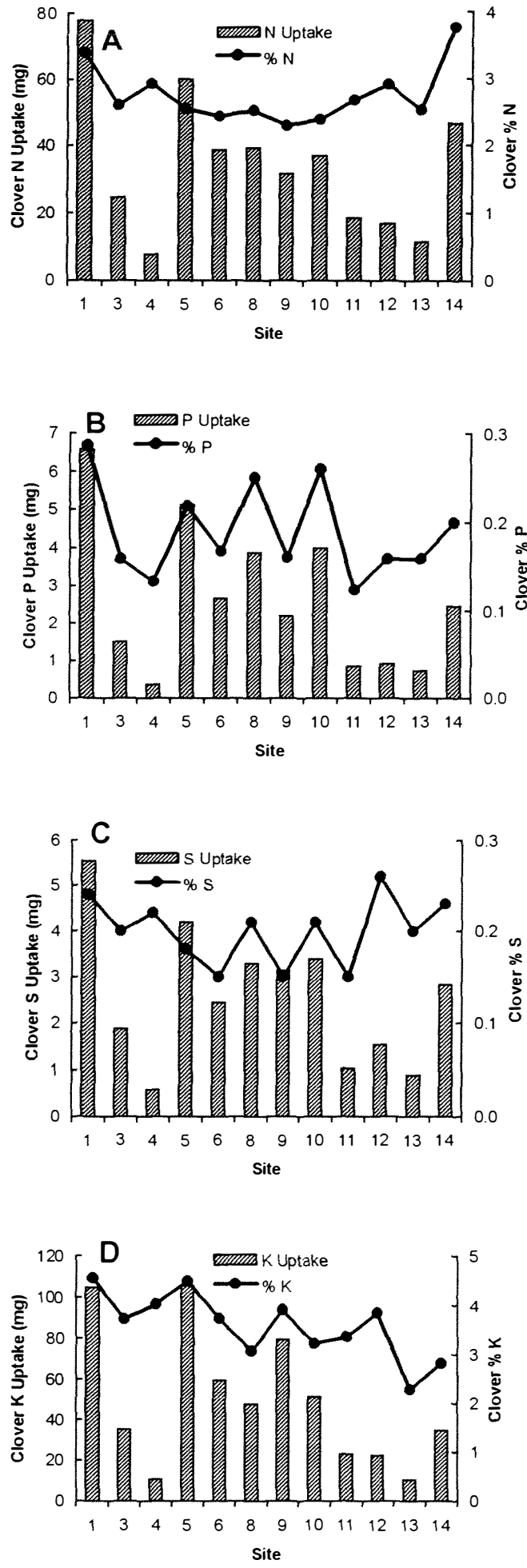


Figure 5.6 Herbage concentration and plant uptake of **A** N, **B** P, **C** S and **D** K by glasshouse grown white clover across all soils.

The percentage of P present in ryegrass (ryegrass % P; $R^2 = 0.55$, Figure 5.7A), P uptake in ryegrass ($R^2 = 0.73$, Figure 5.7B), clover % P ($R^2 = 0.61$, Figure 5.7C), P uptake by clover ($R^2 = 0.75$, Figure 5.7D), clover N uptake ($R^2 = 0.69$, Figure 5.7E) and N uptake in ryegrass ($R^2 = 0.47$, Figure 5.7F) were all strongly correlated to the soil Olsen P test values. The N uptake by glasshouse grown plants (Figure 5.7F) was more strongly correlated with the Olsen P test values for each soil than with field N uptake and field Olsen P test (see Appendix 5.4).

Essentially, as Olsen P status increased, yield, plant P and N uptake increased in a linear fashion (also, refer Appendix 5.4). These relationships were generally stronger for clover than for ryegrass. Although N and P uptake increased with increasing Olsen P test value, legume N:P ratio declined rapidly with increasing % P, strongly indicating that at high herbage P concentrations, clover N uptake was restricted (Appendix 5.5). Very similar, but less strongly correlated trends were observed for field clover, adding evidence to plant N limitations on these soils (Appendix 5.4). The extent of supply of S, N and P from these soils is studied further in the discussion of results from the exhaustive glasshouse trial.

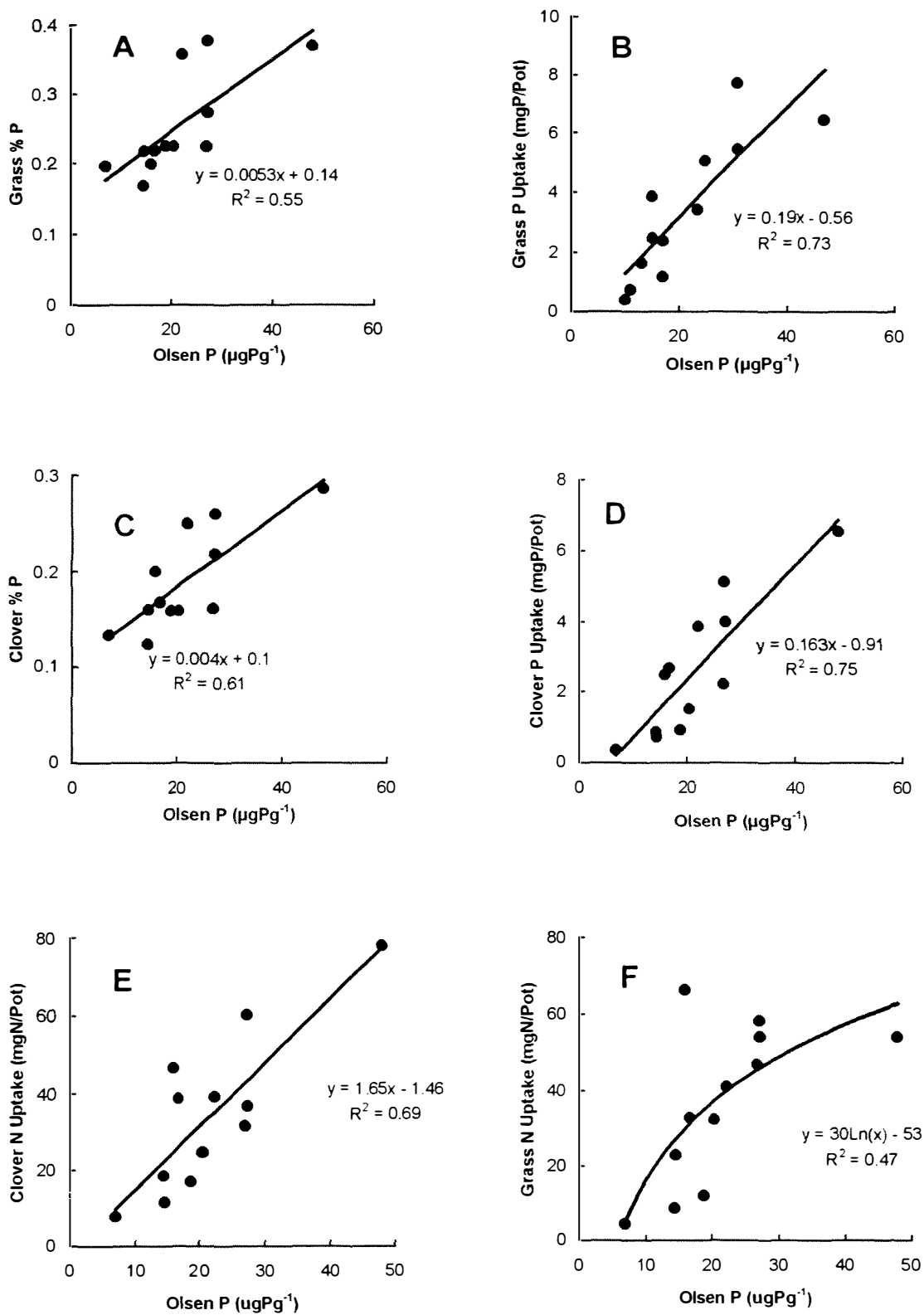


Figure 5.7 Relationship between Olsen P and **A** ryegrass % P, **B** ryegrass P uptake, **C** clover % P, **D** clover P uptake, **E** clover N uptake and **F** ryegrass N uptake.

5.5

RESULTS AND DISCUSSION - EXPERIMENT 2

Minus P treatments produced the greatest yield variation across soils in this experiment, ranging from 2.76 (Soil 4) to 6.43 gDM pot⁻¹ (Figure 5.8). High yielding soils were soils 1, 10, 8 (all high fertility soils; Table 5.6) and 5 (high Olsen P status; Table 5.1). Yields on the -P pots were strongly related to Olsen P ($R^2 = 0.70$, Figure 5.9A), confirming that the Olsen P test is a strong indicator of plant-available P in these soils.

Table 5.6 Mean dry matter yields (gDM pot⁻¹) and statistical analysis in Stanford and DeMent experiment. Total mean yields (within treatments) in columns with different letters are significantly different at $P < 0.05$.

Site	Treatment*									
	- N			- P				- S		
	Cut 1	Cut 2	Total	Cut 1	Cut 2	Cut 3	Total	Cut 1	Cut 2	Total
1	1.71	0.42	2.13 ^A	2.80	1.02	2.61	6.43 ^A	1.90	0.55	2.45 ^A
3	0.95	0.27	1.22 ^B	1.30	0.64	1.31	3.24 ^{EF}	1.45	0.46	1.90 ^{DE}
4	0.27	0.12	0.39 ^E	1.21	0.59	0.96	2.76 ^G	1.32	0.44	1.76 ^F
5	0.86	0.25	1.12 ^B	2.03	0.84	1.78	4.65 ^D	1.57	0.48	2.05 ^{CD}
6	0.56	0.18	0.73 ^C	1.38	0.67	1.24	3.30 ^{EF}	1.57	0.44	2.01 ^{CD}
8	0.60	0.20	0.80 ^C	2.30	0.87	2.17	5.35 ^C	1.70	0.56	2.26 ^B
9	0.86	0.31	1.18 ^B	1.24	0.74	1.48	3.46 ^E	1.57	0.51	2.08 ^C
10	0.89	0.29	1.17 ^B	2.27	0.92	2.70	5.88 ^B	1.57	0.52	2.09 ^C
11	0.30	0.12	0.41 ^E	1.28	0.58	1.19	3.05 ^{FG}	1.34	0.35	1.69 ^F
12	0.39	0.13	0.52 ^D	1.58	0.71	1.21	3.50 ^E	1.56	0.48	2.03 ^{CD}
13	0.58	0.13	0.71 ^C	1.39	0.70	1.15	3.24 ^{EF}	1.34	0.37	1.71 ^F
14	0.99	0.23	1.22 ^B	1.55	0.74	1.14	3.43 ^E	1.48	0.42	1.89 ^{DE}

* ● overall, treatments are significantly different at $P < 0.01$.

For the -N treatment, plant dry matter yields varied significantly across soils (see Plate 5.2), ranging from 0.39 (Soil 4) to 2.13 gDM pot⁻¹ (Soil 1; Figure 5.8). Minus N treatment yield was positively correlated with soil Olsen P (Figure 5.9B, $R^2 = 0.79$), soil mineralisable N ($R^2 = 0.52$, Figure 5.10A), and hydrogen peroxide-extractable N ($R^2 = 0.63$, Figure 5.10B). This result indicates that Olsen P may be a surrogate indicator of plant-available N status across this range of soils because of their history as SSP fertilised clover based pastures.

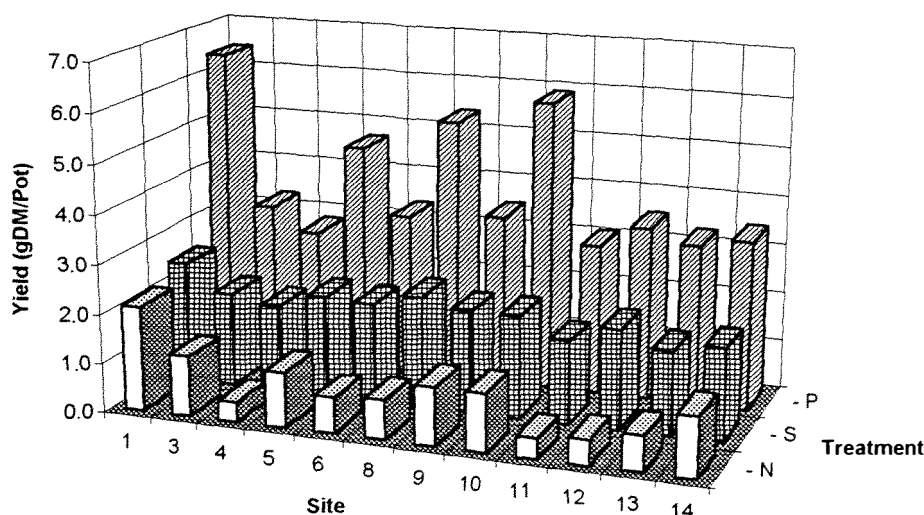


Figure 5.8 Mean dry matter yields across all treatments.

The minus S treatments also showed significantly different, but low yield variation across soils (Plate 5.2). Yields ranged from 1.69 (Soil 11) to 2.45 (Soil 1; Figure 5.8) gDM pot⁻¹. Yields for this treatment were also strongly correlated with peroxide-extractable S ($R^2 = 0.80$, Figure 5.10C), and Olsen P (Figure 5.9C, $R^2 = 0.73$), suggesting that S (and N) extracted by this soil test is closely related to the short-term readily-degradable (mineralisable) pool of S and N in these soils. Minus N and S yields were also strongly related to Olsen P status, strongly reinforcing the thesis that Olsen P is a strong indicator of “general fertility status” (plant-available P, N and S) across these soils.

Overall, the largest reductions in plant yield were caused by supplying N from the soil only (-N treatment), despite the +N treatment containing 2-4 times more soil than the S and P treatments (see Plate 5.2, 5.3). This result indicates that short-term N mineralisation rates are likely to constrain plant growth on these soils, to a greater extent than S mineralisation or P uptake. Results from this experiment differ from that of Mackay et al. (1995), where only small yield variations were reported across soils of wide-ranging fertility status. It should be noted however that these workers used only 25g of air-dry soil for all three treatments (-N, -P and -S), which may have affected the results.



Plate 5.2 Soil effect on plant growth; from left to right HF soil 1, MF soil 5 and LF soil 4. From top to bottom, treatments are -N, -S and -P.

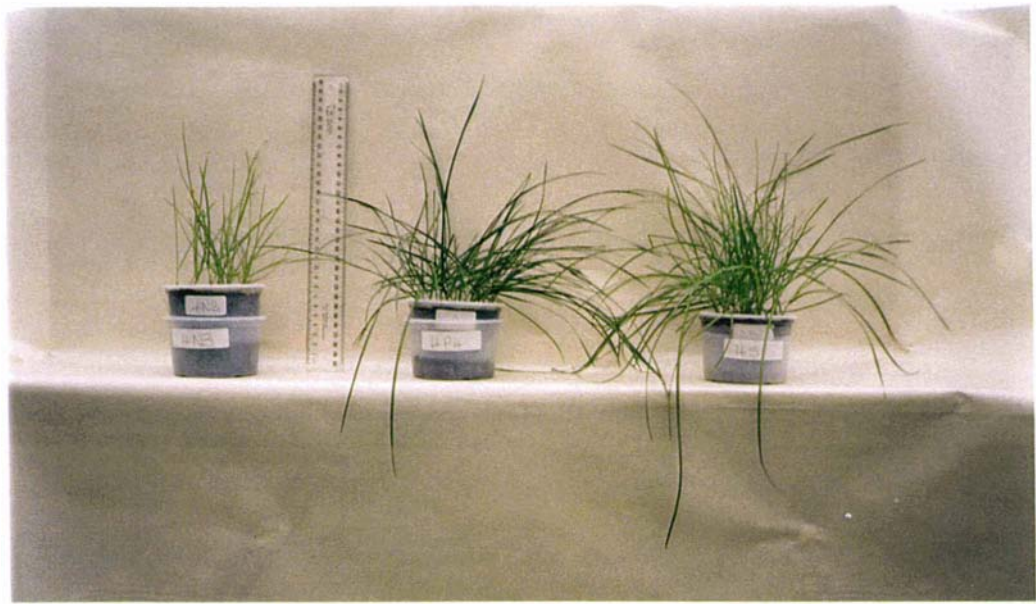


Plate 5.3 Treatment effect across a single soil; HF soil 1 (top), and LF soil 4 (bottom). From left to right, treatments are -N, -P and -S.



Plate 5.4 Some root exposures of the Stanford and DeMent bio-assay pot.

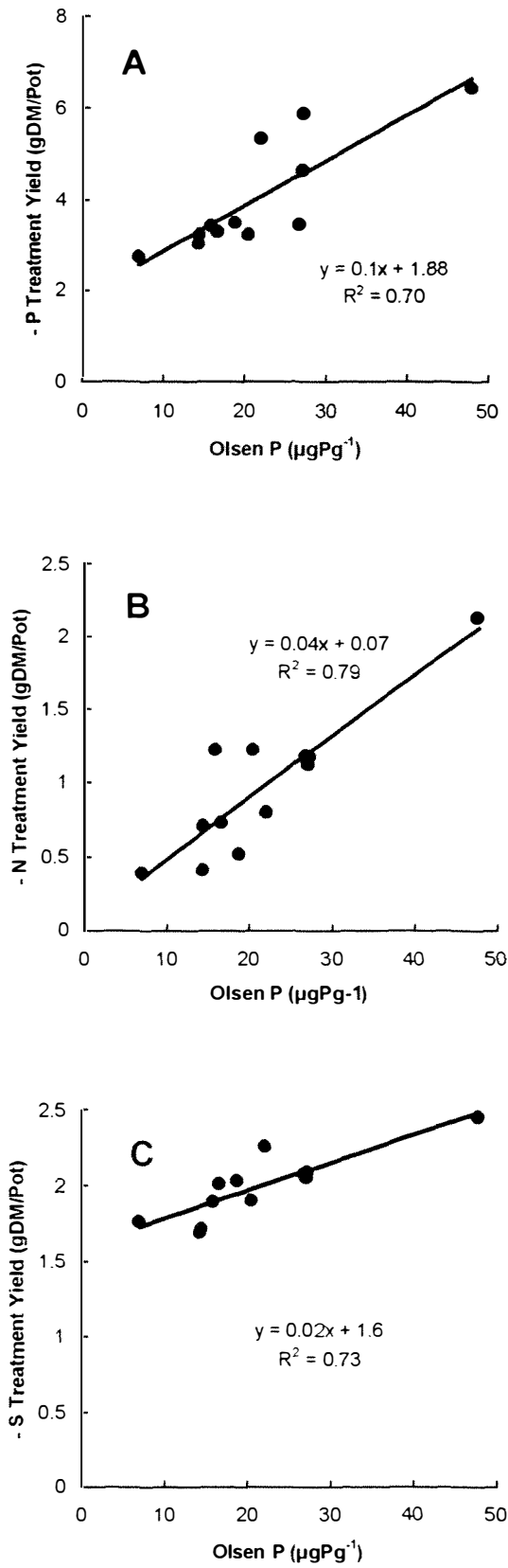


Figure 5.9 The relationship between Olsen P and **A** -P treatment ryegrass yield; **B** -N treatment ryegrass yield; and **C** -S treatment ryegrass yield across all soils.

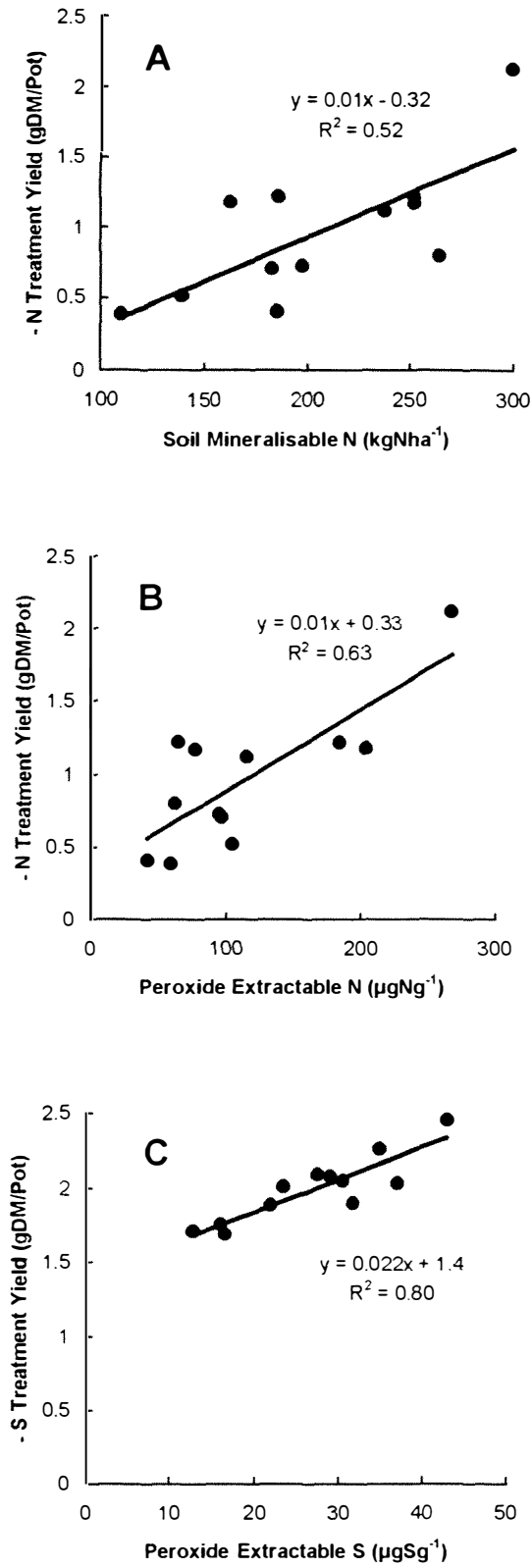


Figure 5.10 The relationship between **A**, soil mineralisable N and -N treatment ryegrass yield; **B**, Peroxide extractable N and -N treatment ryegrass yield ; and **C**, Peroxide extractable S and -S treatment ryegrass yield.

Effect of fertiliser history and climate on N and S pools

Across the range of soils used in this glasshouse study, differences in fertiliser history (fertiliser effect) were best indicated by the change in soil total P concentration (see Chapter 3, Figure 3.1). Olsen P is not useful for this purpose because the current Olsen P value is the product of fertiliser P added and the differential partition of P into organic and inorganic P fractions by the difference in vigour of the biologically driven N and C cycles at each site. Thus Olsen P status is also a product of climate difference.

The -N, and to a lesser extent, -S treatment yields increased in a linear fashion with increasing total P concentration (fertiliser history) (total soil P: Figures 5.11A, $R^2 = 0.60$; and 5.11B, $R^2 = 0.37$ respectively), suggesting that the size of plant-available N and S pools on the soils has increased with increasing SSP applications. The pattern of residuals (observed yield minus predicted yield) of these relationships are plotted against site number in Figure 5.11. There were no clear relationships between site climate and residual value (Figure 5.12).

Therefore, in conclusion, the plant-available N and S pools (as indicated by the Stanford and DeMent, 1957 technique) in these soils have been strongly influenced by long-term SSP applications, but there is no definite pattern of influence by climate. This result agrees with the mineralisable N (anaerobic incubation) status of soils which also showed no definite pattern of influence by climate (Section 5.4.1). In contrast, total soil N values were higher at the lower rainfall coastal sites (Section 3.3.2, Figure 3.1C).

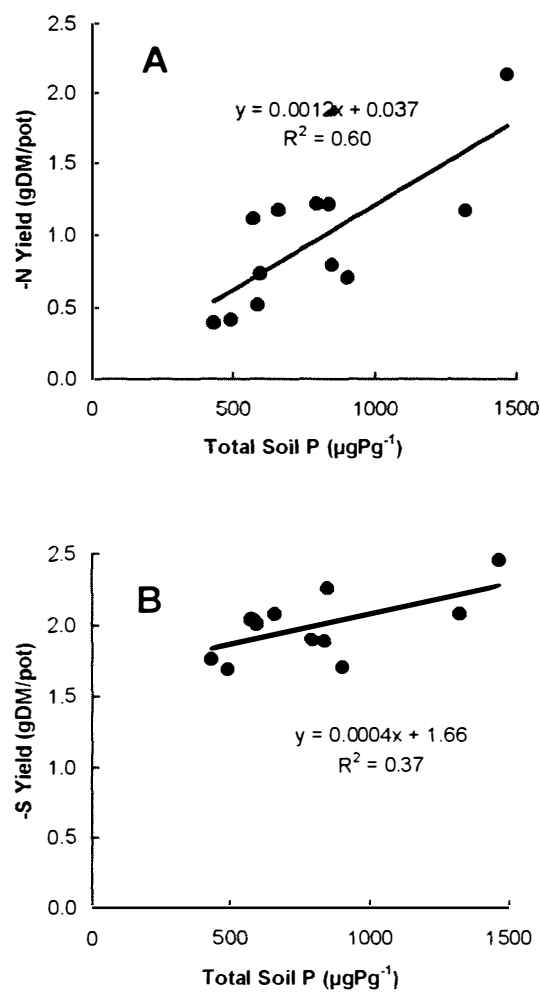


Figure 5.11 The relationship between soil total P content and **A**, -N treatment yield, and **B**, -S treatment yield across all soils.

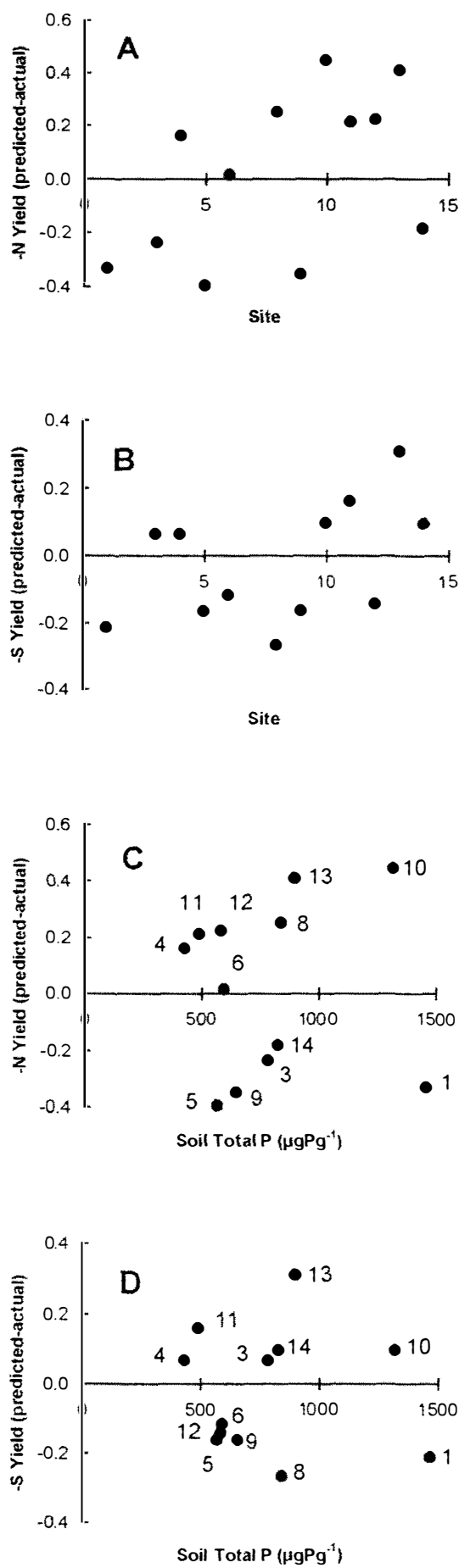


Figure 5.12 Predicted (from relationships in Figure 5.11) minus actual yields (gDM pot⁻¹) for **A**, -N treatment yield, and **B**, -S treatment yield across all soils; and also the relationship between total soil P and **C**, -N , and **D**, -S residual yields.

5.6 SUMMARY AND CONCLUSIONS

Experiment 1

Plant (ryegrass and white clover) yields in the climate-controlled environment of the glasshouse showed large yield differences across soils. Soils from high fertility sites yielded up to ten times that of soils from low fertility sites. Such large differences in yield were not reflected by, nor strongly correlated with, annual and spring dry matter yields at the field sites. Such a result confirms that at field sites yield responses to nutrient availability are strongly modified by climate at each site.

Glasshouse yields were strongly correlated with Olsen P, and were best described by a relationship similar to that seen between field yield (spring) and Olsen P. However, bringing these soils into a climate-controlled environment did not provide a better relationship between Olsen P values and yield for glasshouse grown ryegrass than previously seen between spring field growth and Olsen P values. The relationship between Olsen P values and clover yield was similar to that previously seen for field yields (spring).

Trends in the ratios of nutrients taken up by clover in the glasshouse trials confirmed that P availability in low fertility soils was the major growth-limiting factor, probably followed by S or N, which became limiting (in high fertility soils) when P availability was adequate to high. Potassium availability in all soils was high, and did not constrain clover growth.

Experiment 2

Using the Stanford and DeMent exhaustive cropping technique, the soils examined in this experiment exhibited a large variation (range) in ryegrass yield across -P and -N treatments, which was strongly correlated with various soil tests for N, S and P availability. Olsen P was a better predictor of plant-available soil N supply than soil mineralisable N tests. S availability to plants was less variable across soils than P and N availability to plants. Variation in S limited yield was strongly correlated with the variation in the new Peroxide S test (S. Saggar, pers. comm.). This soil test should be examined further.

The amount of dry matter production, when considered with the quantity of soil used for each treatment (-N, 100g; -P, 50g; -S, 25g), suggest that these soils have large pools of plant-available or mineralisable P and S, and, relative to plant demand, small pools of soil mineralisable N.

The mineralisable N and S pool sizes (as measured by the Stanford and DeMent technique) are correlated to P and S fertiliser history, but show little correlation to the rainfall regimes under which the pasture soils were developed. It is recognised however that the higher pasture yields under the higher rainfall regimes would have induced use of higher SSP inputs. Therefore climate and SSP history probably are not mutually exclusive.

Results from both experiments in this Chapter provide strong evidence that the Olsen P soil test is a valuable soil fertility indicator of plant-available P, N and S on legume-based pastures with a history of superphosphate use. The implications of such a result are that when attempting to model pasture growth in the field, using a single soil fertility component, the Olsen P test is a strong and robust indicator of ‘overall’ fertility status of such hill country soils. This is valuable information to those attempting to model pasture yield.

In the next Chapter (Chapter 6), a new field pasture production model is presented. This model incorporates evapotranspiration / soil moisture climate-based data, along with soil fertility data, in order to accurately predict field pasture growth at our twelve field sites in the Wairarapa. Based on the results of this Chapter, soil Olsen P values have been incorporated as the main fertility variable in the model.

5.7 REFERENCES

Blakemore, L.C., Searle, P.L. and Daly, B.K., 1987. Methods for chemical analysis of soils. Scientific Report 80, New Zealand Soil Science Bureau, Lower Hutt, New Zealand.

- Carran, R.A., 1990. Dynamics of soil and plant nitrogen in cultivated and no-till spring wheat systems following old pasture. *New Zealand Journal of Crop and Horticultural Science*, 18(1): 5-9.
- Cornforth, I.S. and Sinclair, A.G., 1982. Model for calculating maintenance phosphate requirements of grazed pastures. *New Zealand Journal of Experimental Agriculture*, 10: 53-61.
- Cornforth, I.S. and Sinclair, A.G., 1984. Fertiliser and lime recommendations for pastures and crops in New Zealand. Second revised edition, Ministry of Agriculture and Fisheries, Wellington.
- Edmeades, D.C., Feyter, C. and O'Connor, M.B., 1984a. Lime and phosphorus requirements for hill country yellow-brown earths. In: J.L. Brock (Editor), *Proceedings of the New Zealand Grassland Association*. New Zealand Grassland Association, Gisborne, New Zealand, pp. 98-106.
- Edmeades, D.C., Pringle, R.M., Mansell, G.P. and Shannon, P.W., 1984b. Effects of lime on pasture production on soils in the North Island of New Zealand 1. Introduction and description of data base. *New Zealand Journal of Agricultural Research*, 27(3): 349-356.
- Edmeades, D.C., Pringle, R.M., Shannon, P.W. and Mansell, G.P., 1984c. Effects of lime on pasture production on soils in the North Island of New Zealand 4. Predicting lime responses. *New Zealand Journal of Agricultural Research*, 27(3): 371-382.
- Hardy, R.W.F., Holsten, R.D., Jackson, E.K. and Burns, R.C., 1968. The acetylene-ethylene assay for N_2 fixation: Laboratory and field evaluation. *Plant Physiology*, 43: 1185-1207.
- Hoglund, J.H. and Brock, J.L., 1978. Regulation of nitrogen fixation in a grazed pasture. *New Zealand Journal of Agricultural Research*, 21: 79-82.
- Jackman, R.H., 1964. Accumulation of organic matter in some New Zealand soils under permanent pasture 1. Patterns of change in organic carbon, nitrogen, sulphur and phosphorus. *New Zealand Journal of Agricultural Research*, 7: 445-471.
- Johnson, C.M. and Nishita, H., 1952. Microestimation of sulfur in plant material, soil, and irrigation waters. *Analytical Chemistry*, 24: 736-742.
- Kamphake, L.J., Hannah, S.A. and Cohen, J.M., 1967. Automated analysis for nitrate by hydrazine reduction. *Water Research*, 1: 205-216.

- Keeney, D.R. and Bremner, J.M., 1966. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. *Agronomy Journal*, 58: 498-503.
- Lambert, M.G., Clark, D.A., Mackay, A.D. and Costall, D.A., 2000. Effects of fertiliser application on nutrient status and organic matter content of hill soils. *New Zealand Journal of Agricultural Research*, 43: 127-138.
- Lambert, M.G., Mackay, A.D. and Costall, D.A., 1998. Long-term fertiliser application and fertility of hill soils. In: M. Baker (Editor), *Proceedings of the New Zealand Grassland Association*. New Zealand Grassland Association, Nelson, New Zealand, pp. 63-67.
- Mackay, A.D., Lambert, M.G., Trolove, S.N. and Costall, D.A., 1995. Effect of long-term fertiliser use and sheep grazing on the accumulation and distribution of nitrogen, phosphorus and sulphur in a hill soil. In: L.D. Currie and P. Loganathan (Editors), *Fertiliser requirements of grazed pasture and field crops: Macro and micro nutrients*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, pp. 206-219.
- McKenzie, H.A. and Wallace, H.S., 1954. The kjeldahl determination of nitrogen: A critical study of digestion conditions-temperature, catalyst and oxidising agent. *Australian Journal of Chemistry*, 7: 55-70.
- Moir, J.L., 1994. Influences of climate and fertiliser application history on various measures of soil fertility and productivity in Wairarapa hill country. Master of Agricultural Science Thesis, Massey University, Palmerston North, New Zealand, 145 pp.
- Moir, J.L., Hedley, M.J., Mackay, A.D. and Garland, C.B., 1995. Climate, soil fertility and pasture production in Wairarapa hill country. In: L.D. Currie and P. Loganathan (Editors), *Fertiliser requirements of grazed pasture and field crops: Macro and micro nutrients*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 254-260.
- Moir, J.L., Hedley, M.J., Mackay, A.D. and Tillman, R.W., 1996. Effects of various fertiliser histories on nutrient accumulation and pasture production in Wairarapa hill country., *Soil Science - Raising the Profile*. Australian and New Zealand Soil Science Societies, Melbourne University, Melbourne, Australia, pp. 189-190.

- Moir, J.L., Hedley, M.J., Mackay, A.D. and Tillman, R.W., 1997. The effect of fertiliser history on nutrient accumulation and plant-available nutrient supply in legume-based pasture soils. In: J.G. Buchanan-Smith, L.D. Bailey and P. McCaughey (Editors), XVIII International Grassland Congress. International Grassland Association, Winnipeg, Canada, pp. 10, 68-69.
- Murphy, J. and Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27: 31-36.
- Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate, USDA.
- Perrott, K.W., Saggar, S. and Menon, R.G., 1993. Evaluation of soil phosphate status where phosphate rock based fertilisers have been used. *Fertiliser Research*, 35(1/2): 67-82.
- Saggar, S., Hedley, M.J. and White, R.E., 1990. A simplified resin membrane technique for extracting phosphorus from soils. *Fertilizer Research*, 24: 173-180.
- Saggar, S., Hedley, M. J., White, R. E., Gregg, P. E. H., Perrott, K. W., and Cornforth, I. S., 1992. Development and evaluation of an improved soil test for phosphorus: 2. Comparison of the Olsen and mixed cation-anion exchange resin tests for predicting the yield of ryegrass grown in pots. *Fertilizer Research*, 33(2): 135-144.
- Saggar, S., Hedley, M. J., White, R. E., Gregg, P. E. H., Perrott, K. W., and Cornforth, I. S., 1993. Assessment of the relative agronomic effectiveness of phosphate rocks under glasshouse conditions. *Fertilizer Research*, 34(2): 141-151.
- Saggar, S., Hedley, M. J., White, R. E., Perrott, K. W., Gregg, P. E. H., Cornforth, I. S., and Sinclair, A. G., 1999. Development and evaluation of an improved soil test for phosphorus, 3: Field comparison of Olsen, Cowell and resin soil P tests for New Zealand pasture soils. *Nutrient Cycling in Agrosystems*, 55(1): 35-50.
- Saunders, W.M.H., 1965. Phosphate retention by New Zealand soils and its relationship to free sesquioxides, organic matter and other soil properties. *New Zealand Journal of Agricultural Research*, 8: 30-57.
- Schollenberger, C.J. and Simon, R.H., 1945. Determination of exchange capacity and exchange bases in soil-ammonium acetate method. *Soil Science*, 59: 13-24.
- Searle, P.L., 1975. Automated colorimetric determination of ammonium ions in soil extracts with "Technicon Autoanalyzer II equipment". *New Zealand Journal of Agricultural Research*, 18: 183-187.

- Searle, P.L., 1979. Measurement of adsorbed sulphate in soils - effect of varying soil extract ratios and methods of measurement. *New Zealand Journal of Agricultural Research*, 22: 287-290.
- Searle, P.L., 1984. The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen. A review. *Analyst*, 109: 549-568.
- Sinclair, A.G., Johnstone, P.D., Smith, L.C., Roberts, A.H.C., O'Connor, M.B., and Morton, J.D., 1997a. Relationship between pasture dry matter yield and soil Olsen P from a series of long-term field trials. *New Zealand Journal of Agricultural Research*, 40: 559-567.
- Sinclair, A.G., Morrison, J.D., Smith, L.C. and Dodds, K.G., 1996a. Effects and interactions of phosphorus and sulphur on a mown white clover/ryegrass sward 2. Concentrations and ratios of phosphorus, sulphur, and nitrogen in clover herbage in relation to balanced plant nutrition. *New Zealand Journal of Agricultural Research*, 39: 435-445.
- Sinclair, A.G., Morrison, J.D., Smith, L.C. and Dodds, K.G., 1997b. Effects and interactions of phosphorus and sulphur on a mown white clover/ryegrass sward 3. Indices of nutrient adequacy. *New Zealand Journal of Agricultural Research*, 40: 297-307.
- Sinclair, A.G., Smith, L.C., Morrison, J.D. and Dodds, K.G., 1996b. Effects and interactions of phosphorus and sulphur on a mown white clover/ryegrass sward 1. Herbage dry matter production and balanced nutrition. *New Zealand Journal of Agricultural Research*, 39: 421-433.
- Stanford, G. and DeMent, J.D., 1957. A method for measuring short-term nutrient absorption by plants: I. Phosphorus. *Soil Science Society of America Proceedings*, 21(6): 612-617.
- Walker, T.W., Thapa, B.K. and Adams, A.F.R., 1959. Studies on soil organic matter: 3. Accumulation of carbon, nitrogen, sulphur, organic and total phosphorus in improved grassland soils. *Soil Science*, 87: 135-140.
- Wang, H.L., Hedley, M.J. and Bolan, N.S., 1995. Chemical properties of fluidized bed boiler ash relevant to its use as a liming material and fertiliser. *New Zealand Journal of Agricultural Research*, 38(2): 249-256.

- Wang, H.L., Hedley, M.J., Bolan, N.S. and Horne, D.J., 1999. The influence of surface incorporated lime and gypsiferous by-products on surface and sub-surface soil acidity II. Root growth and agronomic implications. *Australian Journal of Soil Research*, 37(1): 181-190.
- Waring, S.A. and Bremner, J.M., 1964. Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. *Nature*, 201: 951-952.
- Watkinson, J.H. and Kear, M.J., 1995. A soil test for mineralisable organic sulphur. In: L.D. Currie and P. Loganathan (Editors), *Fertiliser requirements of grazed pasture and field crops: Macro and micro nutrients*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 144-149.
- Zoysa, A.K.N., 1997. The influence of phosphorus fertiliser forms and rhizosphere processes on the phosphorus nutrition of tea (*Camellia sinensis* L.). PhD Thesis, Massey University, Palmerston North, New Zealand, 268 pp.

CHAPTER 6

A CLIMATE-DRIVEN, SOIL FERTILITY DEPENDENT, PASTURE PRODUCTION MODEL

J. L. Moir¹, D. R. Scotter¹, M. J. Hedley¹ and A. D. Mackay²

¹Soil and Earth Sciences, Institute of Natural Resources, Massey University, Private Bag 11222, Palmerston North, New Zealand.

²AgResearch Land Management, Private Bag 11008, Palmerston North, New Zealand.

New Zealand Journal of Agricultural Research (2000) 43 (4): 491-500.

6.1 ABSTRACT

Field trial data relating pasture growth to measures of soil fertility are confounded by many site-specific environmental factors, particularly the weather. One approach to accommodate this is to express fertiliser responses in terms of relative rather than absolute yields, but this approach places constraints on trial design and is unhelpful when attempting to extrapolate data to estimate actual yields at other sites or in other years. An alternative approach that includes the soil moisture, and so the effect of climate as it influences evapotranspiration is suggested. The model assumes that pasture growth is proportional to evapotranspiration, and that the proportionality constant (k) depends on soil fertility. Evapotranspiration is calculated from a simple daily soil water balance. Values for k varied from 11 to 19 kg DM ha⁻¹ mm⁻¹ of evaporation. The greatest divergence between the measured and modelled production occurred during a prolonged dry period. Possible reasons for this are discussed. With simulated weather data, the model can be used to generate probability-density functions of pasture production. The advantage of the approach is that prediction of “actual” yield is a very helpful measurement for producers, and more valuable to scientists than relative yield when

modelling nutrient cycling. This modelling approach also has potential applications in farm risk management and feed budgeting.

6.2 KEYWORDS

Pasture growth model; soil fertility; climate; evapotranspiration; Olsen P; relative yield; water balance model

6.3 INTRODUCTION

Gandar & Kerr (1980) examined 100 papers reporting on agronomic field trials in New Zealand, and reported that “climatic data were rarely used to aid interpretation of results”. There is little evidence in scientific literature to suggest that the situation has changed much in the last 20 years. This applies particularly to the analysis of field trials assessing the relationship between pasture growth and measures of soil fertility status conducted at different sites and in different years (Sinclair et al. 1997). Rather than the effects of site- or time-specific environmental factors, particularly the weather, being examined explicitly, they are masked. This is done by expressing fertiliser responses in relative, rather than absolute yield terms. Percentage yields relative to the site maximum yield are then pooled and relative yields from all sites (or seasons) graphed against the soil fertility index under investigation. This approach requires some treatments to achieve near maximum yields, which places constraints on trial design and is particularly unhelpful when attempting to extrapolate data to estimate actual yields at other sites or in other years.

For example, Sinclair et al. (1997) found that the fitted curve for the relationship between Olsen P and relative yield for 17 field trials measuring long-term (6-year) pasture growth response to phosphatic fertilisers explained less than 28% of the yield variation. Sinclair et al. (1997) noted that considerable error was involved in calculating relative yield, mostly due to the difficulty of predicting maximum yields at each site. In fact, 28 of the 72 data sets considered by Sinclair et al. (1997) had to be excluded because of unacceptable error (>10%) in predicting maximum yields. Of the remaining data sets for which relationships between relative yield and Olsen P were evaluated, most of the variation in relative yield unexplained by Olsen P was due to inconsistent response

patterns at each site, both within and across years. The inconsistency suggests that climate variation was one of the major sources of variation influencing the shape of the pasture response function.

Moir et al. (1995, 1997) used data from a series of field sites varying from high (Olsen P 33-47 μgPg^{-1}) to low (Olsen P 10-12 μgPg^{-1}) fertility, across three rainfall zones (<750 to 1500 mmyr^{-1}), to demonstrate that as rainfall increased, the size of the pasture response to a unit of Olsen P also increased (Figure 6.1). For example, at the Mauriceville sites (Figure 6.1A), under high rainfall (1500 mmyr^{-1}), the high fertility (HF) site (Olsen 47 μgPg^{-1}) yielded approximately four times that of the low P site (Olsen P 10 μgPg^{-1}). In comparison, at Whareama (rainfall < 750 mmyr^{-1} ; Figure 6.1B) the difference between the low and high fertility sites was only two-fold. Pasture growth stopped when the soil volumetric water content (0-7.5 cm soil depth) dropped to 0.20 (Moir 1994). It is apparent that both rainfall and soil fertility status at the measured sites strongly influenced annual pasture yield.

The interaction between Olsen P and rainfall is further illustrated if the relationship between Olsen P and pasture growth when moisture is not limiting in the spring is compared with annual production, which includes periods when soil moisture limits growth at some sites. Moir et al. (1997) found that pasture yield was strongly related to soil Olsen P status during periods when soil moisture was not limiting plant growth (Figure 6.2). Under these conditions (spring climate) Olsen P was a good indicator of fertility between field sites. However, when the relationship between annual yield and Olsen P at the same sites are plotted (Figure 6.3), the yield data fall into two groups based largely on rainfall. Coastal dry sites had a measurable yield maxima, allowing a relative yield to be calculated. However, the high rainfall sites diverged from the low rainfall sites and showed a linear relationship with increasing Olsen P, with no apparent maxima over the range studied. The variation caused by climate (rainfall) limits the portability of data to other sites for developing generalised relationships.

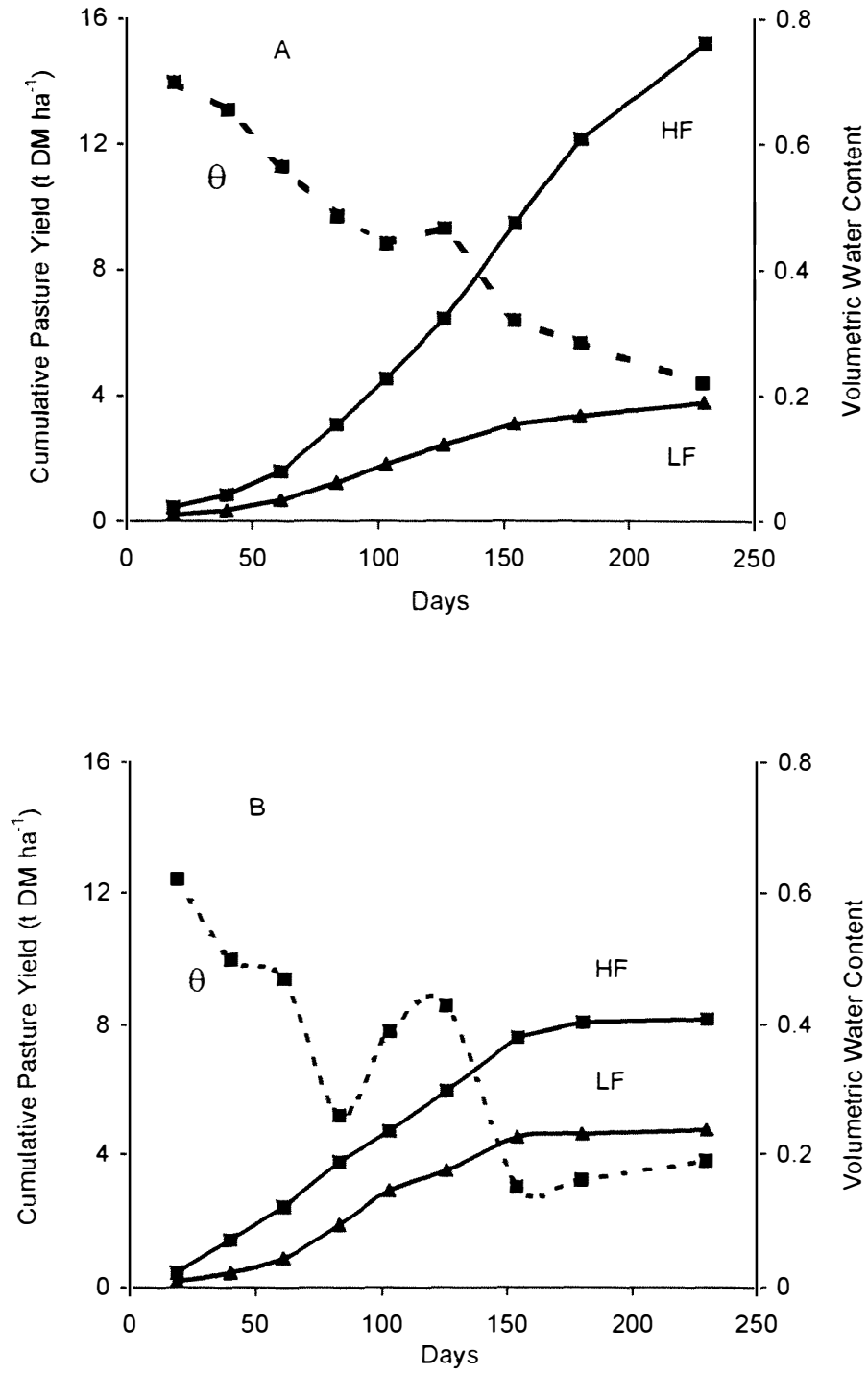


Figure 6.1 Cumulative pasture yield (solid lines) and 0-75 mm depth soil volumetric water content (θ , broken line) at high fertility (HF) and low fertility (LF) sites from August 1993 to April 1994 at **A**, Mauriceville and **B**, Whareama. Data from Moir et al. (1997).

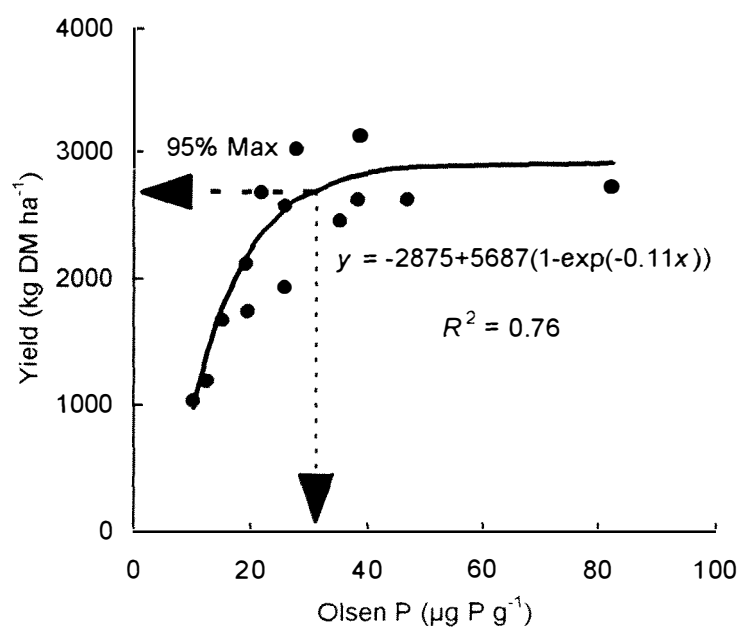


Figure 6.2 The relationship between Olsen P and pasture yield for a non-moisture limiting growth period (spring harvests). Data from Moir et al. (1997).

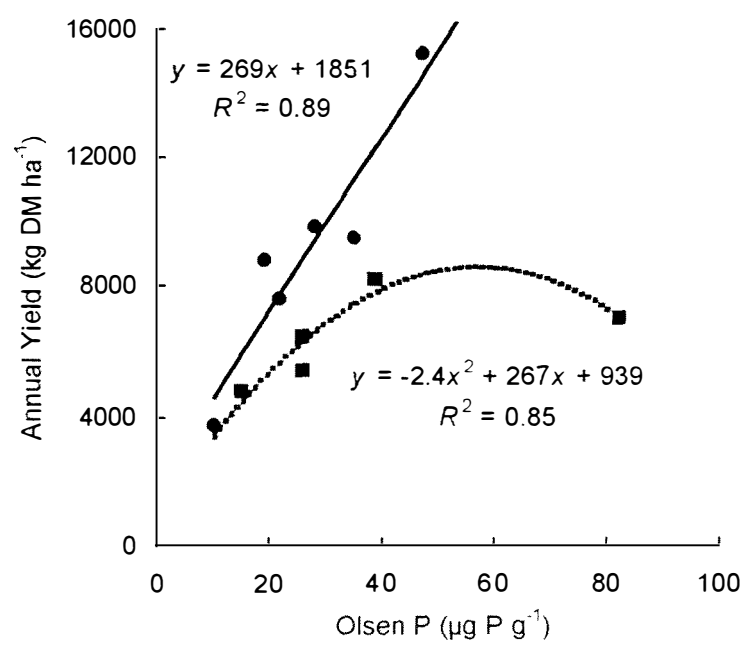


Figure 6.3 The relationship between Olsen P and annual pasture yield at Mauriceville (high rainfall) (●, ---), and Whareama (low rainfall)(■, —) sites.

As a step towards solving the problems outlined above, this Chapter describes and tests a simple pasture production model that explicitly takes into account the effect of climate as it influences soil moisture and evapotranspiration, in addition to the effect of soil fertility. Pasture growth is computed using a spreadsheet, and is based on calculations of soil water balance. Running the model alongside fertility trials to aid interpretation and extrapolation of the results is suggested, and it is demonstrated how this might be done.

6.4 MODEL DEVELOPMENT

The basic model is described here, and justified in a later section. Pasture growth (G) is calculated daily. It is assumed to be proportional to the actual evaporation rate (E) so that

$$G = kE. \quad (6.1)$$

The proportionality constant (k), with units of $\text{kgDMha}^{-1} \text{ mm}^{-1}$, is a site-specific factor which could be an index of soil fertility status.

6.4.1 Calculation of E

The simple soil water balance used to estimate E is that described by Coulter (1973a). When the root zone is at field capacity, it is assumed to hold a certain maximum amount of plant-available water (W_a), typically 75 mm. If there is plant-available water (W) in the root-zone, evaporation is assumed to occur at the reference crop or potential evapotranspiration rate. Once the available water is used up, evaporation is assumed to be zero. Excess water is assumed to be lost as deep drainage or surface runoff if, and only if, the available soil water has been recharged to W_a .

The following equations describe the water balance. Writing the plant-available water stored in the root zone at the conclusion of day j as W_j , then

$$W_j = W_{j-1} + P - E - D \quad (6.2)$$

where P is the rainfall, E the evaporation, and D is the drainage plus surface runoff, all on day j , and W_{j-1} is the value of W at the end of the preceding day. All the terms in Equation (6.2) are equivalent water depths. E is found as

$$E = E_p \quad \text{if} \quad W_{j-1} + P \geq E_p \quad \text{else} \quad E = W_{j-1} + P, \quad (6.3)$$

where E_p is the estimate of reference crop evaporation for day j , calculated as described below. D for day j is found as

$$D = W_{j-1} + P - E - W_a \quad \text{if} \quad W_{j-1} + P - E > W_a \quad \text{else} \quad D = 0. \quad (6.4)$$

Equations (2), (3), and (4) ensure that $0 \leq W \leq W_a$.

Coulter (1973a) used Thornthwaite estimates of E_p , but later (Coulter, 1973b) showed them to be unreliable in New Zealand, and recommended Penman's equation. The Priestley-Taylor estimates of reference crop evaporation are used, rather than more data-intensive Penman estimates, as the two equations are of similar accuracy for well-watered pasture in the Manawatu (Clothier et al., 1982). The Priestley and Taylor (1972) approach calculates the reference crop evaporation as

$$E_p = \frac{1.26sR_n}{\rho_w L(s + \gamma)} \quad (6.5)$$

where s is the slope of the relationship between the saturated vapour density and temperature, γ is the psychrometric constant, R_n is the net radiation over the 24 hour period (MJ m^{-2}), ρ_w is the density of water (1000 kgm^{-3}), and L is the latent heat of vaporisation of water at ambient temperature (2.5 MJkg^{-1}). A quadratic equation fitted to tabulated values for the dimensionless ratio of $s/(s + \gamma)$ (Tanner pers. comm.) at an air pressure of 100 kPa over the temperature range 5 to 20°C gives

$$s/(s + \gamma) = 0.403 + 0.0164T_{av} - 0.00012T_{av}^2 \quad (6.6)$$

where T_{av} ($^\circ\text{C}$) is the average screen air temperature, approximated as the mean of the daily maximum and minimum. The net radiation may be estimated from the daily incoming solar radiation (R_s) (Scotter et al. 1979) as

$$R_n = 0.62R_s - 1.47, \quad (6.7)$$

where both R_n and R_s are in units of MJ m^{-2} . If solar radiation data are not available, values can be estimated from sunshine hour data for the day, as described by Scotter et al. (2000).

A numerical example of the use of the above equations may help the reader, using data obtained from the local newspaper. Consider 1 September 1999 in Palmerston North. At AgResearch Grasslands, near Massey University, the maximum screen temperature was 11.7°C , and the minimum was -1.7°C . A few kilometres away at Palmerston North

Airport, the measured incoming solar radiation was 15.9 MJ m^{-2} . Putting the average daily air temperature of 5°C into Equation (6.6) gives $s/(s+\gamma)$ as 0.48. Putting R_s of 15.9 MJm^{-2} into Equation (7) gives R_n as 8.4 MJm^{-2} . From Equation (5), E_p was then found for the day as 0.0020 m or 2.0 mm.

Flat land is assumed, although it would be simple to add the effects of slope and aspect on solar radiation (McAneney & Noble 1976) and rainfall, and so estimate how they affect the water balance for permeable soil. Processes such as surface runoff induced by compaction or water repellency are also not included in the basic model, as again the relevant information is not yet available.

Running the model with the evaporation equal to E_p provides a useful benchmark, as it simulates production with irrigation. The difference between this and production simulated without irrigation (using E) indicates how much water stress has affected yield.

To quote McAneney & Judd (1983) "Many studies have shown that to an acceptable first approximation, dry matter production is proportional to transpiration." For full-cover pasture ($\text{LAI} > 3$), nearly all of evapotranspiration is transpiration (Kerr et al. 1986; Allen et al. 1998). The soil water balance model used to calculate E here is very simple. It assumes soil water is either readily available or unavailable. In contrast, McAneney & Judd (1983) assumed that evaporation is related to the storage level in the root zone. This approach was not adopted for a number of reasons. First, the relationship will be unique for each soil, and data to evaluate it will not usually be available. Second, the relationship is not unique. A small amount of rain rewetting the topsoil after a drought will allow evaporation rates to return to the reference crop rate, even though water storage in the root zone is still low. Growth is more sensitive to water stress than is transpiration (McAneney & Judd 1983). For example, in a dry summer pasture transpiration may be able to extract over 200 mm from a soil profile, however, growth effectively stops long before this deficit is reached (Scotter et al. 1979). So Equation (6.1) will not be valid under these conditions. An indirect compensation for this was made by making W_a in effect the readily available water storage capacity, rather than the total available storage capacity.

If the main determinants of the site-to-site, and year-to-year, variation in yield are the weather and soil fertility, the constant k should be a function of soil fertility status. It can thus be used to compare soils.

6.5 DATA FOR MODEL DEVELOPMENT AND VALIDATION

The model is applied to the results of a sets of field trials conducted on sheep and beef cattle farms at Whareama, Gladstone, and Mauriceville, representing low, medium and high rainfall regimes, in the Wairarapa hill country (North Island, New Zealand) from 1993 to 1995. The trials assessed pasture growth using movable cages, with cuts taken at approximately monthly intervals, the actual interval depending on growth rate. Sites with slopes less than 20° were selected (Moir et al. 1997).

Pastures contained a wide range and proportion of grass, legume, and weed species, which varied considerably across sites. Swards ranged from ryegrass dominant (HF, Site 1), to mixed swards of low fertility species typical of that found on low fertility North Island hill country (Lambert et al. 1986). However, sward composition at these sites is complex, and often could not be clearly linked to patterns of soil fertility, and climate regime. Other workers (Lambert et al. 1986; Hernández Garay et al. 1997) have indicated that grazing management can influence the botanical composition of pastures. It is suggested that further studies could focus on factors influencing sward composition at these sites.

At each location, pasture growth was measured at sites with different superphosphate fertiliser histories, resulting in high (Olsen soil test values $\geq 25 \mu\text{gPg}^{-1}$ soil) and low (Olsen soil test values $7\text{-}10 \mu\text{gPg}^{-1}$ soil) fertility regimes (Moir et al. 1997). At these sites, Olsen P status was shown to be the best of a number of indicators of soil fertility status (Moir et al. 1997). McCall & Thorrold (1991) have proposed that a fertiliser index approach may also be appropriate. Low soil P status has been the major constraint to developing legume-based grassland at these sites. This has been overcome by frequent use of superphosphate over 25-60 years. Thus, it should be noted that soil-available-P status measured by Olsen P also acts as an indicator of increasing available soil sulphur and nitrogen as successful legume establishment is maintained over a number of years.

Rainfall was measured at the three locations. Sunshine hours and air temperature data from climate stations at Masterton for 1993 and Martinborough for 1994 were used. However, for Gladstone and Mauriceville, a -1°C correction was applied to the temperature data due to the altitude being 200 to 300 m higher than the sites where the temperature data were collected (Aldridge 1982).

6.6 RESULTS AND DISCUSSION

6.6.1 Parametisation of k

A number of studies (Rickard & Fitzgerald 1970; Wright & Baars 1976; Rickard et al. 1986) have shown linear relationships between evapotranspiration and pasture production. Tanner & Sinclair (1983) argued that unless malnutrition is severe, k is not greatly affected by nutrient status. Ritchie (1983) and Power (1983) argued the opposite, and supported their argument with experimental data. Unless the pasture is very sparse and the exposed soil surface dry, evaporation will occur at close to the reference crop rate when adequate soil moisture is present, regardless of the pasture growth rate. Since most New Zealand pasture is not sparse (10000 tillers m^{-2} on sheep-grazed pasture; Barker et al. 1993; Matthew et al. 1996), a growth response to fertiliser will be reflected in a higher value for k . It is argued that the proportionality constant (k), linking growth and evapotranspiration, depends primarily on soil fertility. To test this assertion, data from six sets of field trials, with contrasting fertility and climate, were compared.

The water-balance model was used to calculate daily E values for the Whareama, Gladstone, and Mauriceville sites. Accumulated pasture growth for a low and high fertility site was plotted against cumulative E for each rainfall regime. The relationships found were essentially linear (Figure 6.4). The slope of the line is the k value. There was a marked difference in k values between soil fertility levels at each rainfall regime, but not between rainfall regimes at the same fertility level.

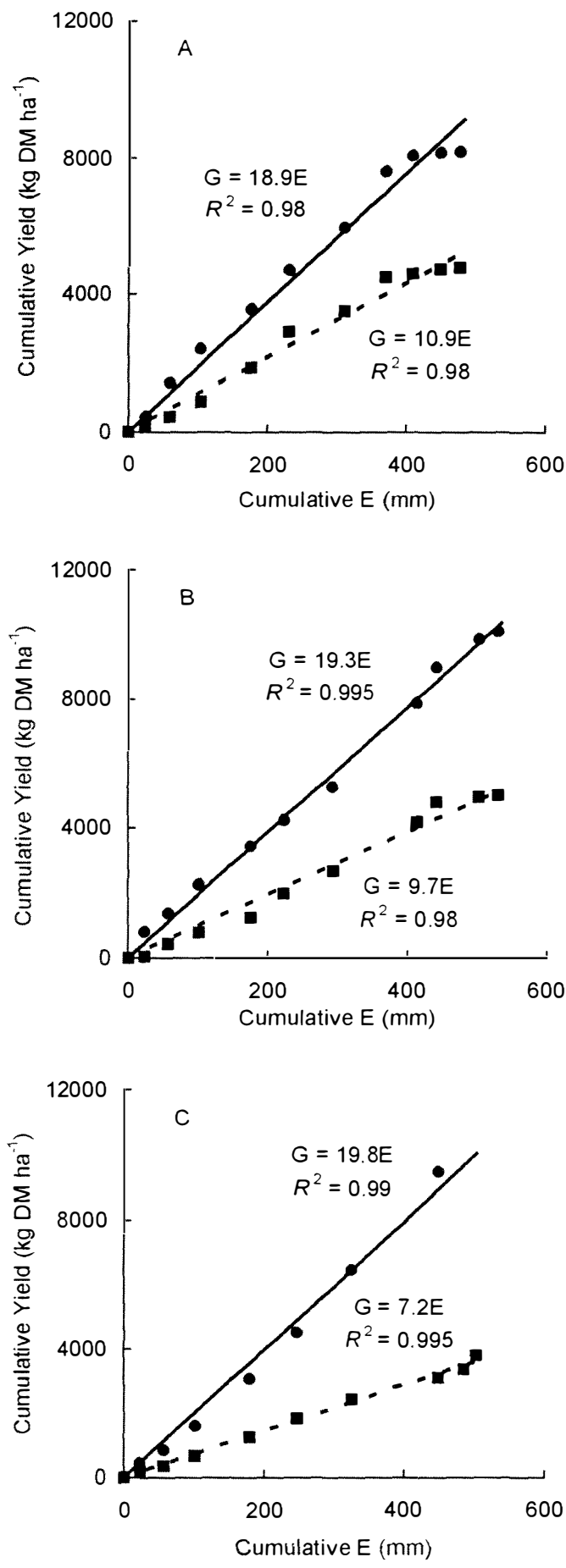


Figure 6.4 Relationships between cumulative evapotranspiration (E) and cumulative pasture yield (G) from August 1993 to April 1994 at high (●, —) and low (■, ---) fertility sites at **A**, Whareama; **B**, Gladstone; and **C**, Mauriceville.

6.6.2 Relationship Between Olsen P and Growth Per Unit E

Data from all 14 sites reported by Moir et al. (1997) were used to examine the relationship between k and Olsen P. There is a strong relationship ($R^2 = 0.89$, Sites $n = 13$) between Olsen P and growth per unit E across most sites (Figure 6.5). The reasons for two sites being outliers (Figure 6.5, ■) are not clear at this stage. Overall, the relationship between Olsen P and growth per unit E was considered to be robust, and suitable for use as a proportionality constant in pasture growth modelling. On this range of long-term farmed sites, it is worthwhile noting that k shows little evidence of reaching an asymptote as Olsen values approach $50 \mu\text{gPg}^{-1}$. This may suggest that pasture growth on these long-established, well-fertilised pastures (reflecting high N status) does not follow previously observed yield plateau's in Olsen P response pattern occurring at Olsen values of less than $30 \text{ mg P kg}^{-1} \text{ soil}$ (Sinclair et al. 1997). It must be noted, however, that the field trial sites used by Sinclair et al. (1997) were less well developed, P-responsive sites, chosen specifically to evaluate the agronomic effectiveness of P fertilisers.

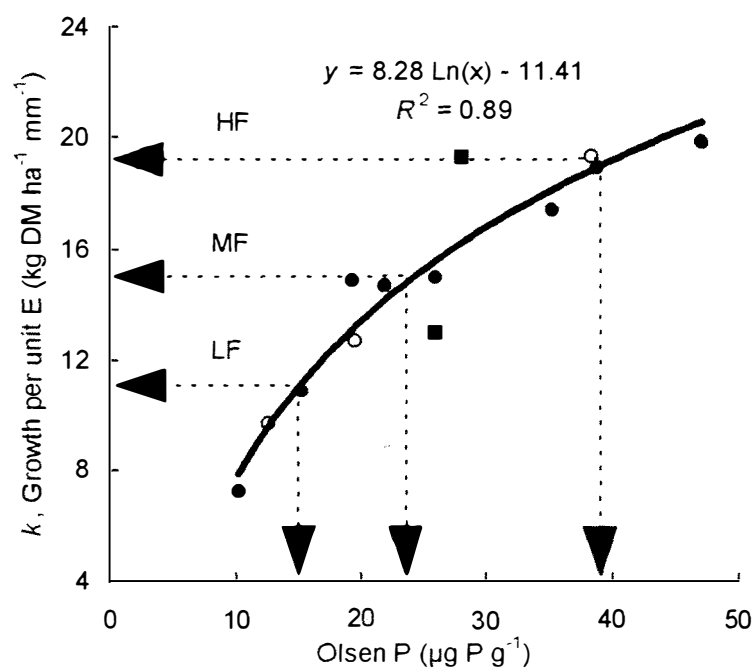


Figure 6.5 The relationship between Olsen P and growth per unit E across all Wairarapa field-trial sites (Gladstone sites = $\circ$, Outliers = $\blacksquare$).

This result (Figure 6.5) also illustrates that the efficiency of soil water use per kg of pasture dry matter increases with increasing soil fertility status at these sites. Higher soil fertility levels (and subsequent increases in plant nutrition) will raise soil water nutrient content and influence plant physiology (photosynthetic efficiency; plant tissue characteristics and related growth properties), root density (Barker et al. 1988), and sward ecology, e.g., species composition (Lambert et al. 1986). Changes in these factors will contribute to changes in the rate of plant growth per unit of water transpired to meet evaporative demand.

6.6.3 Model Validation

To initially test the pasture growth model (Equation 6.1), data from the three Gladstone sites (Figure 6.4B, 6.5) in 1993/94 was used to calibrate the model. The model was then used to predict pasture growth at Gladstone in 1994/5, and at Whareama (low rainfall regime) in both years (where actual pasture growth was measured). By following this procedure, the model was built using data from one region, in one season, then applied it to another season in the same region, and to both seasons in a drier region.

The modelled effect of water stress on production (as indicated by the gap between the top, unlimited moisture, and middle lines in Figure 6.6) is greater at Whareama sites than at Gladstone.

Data for the 1994/95 year show lower pasture production than that of 1993/94, reflecting the drier conditions in both regions until autumn (after Day 203; Figures 6.6B and 6.6C). At Gladstone sites, the measured and modelled productions are in quite close agreement in 1994/5 (Figure 6.6A), and also for Whareama sites for the 1993/94 year (Figure 6.6B). During 1994-5, the measured production at Whareama (Figure 6.6C) was much lower than that modelled, particularly during summer and autumn (i.e., after Day 113). While 45 mm and 85 mm of rain fell on Days 177 and 199, respectively, in February, there was no growth response to this rain from the low fertility pasture, and only limited regrowth from the high fertility site. We can only speculate on the reasons for this. It could be that during the preceding dry period the soil surface became water repellent. The sporadic heavy rain would then have run off rather than soaked in (D.J.

Barker pers. comm.). In addition, the protracted dry spell desiccated most above-ground plant material and left few species capable of vegetative growth. Regrowth upon wetting was severely restricted, recovering only after germination, or from regrowth from buried stolons. Note that the pasture at Gladstone did not dry out as much as at Whareama, and heavy rainfall did not occur during February at Gladstone. This has resulted in predicted growth and actual growth being very similar (Figure 6.6A).

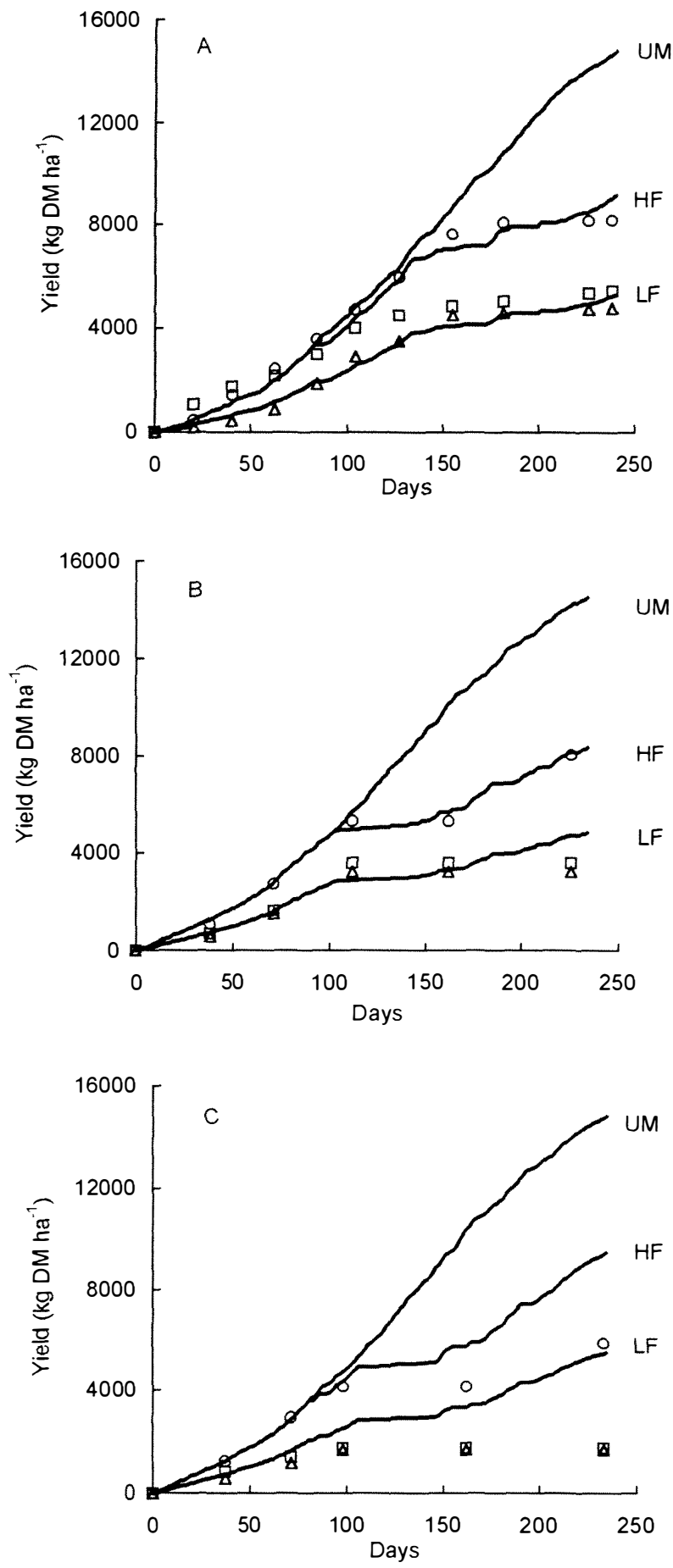


Figure 6.6 Actual (data points) and modelled (solid line) cumulative pasture yields at **A**, Gladstone (1994/5); and Whareama in **B**, 1993/4 and **C**, 1994/95. LF, Low fertility ($\square$ or Δ); HF, high fertility ($\circ$); UM, unlimited moisture.

The activities and dynamics of resident soil microbial populations are also likely to respond to change in soil moisture and temperature (Kieft et al. 1987; Van Gestel et al. 1993) and influence subsequent mineralisation/immobilisation and availability of plant nutrients (Perrott et al. 1992; Ross et al. 1995). On a “rewetted” dry soil, lag phases in pasture growth response (or, alternatively, unaccounted-for “larger than expected” responses) due to increased moisture, may in part be attributed to soil microbial processes and the associated change in nutrient availability to plants. Current understanding of these soil microbiological processes is limited.

6.6.4 Application of Model

The interacting effects of weather and soil fertility on summer and early autumn production are of interest to producers and rural financing agencies. The reasonable agreement between measured and modelled values for Gladstone in Figure 6.6 encouraged us to use the model with 1000 years of simulated daily rainfall and evaporation data for the site. These data were generated, as described by Scotter et al. (2000), from the average monthly rainfall, number of wet days, air temperature, and sunshine hour values given in New Zealand Meteorological Service (1983).

Cumulative probability distributions for simulated pasture production from 30 November to 30 April were obtained for the high ($19 \text{ kg DMha}^{-1}\text{mm}^{-1}$) and low ($11 \text{ kg DMha}^{-1}\text{mm}^{-1}$) fertility sites (Figure 6.7). The y -axis value is the probability that pasture production will be less than the x -axis production value. Thus, one minus the y -axis value is the probability of the production being greater than the x -axis value. High fertility gives an extra 1000 to 3000 kg DMha^{-1} , depending on the weather, but as production is assumed proportional to k , there is also greater year-to-year variability in production. One potential use of this figure would be to estimate the yield benefit of having a higher Olsen P status in, for example, a 1 in 10 drought year, or 1 in 10 wet year. Such information could be valuable to the producer.

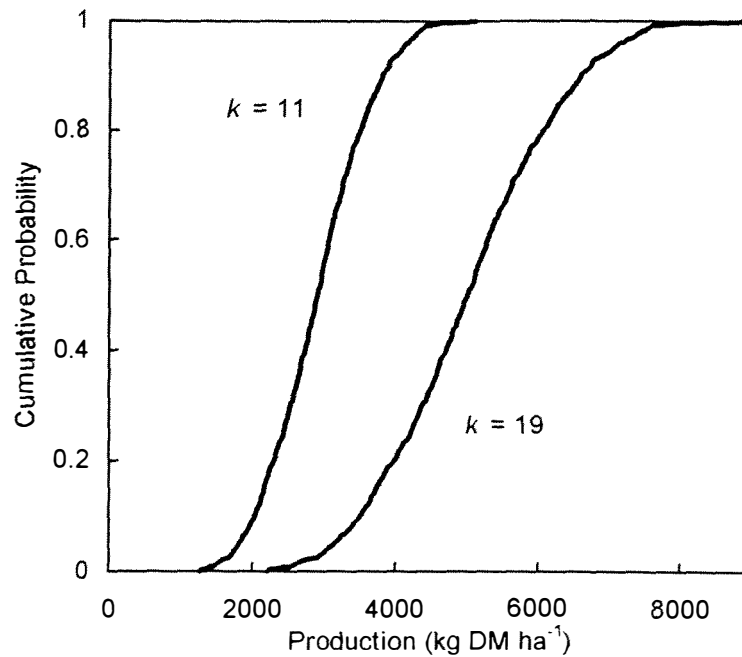


Figure 6.7 The cumulative probability distribution of generated pasture production from 30 November to 30 April at Gladstone, for k values of 11 (low fertility) and 19 (high fertility) $\text{kgDMha}^{-1}\text{mm}^{-1}$.

6.7 CONCLUSIONS

Pasture growth per mm of evapotranspiration was strongly related to available P status at the sites described by Moir et al. (1997). On this basis, the model described in this Chapter estimates the interacting effects of soil fertility and weather (as it influences soil moisture and evapotranspiration) on pasture production. With the exception of growth after severe drought conditions, the model shows potential to closely predict actual pasture yield. A possible weakness of the model is the way in which temperature is treated. In winter, pasture growth is likely to be more temperature sensitive than the reference crop evaporation, while in summer the reverse is likely to be true.

It is hoped that discrepancies between the modelled and measured production may lead to useful speculation and further research on the interacting effects of weather and fertility on pasture growth. For example, does soil fertility affect rooting depth (Matthew et al. 1991) and thus, the readily available soil water storage capacity? If an increase in fertility changed W_a from 75 mm to 125 mm, about two extra weeks of spring growth would be expected before stress set in, with the extra available water

producing an extra 1000 kgDMha⁻¹ if $k = 19 \text{ kg DMha}^{-1}\text{mm}^{-1}$, but this difference would not be observed under irrigation, or in a wet year with little water stress. The behaviour of pasture and soil following a prolonged dry spell is also of interest, and is one of the most under-researched topics in the soil-water-plant area. As mentioned above, during prolonged dry periods soil fertility status is likely to affect plant survival and, perhaps (indirectly), the development of surface hydrophobicity. Soil microbial activity following a dry period and associated nutrient mineralisation and/or immobilisation are also likely to be major contributors to plant growth response to moisture. As more information on these topics becomes available, it could perhaps be built into the model.

6.8 ACKNOWLEDGEMENTS

The authors wish to thank Mr H. B. Toes and Mr M. R. Bretherton, Soil and Earth Sciences, Massey University, for valuable assistance with field work. We wish to acknowledge the farmers involved in this study: B. Christensen, D. Dunlop, N. Kilmister (Mauriceville); A. Day, D. Blackwood (Gladstone); D. Kinnell, K. O'Riley, D. Williams (Whareama). Also Mr C. Garland of D. O. Baker and Associates, Masterton, for assistance with site selection, and local knowledge.

6.9 REFERENCES

- Aldridge, R., 1982. The prediction of soil temperature in New Zealand and application to the temperature regimes of 'Soil Taxonomy'. 54, DSIR, Wellington, New Zealand.
- Allen, R.G., Pereira, L.S., Raes, D. and Smith, M., 1998. Crop evapotranspiration; guidelines for computing crop water requirements. Irrigation and Drainage Paper, FAO, 56.
- Barker, D.J., Lambert, M.G., Dymock, N. and Gray, Y.S., 1993. Introduction, production and persistence of five grass species in dry hill country 5. Central Wairarapa, North Island, New Zealand. New Zealand Journal of Experimental Agriculture, 36: 29-39.

- Barker, D.J., Zhang, D.M. and Mackay, A.D., 1988. Root distribution in a low fertility hill country sward grazed by sheep. *New Zealand journal of experimental agriculture.*, 16(1): 73-76.
- Clothier, B.E., Kerr, J.P., Talbot, J.S. and Scotter, D.R., 1982. Measured and estimated evapotranspiration from well-watered crops. *New Zealand Journal of Agricultural Research*, 25: 301-307.
- Coulter, J.D., 1973a. A water balance assessment of New Zealand rainfall. *Journal of Hydrology (New Zealand)*, 12: 83-91.
- Coulter, J.D., 1973b. Prediction of evapotranspiration from climatological data. In: J.P. Kerr, R.J. Jackson and M.F. Beardsell (Editors), *Proceedings of soil and plant water symposium*. DSIR, Wellington, pp. 38-45.
- Gandar, P.W. and Kerr, J.P., 1980. The efficacy of agronomic research in New Zealand, *Proceedings of the Agronomy Society of New Zealand*, pp. 87-92.
- Hernández Garay, A., Matthew, C. and Hodgson, J., 1997. Effect of spring grazing management on perennial ryegrass-white clover pastures 2. Tiller and growing point densities and population dynamics. *New Zealand Journal of Agricultural Research*, 40: 37-50.
- Kerr, J.P., De Ruiter, J.M. and Hall, A.J., 1986. The magnitude and variability of seasonal water deficits for pasture and crop growth. *New Zealand Agricultural Science*, 20: 13-18.
- Kieft, T.L., Soroker, E. and Firestone, K., 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biology and Biochemistry*, 19(2): 119-126.
- Lambert, M.G., Clark, D.A., Grant, D.A. and Costall, D.A., 1986. Influence of fertiliser and grazing management on North Island moist hill country 2. Pasture botanical composition. *New Zealand Journal of Agricultural Research*, 29(1): 1-10.
- Matthew, C., Hernández Garay, A. and Hodgson, J., 1996. Making sense of the link between tiller density and pasture production. In: M. Baker (Editor), *Proceedings of the New Zealand Grassland Association*. New Zealand Grassland Association, pp. 83-87.

- Matthew, C., Xia, J.X., Chu, A.C.P., Mackay, A.D. and Hodgson, J., 1991. Relationship between root production and tiller appearance rates in perennial ryegrass (*Lolium perenne* L.), Plant root growth: an ecological perspective. Blackwell Scientific Publications, Oxford, UK., pp. 281-290.
- McAneney, K.J. and Judd, M.J., 1983. Pasture production and water use measurements in the central Waikato. *New Zealand Journal of Agricultural Research*, 26: 7-13.
- McAneney, K.J. and Noble, P.F., 1976. Estimating solar radiation on sloping surfaces. *New Zealand Journal of Experimental Agriculture*, 4: 195-202.
- McCall, D.G. and Thorrold, B.S., 1991. Fertiliser history is a useful predictor of soil fertility status. In: M.J. Baker (Editor), *Proceedings of the New Zealand Grassland Association*. New Zealand Grassland Association, Whakatane, New Zealand, pp. 191-196.
- Moir, J.L., 1994. Influences of climate and fertiliser application history on various measures of soil fertility and productivity in Wairarapa hill country. Master of Agricultural Science Thesis, Massey University, Palmerston North, New Zealand, 145 pp.
- Moir, J.L., Hedley, M.J., Mackay, A.D. and Garland, C.B., 1995. Climate, soil fertility and pasture production in Wairarapa hill country. In: L.D. Currie and P. Loganathan (Editors), *Fertiliser requirements of grazed pasture and field crops: Macro and micro nutrients*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 254-260.
- Moir, J.L., Hedley, M.J., Mackay, A.D. and Tillman, R.W., 1997. The effect of fertiliser history on nutrient accumulation and plant-available nutrient supply in legume-based pasture soils. In: J.G. Buchanan-Smith, L.D. Bailey and P. McCaughey (Editors), *XVIII International Grassland Congress*. International Grassland Association, Winnipeg, Canada, pp. 10, 68-69.
- N.Z. Meteorological Service, 1983. Summaries of climatological observations to 1980, N.Z. Meteorological Service Miscellaneous Publication 177. New Zealand Meteorological Service.
- Perrott, K.W., Sarathchandra, S.U. and Dow, B.W., 1992. Seasonal and fertiliser effects on the organic cycle and microbial biomass in a hill country soil under pasture. *Australian Journal of Soil Research*, 30: 383-394.

- Power, J.F., 1983. Soil management for efficient water use. In: H.M. Taylor, W.R. Jordan and T.M. Sinclair (Editors), Limitations to efficient water use in crop production. American Society of Agronomy, Madison, pp. 461-470.
- Priestley, C.H.B. and Taylor, R.J., 1972. On the assessment of surface heat flux and evaporation using large-scale parameters. *Monthly Weather Review*, 100: 81-92.
- Rickard, D.S. and Fitzgerald, P.D., 1970. The effect of soil moisture and irrigation on pasture production in Canterbury, New Zealand, 11th International Grassland Congress. Queensland University Press, Brisbane, Queensland, Australia, pp. 487-492.
- Rickard, D.S., McBride, S.D. and Fitzgerald, P.D., 1986. The effect of soil moisture deficits on pasture yield. *New Zealand Agricultural Science*, 20(1): 7-12.
- Ritchie, J.T., 1983. Efficient water use in crop production: discussion on the generality of relations between biomass production and evapotranspiration. In: H.M. Taylor, W.R. Jordan and T.M. Sinclair (Editors), Limitations to efficient water use in crop production. American Society of Agronomy, Madison, pp. 29-44.
- Ross, D.J., Speir, T.W., Kettles, H.A. and Mackay, A.D., 1995. Soil microbial biomass, C and N mineralisation and enzyme activities in a hill pasture: influence of season and slow release P and S fertiliser. *Soil Biology and Biochemistry*, 27(11): 1431-1443.
- Scotter, D.R., Clothier, B.E. and Turner, M.A., 1979. The soil water balance in a fragiaqualf and its effect on pasture growth in central New Zealand. *Australian Journal of Soil Research*, 17: 455-465.
- Scotter, D.R., Horne, D.J. and Green, S.R., 2000. A procedure for generating daily rainfall and evaporation data: an evaluation and some applications. *Journal of Hydrology (New Zealand)*, 39: 65-82.
- Sinclair, A.G., Johnstone, P.D., Smith, L.C., Roberts, A.H.C., O'Connor, M.B., and Morton, J.D., 1997. Relationship between pasture dry matter yield and soil Olsen P from a series of long-term field trials. *New Zealand Journal of Agricultural Research*, 40: 559-567.
- Tanner, C.B. and Sinclair, T.R., 1983. Efficient water use in crop production: research or re-search? In: H.M. Taylor, W.R. Jordan and T.M. Sinclair (Editors), Limitations to efficient water use in crop production. American Society of Agronomy, Madison, pp. 1-27.

- Van Gestel, M., Merckx, R. and Vlassak, K., 1993. Microbial biomass responses to soil drying and rewetting: the fate of fast and slow-growing microorganisms in soils from different climates. *Soil Biology and Biochemistry*, 25(1): 109-123.
- Wright, A. and Barrs, J.A., 1976. A simulation model of a grazing system with particular reference to soil-plant-climate relationships, *Symposium on Meteorology and Food Production*. New Zealand Meteorological Service, Wellington, NZ, pp. 183-196.

CHAPTER 7

USING BIOLOGTM MICROTITRE PLATES AS A METHOD FOR STUDYING CHANGES IN THE FUNCTION AND CARBON SUBSTRATE USE CHARACTERISTICS OF MICROBIAL POPULATIONS IN PASTURE SOILS: I. INFLUENCE OF SOIL EXTRACT ADDITION ON MICROBIAL GROWTH.

7.1 INTRODUCTION

In the development of soils for agricultural production, the nature and diversity of plant species growing at the soil surface changes markedly from the native forest or grassland. In turn, the amount and form of fixed carbon entering the soil through the decomposition of plant and animal residues is likely to change (Walker and Adams, 1958; Walker and Adams, 1959; Walker et al., 1959; Jackman, 1964a; Jackman, 1964b; Haynes and Williams, 1992; Perrott et al., 1992; Ross et al., 1995). Changes in the amount and form of residues (e.g. C:N ratio, nature of C compound) entering the soil may alter the diversity and or the functional diversity of the soil microflora decomposer population.

In soils with major landuse contrasts i.e. comparison of forest / native forest with grazed pasture and continuously cultivated soils (Ghani et al., 1996; Campbell et al., 1997; Degens and Harris, 1997; Degens et al., 1998; Ghani et al., 1999) changes in the substrate use characteristics of soil microflora with change in landuse have been reported. Some (Ghani et al., 1996; Degens and Harris, 1997; Degens et al., 1998; Bardgett et al., 1999; Ghani et al., 1999; Sparling and Schipper, 1999) have argued that such changes in the substrate use characteristics and the functional diversity of microflora influence decomposition and therefore nutrient cycling. These changes may impact on soil quality.

Attempts have been made to characterise the functional diversity of soil microflora substrate use pattern. Some researchers, have used the BiologTM GN plates for whole community characterisation of soil inocula (Garland and Mills, 1991; Zac et al., 1994;

Harch et al., 1997; Hitzl et al., 1997; Knight et al., 1997; Grayston et al., 1998; Lindstrom et al., 1998;). The BiologTM GN titre plate was designed (Bochner, 1989) for taxonomic characterisation of pre-isolated single bacterial species inocula. BiologTM is a microtitre plate system containing micro culture wells, a control well and 95 others, each containing one of 95 different sole-carbon sources, along with a tetrazolium redox dye. The tetrazolium is reduced (by NADH) as a result of microbial respiration/oxidation of carbon substrate, and produces colour development in the well (Plate 7.1). The rate and extent of colour formation is proportional to the oxidation of a substrate by the microbial inoculum. The resulting C substrate use patterns have then been used to assess the “functionality” or “metabolic diversity” of the cultured soil microbiological communities.

Degens and co-workers, (Degens and Harris, 1997; Degens, 1998a; Degens, 1998b; Degens et al., 1998; Degens, 1999) have modified the substrate induced respiration technique of Anderson and Domsch (1978) to including a narrower range of substrates (similar to those in the BiologTM assay) to report on the substrate use pattern of microflora grown in their natural habitat of the soil.

At present, there is a poor understanding of the ecological significance of these observed changes in microflora functional diversity with landuse, and whether they reflect significant impact on soil quality. In addition, few studies have determined the range of microflora substrate use characteristics that occur within one landuse across one soil type or group.

The series of grazed pasture soils, varying in superphosphate history, rainfall regime and pasture production, studied by Moir et al. (1997), provide a single landuse suite of soils (within 2 soil groups) with which to examine the range of microflora substrate use patterns for pasture soils. In research reported in this, and the following Chapter, the BiologTM GN plates are used to report on the substrate use characteristics of the microbial populations extracted from this range of pasture soils.

During preliminary observations of colour development patterns on the BiologTM plates, it became apparent that a substantial level of colour development (absorbance at 590 nm) occurred in the blank (control) well of the plate. The saline extract from soil may contain (i) coloration; (ii) microbially-respirable C; and (iii) differences in types of micro-

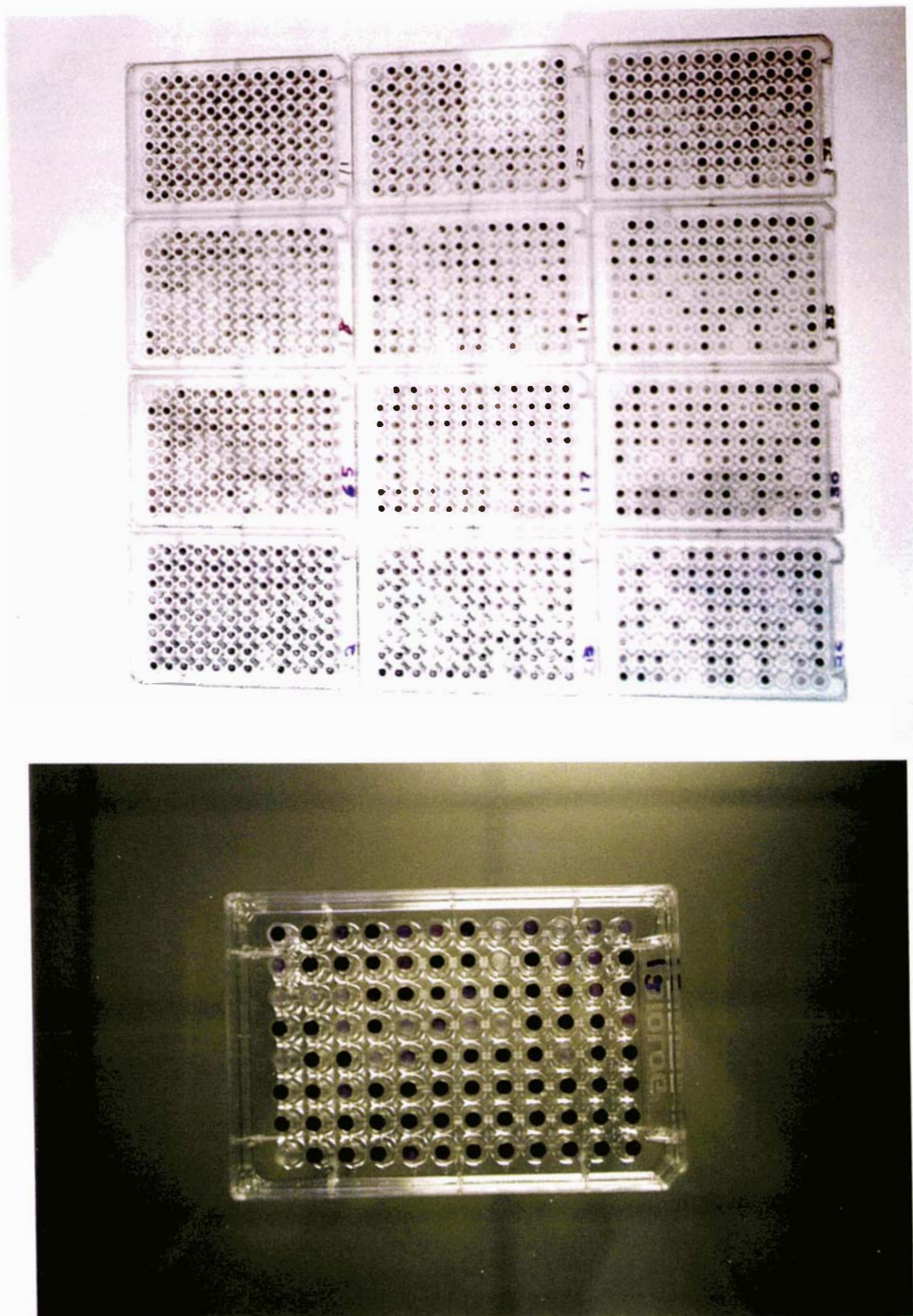


Plate 7.1 BiologTM microtitre plates, showing colour development (extent of C substrate oxidation) of varying degrees across the 96 single C substrates (wells). These microtitre plates have been inoculated with soil microbes and incubated for several days.

organisms present, which may all stimulate (contribute to) a positive result in the control well. Significant amounts of respirable C in the saline inoculum had potential to complicate or cause misinterpretation of the substrate use patterns of the inocula. Therefore, prior to embarking on the experimental work with BiologTM GN assays and substrate use patterns of the soil inocula, a series of additional studies were undertaken to investigate the factors influencing the colour development in the control well (background absorbance). These include concentration of carbon in the soil extract, extract colour, microbial numbers and how these change with soil condition (moisture content).

An examination of whether different application rates of superphosphate fertiliser (applied long-term), and differences in rainfall have influence on the substrate use pattern of the soil microbial population dynamics, as determined by BiologTM GN microtitre plates, is presented in the following Chapter (Chapter 8).

7.2 METHODS AND MATERIALS

7.2.1 Soil Sampling

Undisturbed soil cores 200 x 80 mm (diameter x depth) were sampled from pastures in late summer (March 1998), at a time when the Wairarapa region was experiencing a particularly long dry spell. All study sites had reached permanent wilting point. Cores were taken across slope at approximately 1 to 1.5m intervals, taking care to keep position in slope, soil type and vegetation as consistent as possible. All cores were trimmed flush at the bottom edge and transported to the laboratory in an unrestricted, unbagged state, to allow gaseous exchange to continue. Corresponding soil bulk density samples from each of the 12 sites were taken at this time. It was anticipated that by sampling at this time, there would be some reduction in the inconsistency across sites in microflora variability resulting from different soil moisture contents, normally occurring across the rainfall regimes. All cores were stored in a glasshouse. One core from each site was maintained in a "field-dry" condition, whilst the others were rewetted to a volumetric water content of 0.35.

Soil biological measurements were made at two stages; when soils were field dry (soon after sampling), and on other cores which had been re-wetted and incubated under glasshouse conditions for 28 days .

For more detail of site description of the hill-country pasture sites studied here, refer (Moir, 1994; Moir et al., 1997).

7.2.2 Soil Chemical and Physical Characterisation

The soil fertility of these sites has been characterised and reported in previous papers e.g. Moir et al. (1994), Moir et al. (1995), Moir et al. (1996) and Moir et al. (1997). However, a brief summary of some field characteristics of the 12 sites are given below*.

Table 7.1 Summary of Soil Chemical and Physical Characteristics

Site	Soil	Bulk Density	CEC cmoles kg ⁻¹	pH (H ₂ O)	Total C (%C)	Total N (µgNg ⁻¹)	Total P (µgPg ⁻¹)	Olsen P (µgPg ⁻¹)	Mineralis -able N (µgNg ⁻¹)
1	YBE	0.97	26	5.5	5.8	6057	1466	47	471
3	YBE	1.01	24	5.4	6.0	5555	790	28	395
4	YBE	0.91	25	5.0	6.4	4591	433	10	172
5	YGE/YBE	0.83	32	5.4	6.7	6204	571	26	372
6	YGE/YBE	0.94	27	5.4	5.3	4866	594	15	310
8	YGE/YBE	0.95	32	5.7	6.2	5959	846	33	414
9	YGE/YBE	0.82	34	4.8	8.9	7948	658	26	255
10	Redzina	1.01	20	5.7	5.4	5449	1321	38	395
11	YGE/YBE	0.91	20	5.4	5.8	4281	493	20	291
12	YGE/YBE	0.87	22	5.9	5.4	4889	586	13	219
13	YGE/YBE	0.91	29	5.3	7.2	5810	902	19	287
14	YBE	1.01	26	5.6	5.9	4818	834	22	292

*Adapted from (Moir, 1994),(Moir et al., 1994), (Moir et al., 1995).

Olsen P, pH, and mineralisable N were measured on all 200 mm cores taken from sites used in this study, after sieving for Biolog™ extraction.

In addition, Olsen P (Olsen et al., 1954), and extractable (sulphate) S (Searle, 1979) soil pH (2.5:1 water to soil ratio), mineralisable soil N (Waring and Bremner, 1964: modified; Keeney and Bremner, 1966) and exchangeable cations (Schollenberger and Simon, 1945; Blakemore, 1964; Blakemore et al., 1987) were measured on soil samples (multiple cores 75 mm deep x 25 mm diameter, air-dried, sieved <2mm) taken at each site. Total soil N and P were determined by Kjeldahl digest (McKenzie and Wallace, 1954), and total S by dry oxidation (Landers et al., 1983). Soil total carbon (C) content was determined by the LECO combustion method (Bremner and Tabatabai, 1971). Bulk density and field soil moisture content were measured by drying the bulk density samples at 105°C for 48 hours.

Soil fertility and physical condition

The soil fertility status of these sites has been examined previously, and reported in (Moir et al., 1994; Moir et al., 1996). A brief summary is provided below. For more complete soil fertility information on the soil samples used in Chapters 7 and 8, see Appendix 7.2.

Physically, these soils are mostly silt-loam in texture, with bulk densities ranging from 0.80-1.1 gcm⁻³. Soil pH ranges from 4.8-6.0, and total carbon from 5.3-8.9% C, which fall into the “typical” range for hill country soils of the yellow-grey (Pallic) and yellow-brown (Brown) soil groups.

Total soil phosphorus (P) content ranges widely across these soils, which is strongly related to historical fertiliser inputs of SSP. In general, sites with larger comparative fertiliser inputs have accumulated more P in the 0-7.5 cm horizon, in some cases up to four times the “native” soil P levels were observed. Total soil N and S levels followed the same trend as total soil P, although the range across soils was much narrower, and the relative level of N or S accumulation c.f. “native” soil levels was much lower. This has been attributed in part to leaching losses of N and S. Following this argument, drier coastal sites have probably experienced less nutrient loss in this manner, and hence a higher level of N and S accumulation relative to sites in “wetter” climatic zones.

Plant-available Olsen P levels range widely across these soils from 10-50 µgPg⁻¹. As with total P, Olsen P is strongly related to SSP fertiliser inputs, where higher fertiliser inputs have resulted in higher soil plant-available P levels. Of all soil fertility indices

measured over the course of studies at these sites, soil Olsen P is the index which best predicts plant DM production levels in the field, during periods where soil moisture is non-limiting (Moir et al., 1997). Olsen P is such a strong predictor of DM production because in these legume-based pastures, legume development and the accumulated amount of N fixed are mostly constrained by P and S availability. Olsen P values therefore also reflect the plant-availability of other important nutrients such as nitrogen and sulphur (covariance). Results from glasshouse pot trials (Chapter 5) on these soils also support such suggestions. It also seems reasonable that availability and rate of turnover of such nutrients in these pastoral ecosystem soils is in some way related to the type or “quality” of soil carbon, and related nutrient release and storage properties.

The latter suggestion is supported, in part, by the results of soil mineralisable N levels (Table 7.1). It is interesting to note here that although soil total N has a small range across sites, soil mineralisable N, the fraction of total N which is readily decomposable and more likely to be plant-available, varies widely. Such a result suggests differences in the types of carbon and nitrogen compounds in these soils, and possibly, differences in the activities of soil microbial populations.

7.2.3 BiologTM Substrate Assays

7.2.3.1 Field dry soils

To obtain the soil samples at field-moisture status (one from each site), the galvanised steel cylinder was removed from one soil core per site the day after sampling for use in the first BiologTM experiment. The soil samples were then broken up and 5mm sieved. During sieving, living “above-ground” plant material was removed, while all below-ground root material which passed through the sieve was intentionally included with the soil sample. Less than two hours passed between soil sieving and extraction steps. Soil moisture content was measured at this time.

BiologTM plate methodology was similar to that outlined by (Bochner, 1989), and by (Garland and Mills, 1991; Zac et al., 1994). Soil suspensions were prepared soil with sterile 0.85% w/v NaCl solution in a 10:1 ratio (25 mL NaCl: 2.5 g soil). Each of the 12 soil samples were extracted in triplicate. Soil NaCl suspensions were placed in sterile 50

ml polypropylene centrifuge tubes, and shaken in an end-over-end shaker for 20 minutes. The heavier solids were allowed to settle for 2-3 minutes before carefully decanting the supernatant through a medium, ashless filter paper (Whatman No.40). The Whatman 40 filter paper was used because of its medium filter speed, and a pore diameter of 10 μ m (similar to that used by Garland and Mills, 1991). This pore diameter may allow the following soil carbon sources to pass through: soluble carbohydrate, soluble inorganic C, soil particulate C < 10 μ m, fragments of very fine plant root hairs, bacteria, fungi, spores, and other soil micro fauna and flora, fine clay particles, including resinous carbon coatings, suspended insoluble humus < 10 μ m. It has also been assumed that the No. 40 filter paper would prevent the following material from travelling through the filtration stage: soil-borne plant and root tissue > 10 μ m, soil mineral components > 10 μ m, soil carbon particles > 10 μ m, or those coating mineral components > 10 μ m in diameter, soil macro-fauna and macro-flora.

The collected soil filtrate was then used to inoculate the BiologTM “GN type” microtitre plates. Under aseptic (sterile) conditions, 150 μ L of soil extract was added to each of the 96 wells. This was repeated for all soil extracts, giving a total of 36 BiologTM plates. The concentration of microbes in soil extract was not adjusted before adding to the BiologTM plates, although cell density was measured using plating techniques (see Section 4). All BiologTM plates were incubated at 26°C. Colour development (formation of Formosan) in each cell (see Plate 7.1) was measured using a Dynatech Elisa MR 5000 microplate reader at a wave length of 590 nm. The initial colour development was read after 24 hours, and re-measured every 24 hours thereafter, until absorbance readings reached a “static” growth phase on all plates. Colour development (OD 590 nm) was monitored for 13 days from time of inoculation. To summarise the patterns of optical density change, sigmoidal or exponential functions were fitted to the mean experimentally determined data for each soil extract.

7.2.3.2 Glasshouse Moistened Soils

Three of the four soil cores taken from each of the 12 field sites were re-wetted before further biological observations were made. The experiment was established soon after the soils were sampled from the field.

The soil cores, including the steel sleeve, were placed in individual saucers, and moistened to an initial volumetric water content of 0.4 by weight. Watering weight for each core was calculated using dry soil mass, bulk density and Haynes apparatus measurements of soil water content at given suction potentials. These measurements were made prior to the experiment commencing. A small correction was estimated for the mass of plant matter growing from each core. All cores were watered to weight on a daily basis from above with distilled water only. If noticeable leaching occurred, the leachate was returned to the core from above at a later time, so that any leaching loss of nutrient would be returned to the soil. During the incubation, soils were kept at a constant volumetric water content of 0.35, which is the field moisture content of these soils in autumn, measured in previous studies (Moir et al., 1995).

Once moistened, all cores were randomised and incubated under glasshouse conditions of 25°C daily maximum temperature, and 10°C minimum (night temp), which is similar to field conditions at this time of year (March). It was suggested that an incubation period of 2-3 weeks would be a suitable time period to biologically equilibrate these soils under glasshouse conditions (Saggar, 1998). Therefore, the soils were moistened and incubated for 28 days, before being dismantled and used in BiologTM and agar plating experiments.

7.2.4 Plating Studies to Determine Numbers of Colony Forming Units (CFUs)

Aliquots of the soil extracts used for BiologTM studies were also used to determine the number of CFUs present. The objective was to determine initial inoculum density of viable bacterial and fungal organisms, and also to observe the diversity of organisms which appeared. It was considered that such information would be important to understanding the richness of the microbial population in these soils, and also the interpretation of the BiologTM data.

Each of the 36 saline soil extracts were plated on two separate general nutrient agar mediums, one plate to determine bacterial growth, the other fungal. The bacterial plates were treated with the fungicide cycloheximide at 75 µg mL⁻¹, and fungal plates were treated with the bactericide streptomycin at 75 µg mL⁻¹. Soil extracts were diluted at

three levels with sterile saline, and then plated on the agar medium. Dilution factors were 10^{-4} , 10^{-6} and 10^{-8} for both bacterial and fungal plates. A preliminary plating study involving two of the soil extracts was conducted prior to the main plating experiment, in order to determine the appropriate dilution factors.

After inoculation, the plates were incubated at 25°C for the duration of the BiologTM experiment (13 days). All plates were counted daily, and at completion of the incubation, a visual assessment of culturable colony “diversity” was made. Finally, at this stage, most colony “types” were gram stained and observed under microscope in order to partially classify the types of dominant colonies.

7.2.5 Carbon in Soil Solution

The organic carbon content of extracts used in BiologTM inoculation were measured. The soils were re-extracted to produce a solution presumed to be identical to that used to inoculate the BiologTM plates. This extract (0.85% w/v NaCl) was then analysed (undiluted) for total organic and total inorganic carbon contents using a LECO TOC auto analyser (model TOC-5000). The resulting values were used to calculate carbon input to the BiologTM wells from soil extract.

Saline BiologTM soil extracts were also analysed for total C content using an automated LECO combustion analyser (1050 °C; model FP-2000). Liquid extracts (approximately 2.5 mL), was added to LECO brand “com-aid” powder, to prevent loss of liquid during combustion. Total C values for each sample were then compared to TOC analysis values in order to test the reliability of the method. Due to the low C concentrations in the saline soil extracts, and the relatively high C sensitivity range of the LECO combustion analyser, samples were concentrated (10:1 concentration) by freeze-drying at -30°C, and reconstituting in deionised water. This brought the assay sample C values within the measurable range of the LECO combustion analyser.

7.2.6 Estimation of Biologically Respirable C Contained in Saline Soil Extract

Further BiologTM plates were inoculated in order to determine the effect of varying soil extract carbon additions to BiologTM wells, and hence tetrazolium redox dye. Nine BiologTM GN type plates were inoculated with a 0.85% w/v NaCl solution, with a isolated single-strain inoculum. On this occasion, whole soil microbia were not used as the inoculum. Instead an isolated “wild-type” soil bacterium was used, species *Pseudomonas*. This bacterium was cultured from stock solution onto nutrient agar, then re-cultured. Fresh colonies were used to obtain a cell density of 3×10^2 cells/mL, calibrated using a spectrophotometer (590 nm). This solution was then diluted to the final inoculum density of 10^6 cells mL⁻¹. The blank plate was inoculated with sterile saline only.

To determine the effect of varying C levels in soil inoculum, each plate also had varying carbon additions, in the form of sterile glucose. Each plate received a different carbon concentration, ranging from 0 to 330 ppm C. This range was selected on the basis of results from soil extract C analysis, which coincide with this range. It should be noted at this point that initial cell density for this experiment was selected on the basis of results of the plating of “whole soil inoculum”, which suggested a mean cell density of 10^6 cells mL⁻¹ (refer plating studies).

BiologTM plates were inoculated with one of the following carbon concentrations (in the form of glucose, C₆H₁₂O₆): 0, 6.7, 13.3, 33.3, 66.7, 100, 133.3, 200 and 330 ppm C. All plates, once inoculated, were again incubated at 26°C, and colour development in the wells read at regular intervals on a Dynatech Elisa MR 5000 microplate reader at a wave length of 590 nm.

7.2.7 Carbon Content of BiologTM Microtitre Plate Substrates

In order to fully examine the influences of microbial growth patterns, and understand the implications therein, all supplied carbon energy sources were considered. In the BiologTM microtitre technique, carbon was supplied from two sources - the soil extract, and the BiologTM supplied carbon substrate. Therefore it was important to estimate not

only the “degradability” of the sources supplied, but also the quantities of carbon supplied from each source, for C mass balance purposes. Selected substrates from the “GN” type BiologTM microtitre plate were individually removed and washed from the microtitre plate, and made to volume. Total carbon content was measured by the method of LECO TOC analysis.

7.3 RESULTS AND DISCUSSION

7.3.1 Colour Development in Control Well Inoculated with Soil Extract

In order to analyse the patterns of absorbance change (Figures 7.1 and 7.2) occurring with saline extracts from different soils, the mean maximum absorbance (Abs_{max}) was estimated for each soil extract. This was achieved by best fitting the data to sigmoidal or exponential growth functions. All fits achieved coefficients of variation that exceeded 0.970 (refer Appendix 7.1). Differences in absorbance values across soils was statistically assessed using Duncan and Tukey (ANOVA) multiple comparison procedures. Abs_{max} values (Figures 7.1 and 7.2) in the control well were strongly influenced ($P < 0.001$) by different soils and by moistening and incubation in the glasshouse (glasshouse moistened). Overall, glasshouse moistened soils (Figure 7.2) produced significantly higher Abs_{max} values than their field-dry counterparts. A significant conditioning by soil interaction was also observed ($P < 0.038$).

Abs_{max} also varied significantly with soil type across both field-dry ($P < 0.001$) soils (Figure 7.1), and to a lesser degree on glasshouse-moistened ($P < 0.061$) soils (Figure 7.2). At the 5% significance level, soil 1 had significantly higher Abs_{max} values than other field-dry soils. In contrast, soils 9, 3 and 4 had significantly lower colour development compared with other soils. This suggests that the extract from field-dry soils 9 and 4 contained less useable C than extracts from other soils, especially soil 1. The amount of useable C in a soil extract appeared to be a variable characteristic because, after moistening in the glasshouse, the influence of different soils, on control well Abs_{max} values, altered. Glasshouse-moistened soils 3 and 6 produced significantly higher Abs_{max} values compared with the other soils, while soil 10 yielded a significantly lower Abs_{max} (compare plateaux in Figures 7.1 and 7.2).

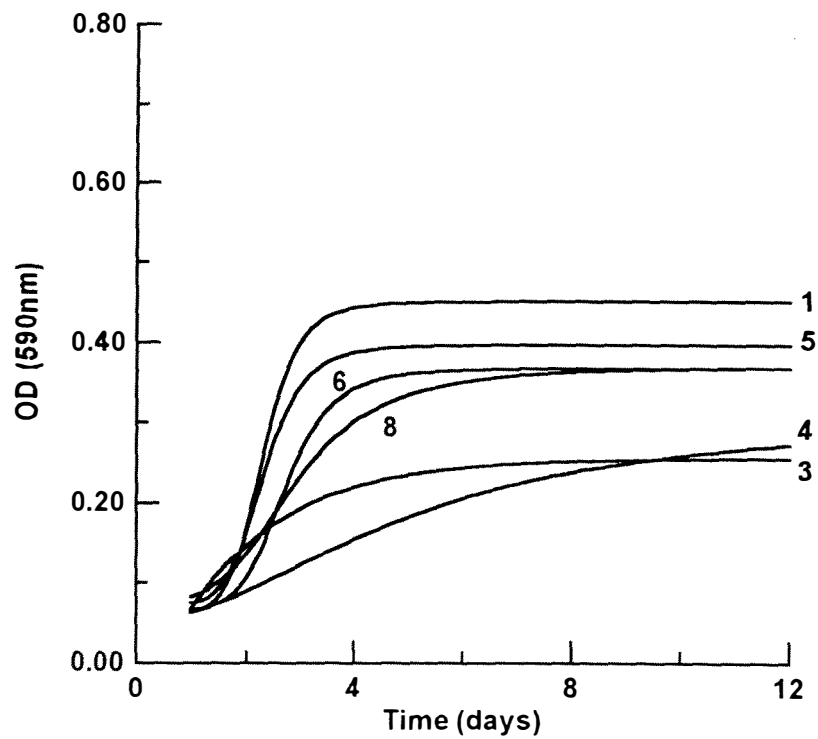


Figure 7.1A The pattern of background absorbance (590nm) over time of BiologTM microtitre plate inoculated assays with extracts from field-dry soils 1-8.

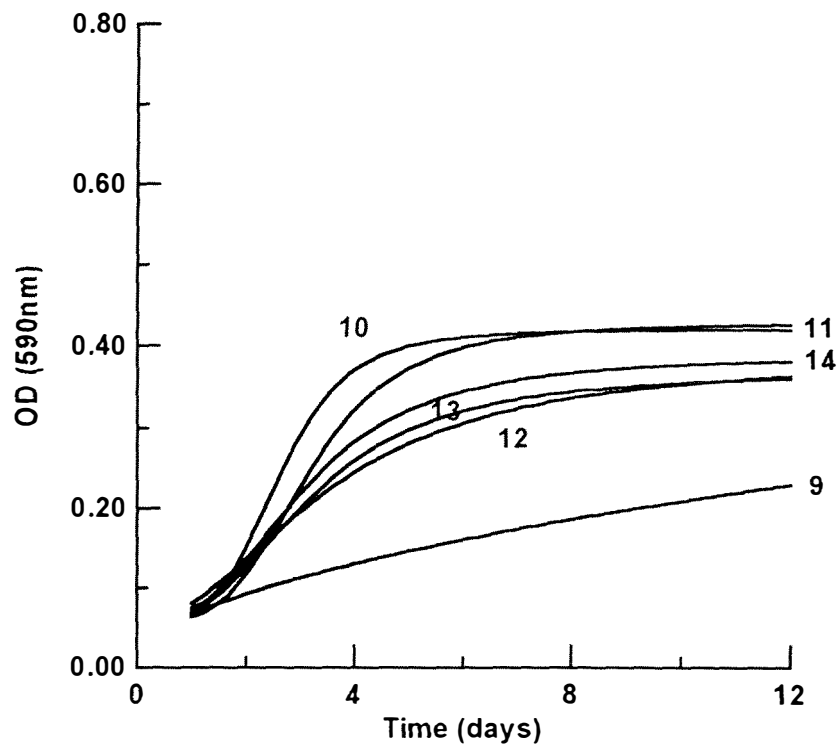


Figure 7.1B The pattern of background absorbance (590nm) over time of BiologTM microtitre plate inoculated assays with extracts from field-dry soils 9-14. Absorbance values are mean values of three replicate extractions.

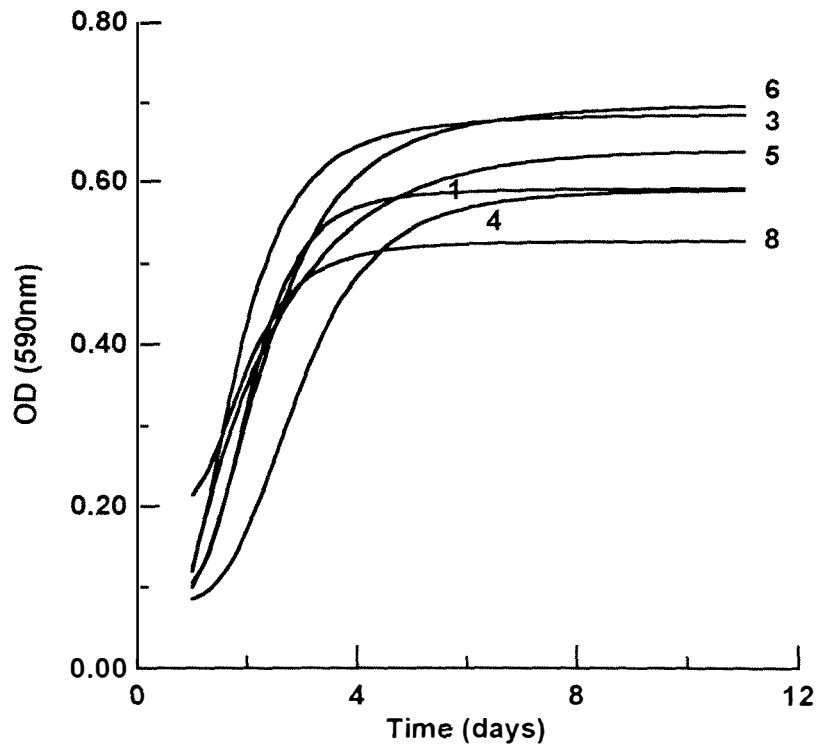


Figure 7.2A The pattern of background absorbance (590nm) over time of Biolog[™] microtitre plate inoculated assays with extracts from glasshouse-moistened soils 1-8.

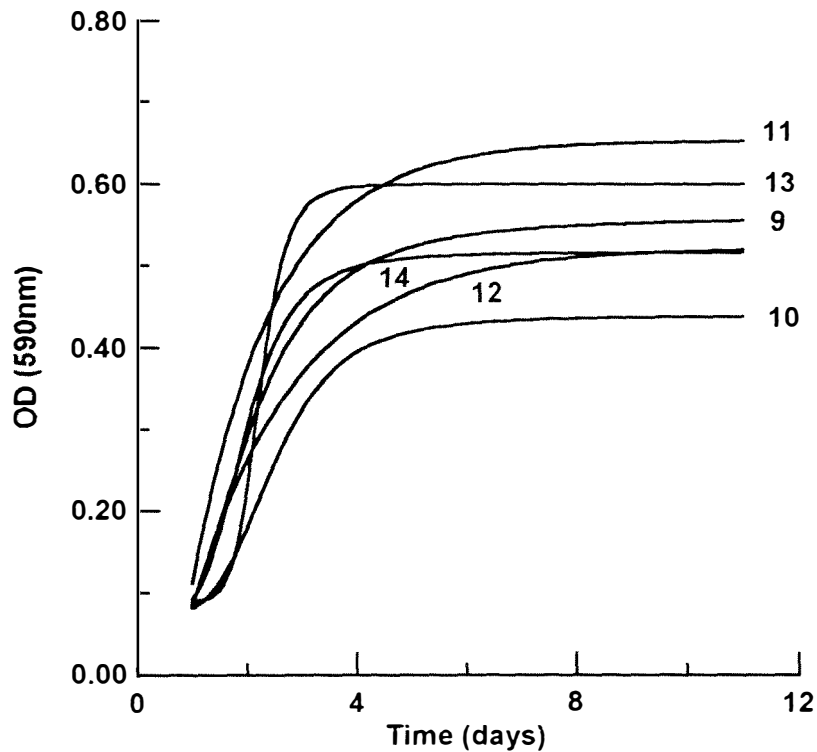


Figure 7.2B The pattern of background absorbance (590nm) over time of Biolog[™] microtitre plate inoculated assays with extracts from glasshouse-moistened soils 9-14. Absorbance values are mean values of three replicate extractions.

7.3.2 Factors Likely to Affect Absorbance in the Control Well Inoculated with Soil Extract

7.3.2.1 Influence of Colour of Soil Extract on Control Well Absorbance

The absorbance (590nm) of empty BiologTM plates were measured on a scanning plate-reader. Several replicates gave mean absorbance values of 0.03 for a blank plate (no substrate). This value is either a function of plate “clarity”, or the lower limit of the Elisa plate reader, or both.

The absorbances (590nm) of BiologTM plates immediately after inoculation with soil extracts (T_0) were measured on a scanning plate-reader. Four replicates gave mean absorbance values ranging from 0.05 to 0.12 (Table 7.2) across all soils. It was expected that water-soluble soil OM and fine (<10 μ m) colloidal material (including clay and microorganisms) would contribute to these values. These initial absorbance levels were low, and for this reason, the impact on most Abs_{max} values may be minor. However, where Abs_{max} is low, absorbance at T_0 may be influential. A soil extract absorbance value of 0.07 represents 10% of the background Abs_{max} for glasshouse-moistened soil (6), and 35% of the background Abs_{max} for field-dry soil (9) of those examined. For future reference it was noted that an absorbance of 0.07 would represent < 3% of Abs_{max} of soil inoculum cultured with some BiologTM substrates (See Chapter 8).

Mean initial absorbances values for saline extracts were a little higher, and more variable, on extracts from the glasshouse moistened soils than those from the field-dry soils. For the glasshouse moistened soils the initial absorbance represented 20% or more of Abs_{max} values. Possible explanations include a higher initial cell count, or that more colloidal mineral material passed through the filter paper at the filtration step during extraction, or the organic/inorganic compounds extracted from these soils differ in colour from their field-dry (moist) counterparts (i.e. decomposition products have more colour, or pH decrease under decomposition solubilizes more Fe containing compounds). It is possible that organo-metal complexes binding soil mineral particles degraded to some extent during the wetting-up cycle (Kieft et al., 1987), causing aggregates to be less stable, and fines (clay-sized particles) to be more mobile. A reduction in extractable / soluble (TOC)

C in the soils during incubation indicates that some C mineralisation occurred, and supports such a scenario (see Section 7.3.2.2). Such degradation may also influence the nature of soil organic C in soil extracts, and, in turn, will influence microbial growth in the BiologTM wells.

Table 7.2 Absorbance (590nm) of 0.85% w/v NaCl soil extracts at time of extraction (T₀). Absorbance values are means of four replicates.

Mean absorbance		
Soil	Field-Dry	Glasshouse Moistened
1	0.051	0.056
3	0.050	0.063
4	0.050	0.073
5	0.071	0.122
6	0.055	0.098
8	0.057	0.087
9	0.060	0.105
10	0.076	0.085
11	0.066	0.124
12	0.067	0.097
13	0.053	0.051
14	0.064	0.075

7.3.2.2 Influence of Carbon Input from Saline Extracts of Soil.

The interpretation of substrate use patterns using BiologTM is complicated by the contents of the soil extract inoculum. The weak saline soil extract inocula from different soils add not only a diverse range of functional microbes (bacteria, fungi, protozoa, algae) to the BiologTM plate, but also a diverse range of nutrients and carbon from which

these organisms can derive energy for growth. To accurately interpret substrate use patterns from a BiologTM plate, there must be clear information about the concentration and availability of carbon energy sources supplied in the inoculum prepared from the soil. Using whole soil community inoculum, there are two separate carbon sources, carbon substrate of a single compound type, supplied with the BiologTM plate, and secondly, a wide range of soil carbon compounds, which would be expected to vary in quantity, and ease of degradability. The total C contents of soil extract inocula are presented in Table 7.3A and 7.3B.

7.3.2.2.1 Total carbon content of saline extracts of soil

Total C content of soil extracts were measured by method of LECO TOC analysis, and also by LECO combustion (Table 7.3). For field-dry soils, the TOC analyser produced slightly lower results than the combustion method (Table 7.3A). For the glasshouse-moistened soils, the C concentrations measured in the saline extracts by LECO combustion were much lower than values produced by TOC. Soil samples used for the LECO combustion method were stored at 4°C in a refrigerator for 4 weeks after the TOC analysis. The longer storage is believed to be the factor causing the marked differences in C concentration. For comparative purposes, TOC determined carbons are used hereafter.

The total carbon concentration in saline extracts of field-dry soils ranged from 344-745 μgCg^{-1} (Figure 7.3). Soils with low values (335-452 μgCg^{-1}) included sites 4 and 11 (low fertility), while sites 5 and 12 had high values. Total nitrogen concentrations in saline extracts ranged from 56-187 μgNg^{-1} (See Appendix 7.2, Table 7.3^A). High values were associated with high extract carbon concentrations. However, the C:N range of the extract did vary somewhat, where sites 10, 13 and 14 had comparatively higher ratios, due to lower nitrogen contents.

Extract carbon levels of glasshouse-moistened soils were much lower than for field-dry soils, ranging from 291-417 μgCg^{-1} . This decrease in soluble carbon concentration was probably caused by increased microbial growth and respiration after the soils were wetted and incubated in the glasshouse. Soils with low values included sites 4 and 13, while sites 10 and 11 had higher values. Nitrogen concentrations in saline extracts

ranged from 17-88 μgNg^{-1} , a marked reduction from the dry soil concentration. The C:N ratios of glasshouse-moistened saline soil extracts (1.3-5.7) were lower than for field-dry soils (4.0-10.3), indicating a greater relative decrease in saline extractable carbon.

For field-dry soils, TOC content of saline extracts was linearly related to the initial (T_0) absorbance at 590nm (Figure 7.4A). This relationship was not apparent for glasshouse-moistened soil extracts (Figure 7.4B). Initial absorbances from field-dry soils were low ($\text{Abs} < 0.1$), and suggest that the Abs at T_0 is a product of the dark colour associated with Fe-associated soil-based organic compounds. Lower TOC values, and a narrower range of TOC values, in the glasshouse-moistened soils may explain why no relationship exists.

Table 7.3A and B Total carbon contents of soil saline extracts for field-dry and glasshouse-moistened soils, by methabsorbance of LECO and Total Organic Carbon (TOC) Analyser (μgCg^{-1} soil, oven-dry basis).

Table 7.3A Field-dry soils

Soil From Site:	TOC			LECO		
	μgCg^{-1} soil	μgCmL^{-1} extract	μgC^{-1} well	μgCg^{-1} soil	$\mu\text{gC/mL}^{-1}$ extract	μgC^{-1} well
1	359.1	35.9	5.39	539.0	53.9	8.09
3	413.0	41.3	6.19	578.2	57.8	8.67
4	334.9	33.5	5.02	344.2	34.4	5.16
5	665.2	66.5	9.98	702.4	70.2	10.54
6	418.5	41.8	6.28	539.4	53.9	8.09
8	678.5	67.8	10.18	687.7	68.8	10.32
9	589.5	59.0	8.84	604.4	60.4	9.07
10	669.1	66.9	10.04	535.8	53.6	8.04
11	508.2	50.8	7.62	445.1	44.5	6.68
12	738.8	73.9	11.08	745.3	74.5	11.18
13	402.3	40.2	6.03	572.8	57.3	8.59
14	451.5	45.1	6.77	543.7	54.4	8.16

Table 7.3B Glasshouse-moistened soils

	TOC			LECO		
Site	μgCg^{-1} soil	μgCmL^{-1} extract	μgC^{-1} well	μgCg^{-1} soil	μgCmL^{-1} extract	μgC^{-1} well
1	340.0	34.0	5.10	109.5	10.9	1.64
3	319.8	32.0	4.80	126.2	12.6	1.89
4	291.4	29.1	4.37	139.3	13.9	2.09
5	362.2	36.2	5.43	155.9	15.6	2.34
6	310.0	31.0	4.65	67.9	6.8	1.02
8	378.0	37.8	5.67	201.9	20.2	3.03
9	368.3	36.8	5.52	130.0	13.0	1.95
10	406.2	40.6	6.09	113.9	11.4	1.71
11	417.1	41.7	6.26	185.8	18.6	2.79
12	376.1	37.6	5.64	136.7	13.7	2.05
13	307.8	30.8	4.62	91.2	9.1	1.37
14	336.0	33.6	5.04	156.3	15.6	2.34

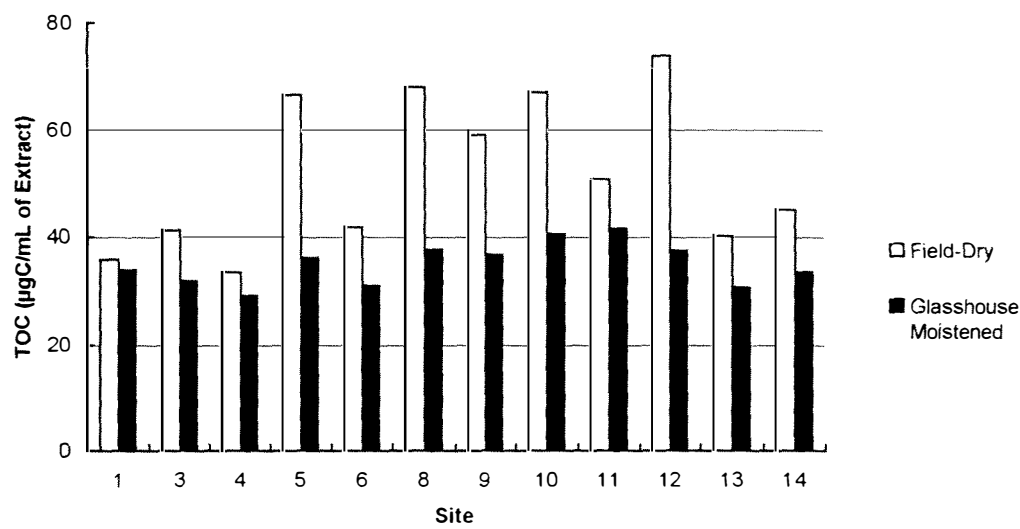


Figure 7.3 TOC contents of all soils

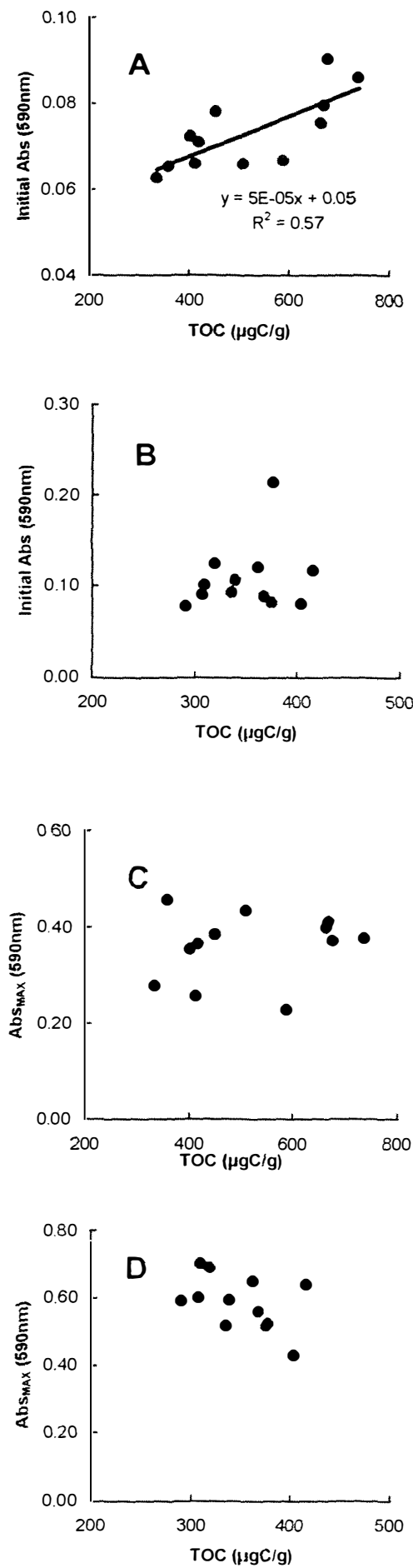


Figure 7.4 The relationship between initial absorbance (Abs) and C concentration of saline soil extracts from **A** field-dry, and **B** glasshouse-moistened soils; and the relationship between Abs_{max} and C concentration of saline soil extracts from **C** field-dry, and **D**, glasshouse-moistened soils.

When considering the maximum absorbance values for each soil, there was no relationship between Abs_{max} and TOC content of saline soil extracts for both field-dry (Figure 7.4C), and glasshouse-moistened (Figure 7.4D) soils. One explanation for this result may be that not all TOC supplied in the soil extract is available for microbial growth (reduction activity). This raises the question of how absorbance at 590 nm responds to available C. This question has been addressed in the following Section by examining microbial growth patterns under differing regimes of carbon (glucose) addition.

7.3.2.2.2 Bioassay to determine bio-available C content of saline soil extract

Calibration curves for examining the relationship between absorbance at 590 nm in BiologTM wells and available C in the inocula are developed in this section. Following this, these calibration curves are used to interpret available C content of soil extracts.

Two bioassays were conducted. The first used a pure single-species (*Pseudomonas fluorescens*) culture. The advantages of such an assay are that it is simple, and has no complicating background effects such as variable C content, and variable coloration. However, such an assay may exclude some important inter-species effects that may occur in a mixed population. The second bioassay used a mixed culture of soil inoculum. This provided a comparison with the first bioassay, in order to examine the background effect of soil extract colour and any interactions between soil C and added single C substrates. Inoculum from soil 8 was used because in later studies (Chapter 8) inocula from this soil were shown to have the ability to utilise a wide range of BiologTM C substrates, perhaps indicating a diverse range of microbial species, capable of vigorous growth.

This bio-assay uses a single bacterium species found commonly in soil species (*Pseudomonas fluorescens*) and an easily-degraded C energy source (glucose) of varying concentrations (the range of which is based on the general range of extracted soil soluble C levels). The available C versus absorbance calibration curves are presented in Figures 7.5A and 7.5B. Both the pseudomonas and the soil derived inoculum (Figure 7.5A) produce straight line relationships between available C and absorbance. The mixed population (slope 0.0202 Abs/ μ gC, Figure 7.5B) were slightly more efficient at turning increases in C into increases in absorbance than the pseudomonas culture (slope 0.013 Abs/ μ gC, Figure 7.5A).

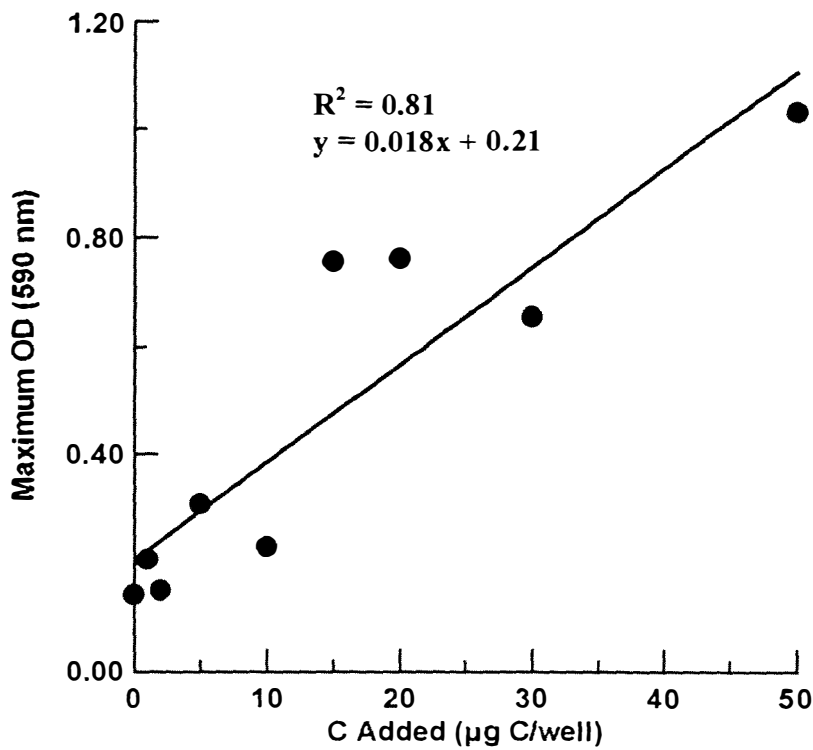


Figure 7.5A Bioassay Standard Curve - Maximum absorbance with Increasing concentration, single species (WT) inoculum.

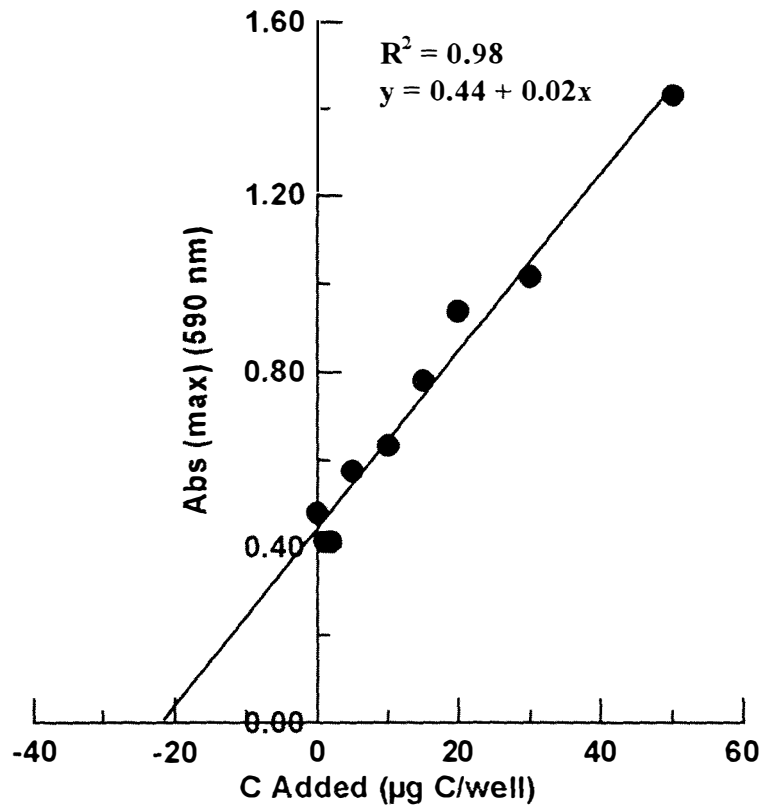


Figure 7.5B Bioassay Standard Curve - Maximum absorbance with Increasing C concentration, using a (whole community) saline extract inoculum from soil number 8.

Using the glucose standard curve generated in the mixed soil culture bio-assay, the quantity of readily available C in the soil extracts was estimated. Soil extract C is low, e.g. 60 ppm C, and because C input to the BiologTM well will be accordingly low (<10 µgC/well), then all soil extract C values fall at the low range of the standard curve produced in our bio-assay experiment. This may compromise the accuracy of estimating the available C fraction of total C in soil solution from such a curve.

Each soil extract will have a different background absorbance at 590 nm due to soil extract colour. The following relationships have been used to calculate available C in soil extract. Available C values for field-dry and glasshouse-moistened soils are presented in Figure 7.6.

Corrected absorbance_{max} = Soil Extract absorbance_{max} - Soil T₀ absorbance (7.1)

Biologically Available C in soil extract = Corrected absorbance_{max} / 0.0202 (7.2)
(slope, glucose soil inoculum std curve)

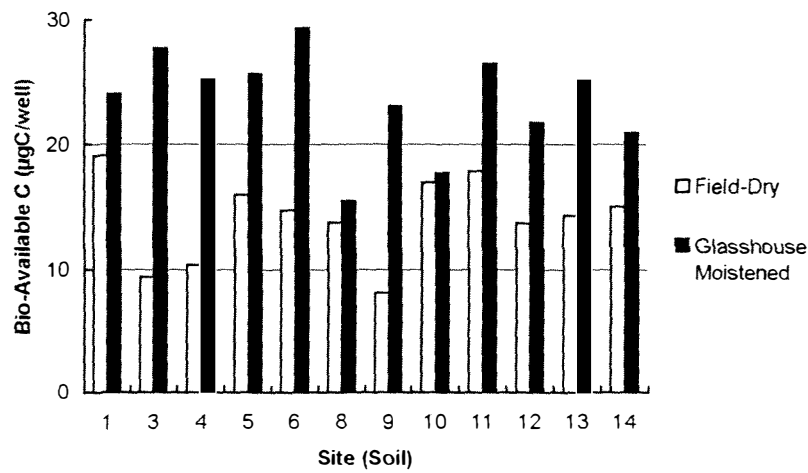


Figure 7.6 Estimated bio-available C in saline soil extracts from field-dry and glasshouse moistened soils.

Using equation 7.2, bio-available C levels ranged from 7.9-19.4 µgC/well in field-dry soils, and from 16.3-28.8 µgC/well in glasshouse-moistened soils. These values are

considerably higher than the total C values obtained by TOC measurement, or by LECO combustion of soil extract. This result is a surprising one, and difficult to explain.

There are several possible reasons for such a result. Experimental error may have resulted in an underestimation of available C in the saline soil extract. Errors may have occurred in TOC/LECO C measurement, or perhaps C levels in the extract were so low that they are beyond accurate sensitivity ranges of a credible standard curve. This is possible, although is a less likely explanation. It appears that C levels of 3-5 $\mu\text{C}/\text{well}$ are well within sensitivity range of instruments, and BiologTM plate sensitivity, and at this level, are at least equal to the lowest soil extract C levels measured by TOC or LECO methods.

Also, glucose may be used less efficiently to produce NADH than TOC in saline soil extracts or conversely, soil C may contain a diverse range of C compounds which lend themselves to efficient microbial growth. This would explain why there is more absorbance per unit C on soil extract C cf. glucose C. There is some evidence to support such a theory (Saier, 1989).

Alternatively, the extract C content varied over time between the TOC and available C assay. The TOC and LECO values presented in this Chapter were from soil extracts taken one or two days after the BiologTM plate inoculation. They were not the same extract, but a extract produced one day later, using the same soil (stored in a chiller at 4°C), and using an identical extraction process. It is possible that the quantity of C in the soil extract may be variable, depending on which point in time the extraction is made. This variation in extract C content is evident if the TOC values of Soil 8, the soil used for the low C bio-assay standard calibration curve are compared. At the time of extraction for the low-C bioassay experiment, the TOC for this soil extract was 63.1 ppm C (9.5 $\mu\text{gC}/\text{well}$). However, when the same soil was extracted in the same way several weeks prior, the TOC in extract was only 37.8 ppm C (5.7 $\mu\text{gC}/\text{well}$). Because extraction procedures were identical for both extractions, this variability can only be attributed to changes in soil attributes over storage time.

Therefore, in future experiments, it is suggested that C measurements on soil extracts be made on the same extract which is used to inoculate the BiologTM plate. This may prove

to be critical where the soil extract C, and its influence on microbial growth in the plate, are of interest to the researcher. Further investigation in this area is required in order to fully explain this result, and increase the understanding of the influence of soil soluble C on microbial growth in the BiologTM plate.

7.3.2.2.3 Other factors related to available C concentrations in soil extracts

The relationships between available C in saline extracts of soil and various soil indices e.g. Olsen P, mineralisable N, CFUs were examined. The amount of available C in the extract was positively related to CFUs in soil extracts and available C in the field-dry soils (Figure 7.7). This may indicate that much of the available C was microbial C. No such relationship was apparent where glasshouse-moistened soil extracts were used. No relationships existed between the amount of available C in saline extracts and to Olsen P or mineralisable N values for each soil.

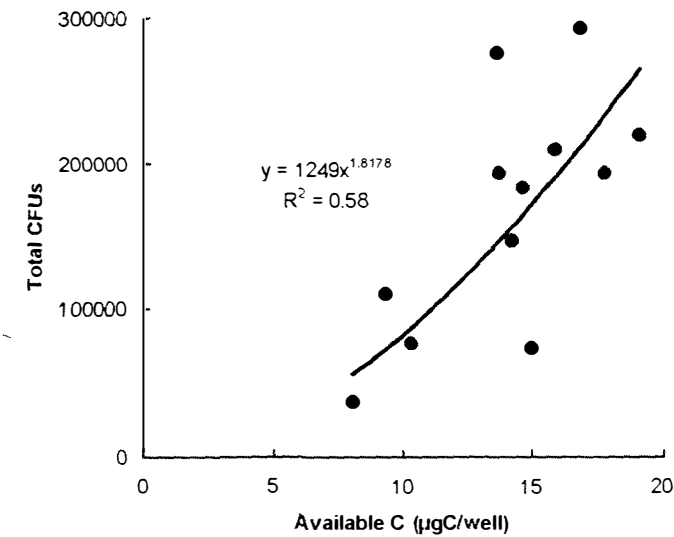


Figure 7.7 The relationship between estimated bio-available C in saline soil extract, and soil-extracted CFUs, for field-dry soils

7.3.2.2.4

Mass balance of C input to BiologTM wells

Carbon compounds available for microbial growth in the microtitre plate wells originate from one of two sources, or both. These sources are:

- (i) The carbon substrate supplied with the BiologTM microtitre plate (single compound)
- (ii) The carbon compounds present in the saline soil extract

There is also a third C source, which is the tetrazolium redox dye. This contributes around 60µg of C to each BiologTM well. However, this carbon source is not available to microbes as an energy source, and so does not influence microbial growth. It is possible, however, that if enough growth occurs in the well, there may be some potential for the redox dye source to be exhausted, which would in turn influence the maximum absorbance level in the well. Such a scenario is unlikely.

In order to assess the nature and pattern of microbial growth in a BiologTM well, it is important to examine the relative contribution that C in the soil saline extract makes to the total amount of C available for growth. This involves measuring not only the total C input from both soil extract and BiologTM growth medium, but also an assessment of the potential degradability of these C sources, or their viability as an energy source that soil microbes can effectively utilise.

The total C input from both soil extract, and BiologTM microtitre plate carbon sources were measured. Total C contents of the saline soil extracts are presented in tables 7.3A and 7.3B, and ranged from 291-739 µgCg soil⁻¹, or 29-74 µC mL⁻¹ (LECO C = 68-745 µgCg soil⁻¹, or 7-75 µC mL⁻¹) of saline extract. A mean value of mean value is around 44ppm C, or an input of 6.6 µgC/well. Since 150 µL of this extract is used to inoculate a BiologTM well, this C concentration range represents a total C input of only 1-11 µgC per well.

A selection of the 96 C substrates were removed from the microtitre plate, and their C contents measured in a TOC analyser. The results are presented in Table 7.3. Values ranged from 72 to 380 µgC per well or 12-320 µgC per well if the contribution made by the dye is removed. A representative value being around 230 µgC per well. Therefore an estimated total C input (substrate + soil extract) to the BiologTM well would be (12-

320) + (1-11) = (13-331) $\mu\text{gC/well}$, of which 0.3-3.3 % of C can be sourced from the soil extract. Given the range of C contributions made by different soil extracts and the significant amount of growth occurring on this carbon (see Figures 7.1 and 7.2), it is likely that the amount of mineralisable C in the inoculum will influence the BiologTM plate substrate use pattern.

Table 7.4 Carbon contents of selected BiologTM substrates, total C ($\mu\text{gC/well}$)

Well Code	Compound	Formula	Total C $\mu\text{gC well}^{-1}$
A3	dextrin	$\text{C}_6\text{H}_{10}\text{O}_5$	276.4
A5	tween 40		202.3
B1	i-erythritol	$\text{C}_4\text{H}_{10}\text{O}_4$	380.5
B3	L-fucose	$\text{C}_6\text{H}_{12}\text{O}_5$	332.6
B5	gentiobiose	$\text{C}_{12}\text{H}_{22}\text{O}_{11}$	229
C1	D-melibiose	$\text{C}_{12}\text{H}_{22}\text{O}_{11}$	238.7
C3	D-psicose	$\text{C}_6\text{H}_{12}\text{O}_6$	294.5
C5	L-rhamnose	$\text{C}_6\text{H}_{12}\text{O}_5$	259.1
D1	acetic acid	$\text{C}_2\text{H}_4\text{O}_2$	72
D3	citric acid	$\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$	219.8
D5	D-galactonic acid lactone		191.1
E1	p-hydroxyphenylacetic acid	$\text{C}_8\text{H}_8\text{O}_3$	231.8
E3	α -ketobutyric acid		208.4
F1	bromosuccinic acid	$\text{C}_4\text{H}_5\text{BrO}_4$	132.8
F3	glucuronamide		262.5
G1	L-histidine	$\text{C}_6\text{H}_9\text{N}_3\text{O}_2$	154.2
G3	L-leucine	$\text{C}_6\text{H}_{13}\text{NO}_2$	302.9
H1	urocanic acid	$\text{C}_6\text{H}_6\text{N}_2\text{O}_2$	205.8
H3	uridine	$\text{C}_9\text{H}_{12}\text{N}_2\text{O}_6$	200.6

For example, microbial growth on available C from the soil extract provides energy and allows time for adaptive hydrolytic enzymes to be produced that would allow a positive result for a carbon source otherwise not readily degraded. Variation in soil condition, influencing extractable C contents, could have a marked effect on the substrate use pattern and conclusions drawn on the functional diversity of a soils microbial population. This is investigated in more detail in Chapter 8.

7.3.3 Changes in Microbial Population

Differences in numbers and types of micro-organisms in the soil saline extracts may be an additional factor influencing the absorbance values produced by different soil inocula in the BiologTM assay. Dilution plate cultures of saline extracts were established on nutrient agar media to evaluate this possibility.

Final agar plate counts for field-dry and glasshouse-moistened soils are given in table 7.5. Total counts ranged from 37 (soil 9) to 293 (soil 12) colonies per plate. In general, bacterial counts were much higher than fungal counts, which may have been influenced by the dry nature of the soil when taken from the field. The ratio of bacterial: fungal colonies ranged widely across soils, mostly due to variation in total counts, and in some instances, low fungal counts.

An index of visual species diversity was also made. The following criteria was used to assess microbial species diversity:

D = Diverse (>4 different colony types)

L = Low Diversity (2-4 different colony types)

S = Single or bi-colony types

Some soils had more diverse culturable species than others. For example, Soils 1 and 6 had high total counts, but low bacterial and fungal diversity. In contrast, site 10 had high counts and high diversity, while site 4 had low counts and high diversity. Site 9 had both low diversity and low colony counts. Total colony counts were linked with soil fertility status (higher mineralisable N levels), with higher counts observed on higher fertility soils (Figure 7.8D). However, the effect of fertility status on microbial diversity is unclear.

Table 7.5 Nutrient agar plate counts of bacteria and fungi colonies from whole soil inoculum (field-dry and glasshouse-moistened soils). Values quoted are cells mL⁻¹ (culturable-colonies) at 10⁻³ dilution.

Soil	Field-Dry Soils (final counts)					
	Bacteria	Fungi	Total	Ratio	Diversity Index	
					Bacteria	Fungi
1	206.7	13.3	220.0	15.5	L	S
3	103.3	6.7	110.0	15.5	L	S
4	63.3	13.3	76.7	4.8	D	L
5	186.7	23.3	210.0	8.0	D	L
6	180.0	3.3	183.3	54.0	L	S
8	186.7	6.7	193.3	28.0	L	S
9	30.0	6.7	36.7	4.5	L	L
10	276.7	16.7	293.3	16.6	D	D
11	186.7	6.7	193.3	28.0	D	S
12	263.3	13.3	276.7	19.8	D	L
13	143.3	3.3	146.7	43.0	D	S
14	70.0	3.3	73.3	21.0	D	S
Glasshouse-Moistened Soils (final counts)						
1	168.0	47.7	215.7	3.5	D	L
3	81.3	32.0	113.3	2.5	D	D
4	51.3	13.3	64.7	3.9	D	D
5	125.0	14.0	139.0	8.9	D	D
6	128.0	22.3	150.3	5.7	D	D
8	246.7	58.7	305.3	4.2	D	D
9	140.0	30.7	170.7	4.6	D	D
10	160.0	9.7	169.7	16.6	D	L
11	236.7	11.0	247.7	21.5	D	D
12	109.0	28.3	137.3	3.8	L	D
13	189.3	47.7	237.0	4.0	D	D
14	152.3	90.3	242.7	1.7	D	D

Total counts on glasshouse-moistened soils ranged from 65 (soil 4) to 305 (soil 8). Bacterial counts were generally high, but ranged from 51 (soil 4) to 247 (soil 1). Fungal counts ranged widely across soils, and in general numbers were consistently higher than those observed on field-dry soils. Values ranged from 10 (soil 11) to 90 (soil 14).

In contrast to the field-dry soils, glasshouse-moistened soils had a diverse range of both bacteria and fungal populations on most soils. Exceptions were soil 12, which had low bacterial diversity, and soils 1 and 10, which had low fungal diversity. Total counts were not related to soil fertility status measures such as soil Olsen P (Figure 7.8C), but were linearly related with mineralisable N levels (Figure 7.8D, $R^2 = 0.50$). In general, it appeared that on glasshouse-moistened soils, fungal counts increased with both soil fertility status, and increasing rainfall regime (climate region from whence they came)(Figure 7.9B). Because all soils were conditioned in the glasshouse, it is difficult to explain this result.

Statistical comparison of the site effect and soil condition treatments was performed using single or two-way ANOVA where appropriate, and Duncan's and Tukey's multiple comparison tests.

Culturable bacterial populations were measured in field-dry and glasshouse-moistened soils, and a statistical comparison made. Results showed no significant difference at the 5% significance level in bacterial numbers as a result of moistening / glasshouse conditioning of these soils.

However, at the same significance level, there was strong evidence ($P < 0.001$) to suggest that bacterial numbers varied across soils. Within moisture regimes (field-dry, glasshouse moistened), the significance levels for differences in bacterial numbers were $P < 0.006$, and $P < 0.002$ for field-dry and glasshouse-moistened soils respectively. Field-dry soils 10 and 12 had significantly higher, and soil 9 significantly lower, counts than all other soils. When moistened, soils 8 and 11 had significantly higher, and soils 4 and 3 significantly lower, counts than all other soils.

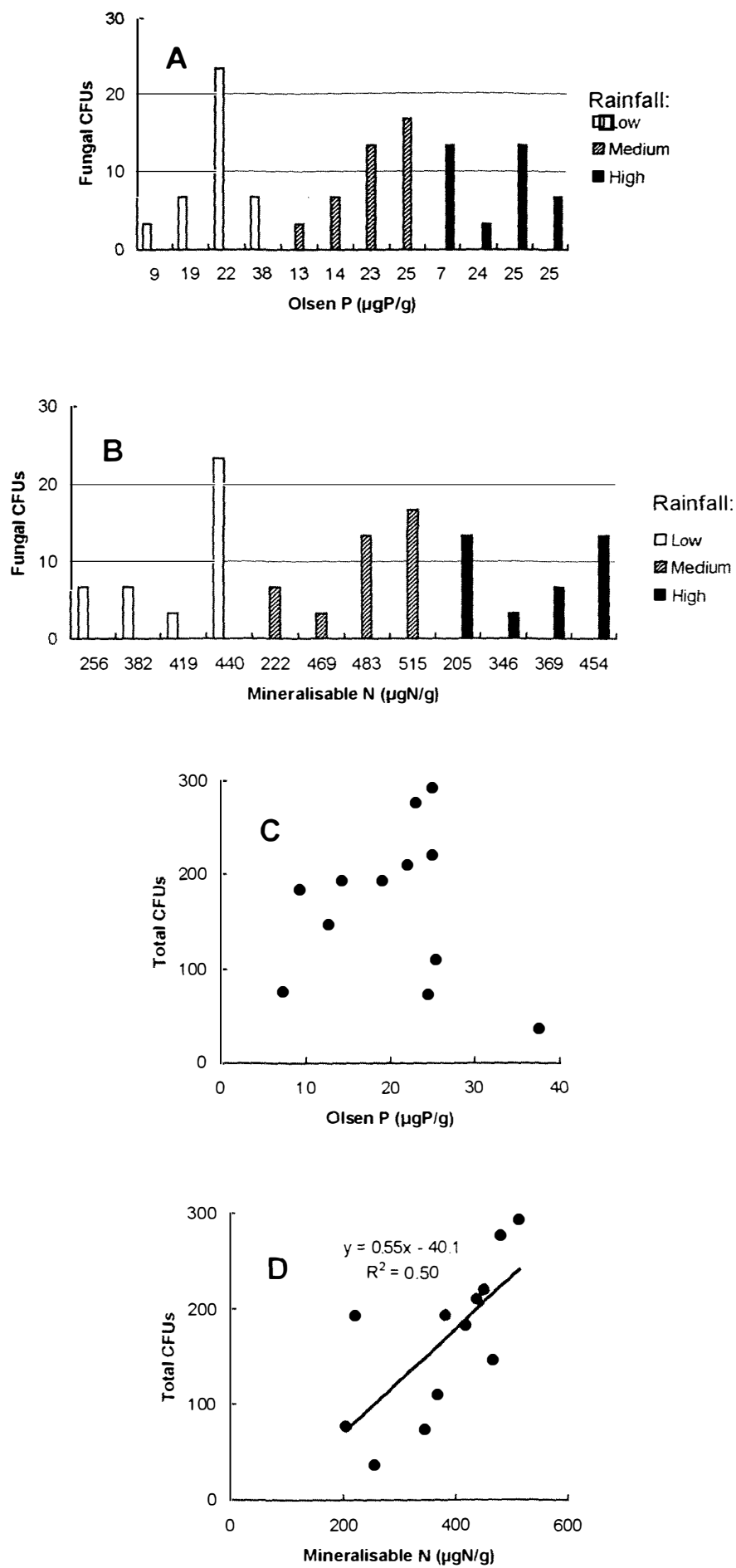


Figure 7.8 The relationship between fungal colony counts and **A** Olsen P, and **B** soil mineralisable N; and the relationship between total colony counts and **C** Olsen P, and **D** Mineralisable N, across all field-dry soils.

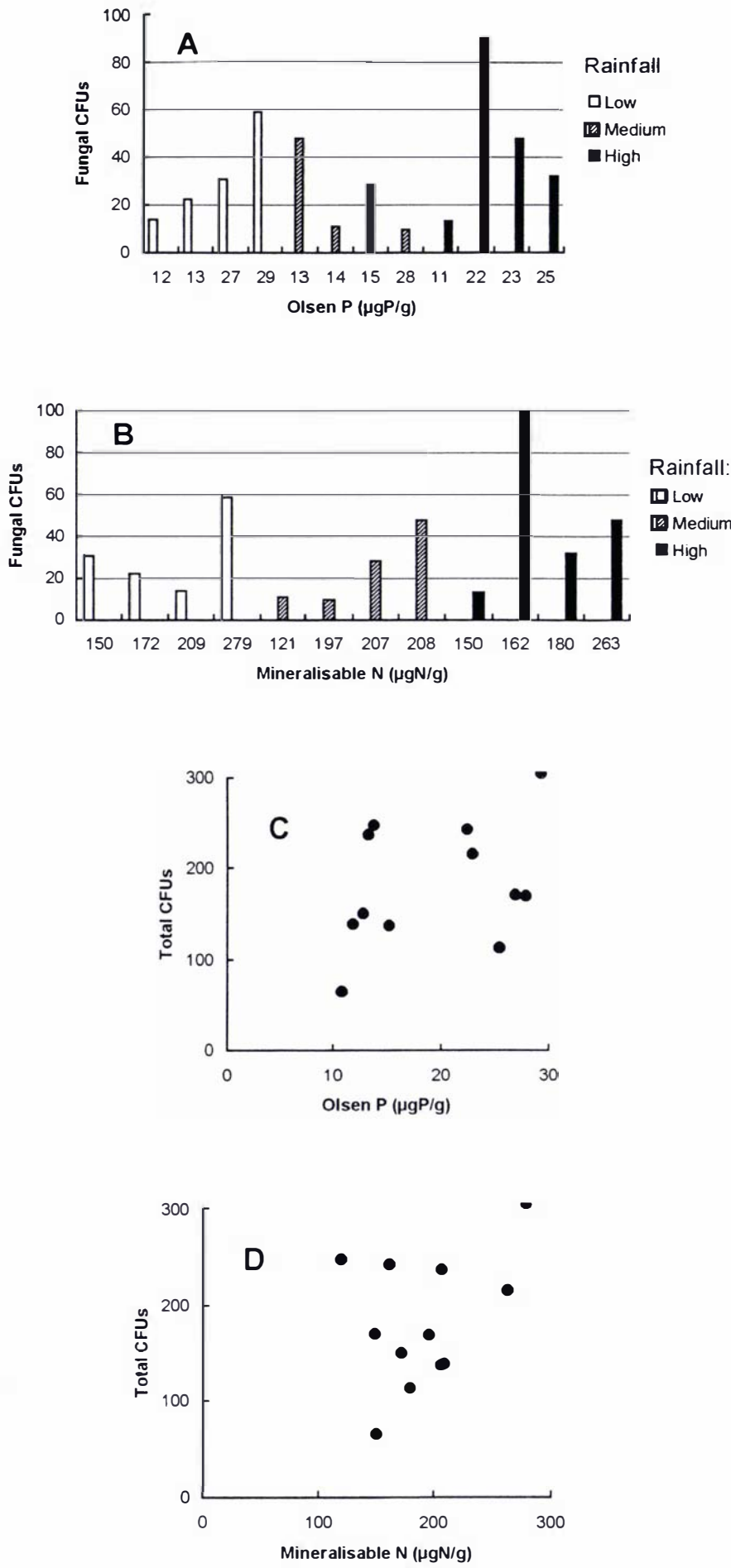


Figure 7.9 The relationship between fungal colony counts and **A** Olsen P, and **B** soil mineralisable N; and the relationship between total colony counts and **C** Olsen P, and **D** Mineralisable N, across all glasshouse moistened soils.

Soil conditioning also affected culturable fungal colony numbers ($P < 0.001$). Field dry soils did not have significantly different fungal counts, which, may in part, be explained by low numbers of colony forming units. Glasshouse moistened soils showed large variance in fungal numbers ($P < 0.001$). Of this group, soils 14 and 8 had significantly high counts, while soils 10, 11, 4 and 5 were significantly low. The relationship between bacterial: fungal ratio in all soils was unclear. The variation in bacterial counts may be responsible for different initial rates of absorbance development in the BiologTM assays.

Bardgett et al. (1999) reported higher microbial and fungal activity on unfertilised grasslands cf. intensively managed, fertilised systems. In this study higher fungal activity was not linked to the lower fertility pasture soil. Whereas associations between microbial population and pastoral soil fertility were not evident, it is clear that changes in microbial populations with soil moisture condition could be responsible for differences in the C content of soil extracts used for BiologTM assays and temporal differences in the substrate use patterns of the same soil.

7.4 CONCLUSIONS

Saline extracts of different fertility status pasture soils used for BiologTM microtitre plate assay inoculation contain significant amounts of readily available carbon causing high absorbance readings in all cells. To interpret the substrate use pattern during a BiologTM assay, this effect must be corrected for. Bioassays with known glucose C input showed that tetrazolium dye reduction was linearly related to available C content of the inoculum. A useful available C bioassay for waters and soil extracts may be developed using tetrazolium dye reduction in microtitre plates.

The amount of dye reduction, indicated by control well absorbance, was not associated with the total carbon content of the soil saline extracts or the number of colony forming micro-organisms in the extract. Changes in soil moisture condition markedly affected the control well absorbance, the carbon content of the saline extracts, and the ratio of fungal:bacterial colony forming units in the extract. No relationships were found between these measurements that could be used to explain control well absorbance values and therefore allow an across soil correction procedure to be developed.

It is highly probable that temporal differences in the amounts of available C in inocula derived from soils will influence the substrate use pattern on BiologTM plates. Therefore the interpretations about soil microbial functional diversity drawn from the substrate use pattern should be treated with caution.

The C bioassay studies indicated that a useful available C bioassay for waters and soil extracts could be developed using tetrazolium dye reduction in microtitre plates. Future research on available C bioassays of soil water and extracts should consider the temporal variation of solution carbon concentration in moist soils. Bioassays and solution carbon concentrations should be measured concurrently on the same sample.

7.5 REFERENCES

- Anderson, J.P.E. and Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry*, 10: 215-221.
- Bardgett, R.D., Lovell, R.D., Hobbs, P.J. and Jarvis, S.C., 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. *Soil Biology and Biochemistry*, 31: 1021-1030.
- Blakemore, L.C., 1964. Effect of adsorbed sulphate on the determination of total exchangeable bases in soils. *New Zealand Journal of Agricultural Research*, 7: 435-438.
- Blakemore, L.C., Searle, P.L. and Daly, B.K., 1987. Methods for chemical analysis of soils. Scientific Report 80, New Zealand Soil Science Bureau, Lower Hutt, New Zealand.
- Bochner, B., 1989. Breathprints at the microbial level. *ASM News*, 55: 536-539.
- Bremner, J.M. and Tabatabai, M.A., 1971. Use of automated combustion techniques for total carbon, total nitrogen and total sulphur analysis of soils. In: L.M. Walsh (Editor), *Instrumental methods for analysis of soils and plant tissue*. Soil Science Society of America, Madison.

- Campbell, C.D., Grayston, S.J. and Hirst, D.J., 1997. Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. *Journal of Microbial Methods*, 30: 33-41.
- Degens, B.P., 1998a. Decreases in microbial functional diversity do not result in corresponding changes in decomposition under different moisture conditions. *Soil Biology and Biochemistry*, 30: 1989-2000.
- Degens, B.P., 1998b. Microbial functional diversity can be influenced by the addition of simple organic substrates to soils. *Soil Biology and Biochemistry*, 30: 1981-1988.
- Degens, B.P., 1999. Catabolic response profiles differ between microorganisms grown in soils. *Soil Biology and Biochemistry*, 31(3): 475-477.
- Degens, B.P. and Harris, J.A., 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. *Soil Biology and Biochemistry*, 29(9/10): 1309-1320.
- Degens, B.P., Schipper, L., Sparling, G. and Vojvodic-Vukovic, M., 1998. Changes in land use can influence microbial diversity through changes in soil organic resources, NZSS Conference. New Zealand Society of Soil Science, Gisborne, New Zealand, pp. 159-161.
- Garland, J.L. and Mills, A.L., 1991. Classification and characterisation of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and Environmental Microbiology*, 57(8): 2351-2359.
- Ghani, A., Sarathchandra, U., Perrott, K. W., Wardle, D. A., Singleton, P., and Dexter, M., 1996. Biological and biochemical quality of pastoral soils: spatial and temporal variability. In: M.J. Baker (Editor), *Proceedings of the New Zealand Grassland Association*. New Zealand Grassland Association, Waitangi, New Zealand, pp. 211-218.
- Ghani, A., Sarathchandra, U., Perrott, K. W., Singleton, P., Wardle, D., Dexter, M., and Ledgard, S. F., 1999. Are soil microbial and biochemical indicators sensitive to pastoral soil management? In: L. D. Currie (Editor), *Best Soil Management Practices for Production*. Fertiliser and Lime Research Centre, Massey University, New Zealand, , pp. 105-115.
- Grayston, S.J., Wang, S., Campbell, C.D. and Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry*, 30(3): 369-378.

- Harch, B.D., Correll, R.L., Meech, W., Kirkby, C.A. and Pankhurst, C.E., 1997. Using the Gini coefficient with BIOLOG substrate utilisation data to provide an alternative quantitative measure for comparing bacterial soil communities. *Journal of Microbial Methods*, 30: 91-101.
- Haynes, R.J. and Williams, P.H., 1992. Accumulation of soil organic matter and the forms, mineralisation potential and plant-availability of accumulated organic sulphur: Effects of pasture improvement and intense cultivation. *Soil Biology and Biochemistry*, 24(3): 209-217.
- Hitzl, W., Rangger, A., Sharma, S. and Insam, H., 1997. Separation power of the BIOLOG system determined in various soils. *FEMS Microbiology Ecology*, 22: 167-174.
- Jackman, R.H., 1964a. Accumulation of organic matter in some New Zealand soils under permanent pasture 1. Patterns of change of organic carbon, nitrogen, sulphur and phosphorus. *New Zealand Journal of Agricultural Research*, 7: 445-471.
- Jackman, R.H., 1964b. Accumulation of organic matter in some New Zealand soils under permanent pasture 2. Rates of mineralisation of organic matter and the supply of available nutrients. *New Zealand Journal of Agricultural Research*, 7: 472-479.
- Keeney, D.R. and Bremner, J.M., 1966. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. *Agronomy journal*, 58: 498-503.
- Kieft, T.L., Soroker, E. and Firestone, K., 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biology and Biochemistry*, 19(2): 119-126.
- Knight, B.P., McGrath, S.P. and Chaudri, A.M., 1997. Biomass carbon measurements and substrate utilisation patterns of microbial populations from soils amended with Cadmium, Copper, or Zinc. *Applied and Environmental Microbiology*, 63(1): 39-43.
- Landers, D.H., David, M.B. and Mitchell, M.J., 1983. Analysis of organic and inorganic sulphur constituents in sediments, soils and water. *International Journal of Environment , Analytical Chemistry*, 14: 245-256.
- Lindstrom, J.E., Barry, R.P. and Brsddock, J.F., 1998. Microbial community analysis: a kinetic approach to constructing potential C source utilisation patterns. *Soil Biology and Biochemistry*, 30(2): 231-239.

- McKenzie, H.A. and Wallace, H.S., 1954. The kjeldahl determination of nitrogen: A critical study of digestion conditions-temperature, catalyst and oxidising agent. *Australian Journal of Chemistry*, 7: 55-70.
- Moir, J.L., 1994. Influences of climate and fertiliser application history on various measures of soil fertility and productivity in Wairarapa hill country. Master of Agricultural Science Thesis, Massey University, Palmerston North, New Zealand, 145 pp.
- Moir, J.L., Hedley, M.J., Garland, C.B. and Mackay, A.D., 1994. Influences of climate and fertiliser application history on soil fertility and productive capability of Wairarapa hill country. In: J.A. Adams, A. Metherell, P.M. Fraser, A.W. Rate and L.M. Condon (Editors), *Soil science: Current research and future opportunities*. New Zealand Society of Soil Science, Lincoln University, Lincoln, New Zealand, pp. 14.
- Moir, J.L., Hedley, M.J., Mackay, A.D. and Garland, C.B., 1995. Climate, soil fertility and pasture production in Wairarapa hill country. In: L.D. Currie and P. Loganathan (Editors), *Fertiliser requirements of grazed pasture and field crops: Macro and micro nutrients*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 254-260.
- Moir, J.L., Hedley, M.J., Mackay, A.D. and Tillman, R.W., 1996. Effects of various fertiliser histories on nutrient accumulation and pasture production in Wairarapa hill country., *Soil Science - Raising the Profile*. Australian and New Zealand Soil Science Societies, Melbourne University, Melbourne, Australia, pp. 189-190.
- Moir, J.L., Hedley, M.J., Mackay, A.D. and Tillman, R.W., 1997. The effect of fertiliser history on nutrient accumulation and plant-available nutrient supply in legume-based pasture soils, XVIII International Grassland Congress, Winnipeg, Canada, pp. 10, 68-69.
- Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate, USDA.
- Perrott, K.W., Sarathchandra, S.U. and Dow, B.W., 1992. Seasonal and fertiliser effects on the organic cycle and microbial biomass in a hill country soil under pasture. *Australian Journal of Soil Research*, 30: 383-394.

- Ross, D.J., Speir, T.W., Kettles, H.A. and Mackay, A.D., 1995. Soil microbial biomass, C and N mineralisation and enzyme activities in a hill pasture: influence of season and slow release P and S fertiliser. *Soil Biology and Biochemistry*, 27(11): 1431-1443.
- Saggar, S., 1998. Landcare Research, Palmerston North, New Zealand.
- Saier, M.H., 1989. Protein phosphorylation and allosteric control of inducer exclusion and catabolite repression by the bacterial phosphoenolpyruvate: sugar phosphotransferase system. *Microbiological Reviews*, 53(1): 109-120.
- Schollenberger, C.J. and Simon, R.H., 1945. Determination of exchange capacity and exchange bases in soil-ammonium acetate method. *Soil Science*, 59: 13-24.
- Searle, P.L., 1979. Measurement of adsorbed sulphate in soils - effect of varying soil extract ratios and methods of measurement. *New Zealand Journal of Agricultural Research*, 22: 287-290.
- Sparling, G. and Schipper, L., 1999. Soil quality changes under intensive and extensive land use in New Zealand. In: L.D. Currie (Editor), *Best Soil Management Practices for Production. Fertiliser and Lime Research Centre*, Massey University, New Zealand, pp. 55-63.
- Walker, T.W. and Adams, A.F.R., 1958. Studies on soil organic matter: 1. Influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulphur, and organic phosphorus in grassland soils. *Soil Science*, 85: 307-318.
- Walker, T.W. and Adams, A.F.R., 1959. Studies on soil organic matter: 2. Influence of increased leaching at various stages of weathering on levels of carbon, nitrogen, sulphur and organic and total phosphorus. *Soil Science*, 87: 1-10.
- Walker, T.W., Thapa, B.K. and Adams, A.F.R., 1959. Studies on soil organic matter: 3. Accumulation of carbon, nitrogen, sulphur, organic and total phosphorus in improved grassland soils. *Soil Science*, 87: 135-140.
- Waring, S.A. and Bremner, J.M., 1964. Ammonium production in soil under waterlogged conditions as an index of nitrogen availability. *Nature*, 201: 951-952.
- Zac, J.C., Willig, M.R., Moorhead, D.L. and Wildman, H.G., 1994. Functional diversity of microbial communities: a quantitative approach. *Soil Biology and Biochemistry*, 26(9): 1101-1108.

CHAPTER 8

USING BIOLOGTM MICROTITRE PLATES AS A METHOD FOR STUDYING CHANGES IN CARBON SUBSTRATE USE CHARACTERISTICS OF MICROBIAL POPULATIONS IN PASTURE SOILS: II. INFLUENCE OF SOIL LOCATION AND FERTILISER HISTORY

8.1 INTRODUCTION

Regional Authorities in New Zealand, charged with responsibility of overseeing the Resource Management Act (Gordon, 1991) are currently searching for indicators of soil health and quality, which may be suitable for assessing the effects (positive or negative) of current land management (Doran, 1996). Whilst current soil chemical fertility indicators (e.g. Olsen P status, pH) seem appropriate for adaptation to this purpose, much research is being conducted to develop and evaluate suitable indicators of soil biological health and quality (Doran, 1996; Bardgett et al., 1999; Ghani et al., 1999; Sparling and Schipper, 1999). In this Chapter the possibility that single carbon (C) source substrate use patterns (BiologTM) can be used as indicators of functional microbial diversity in pastoral soils is examined. The methods used in this Chapter were developed and referenced in Chapter 7, Section 7.2.

Soil carbon degradation and cycling, facilitated by soil microbes, are key factors influencing the turnover and availability to plants of nutrients in grazed pastures. Population size and diversity of soil microbes may have major influence upon the supply of mineral nutrient ions for plant growth. Information on the influence of soil fertility status and climate on soil microbial diversity and activity may allow better understanding of the complexities of nutrient cycling in grazed pasture ecosystems. Few studies (Bardgett et al., 1999; Ghani et al., 1999; Sparling and Schipper, 1999) have examined how soil microbial populations change, or differ, as a direct result of soil fertility status (historical fertiliser input) and climatic conditions (Doran, 1996).

Soil biochemical properties, as influenced by soil fertility status, have been examined by a number of workers. Ross et al. (1995a, 1995b) studied the effects of slow-release phosphorus (P) and sulphur (S) fertilisers on microbial respiration and enzyme activities in grazed hill soils, while Bolan et al. (1996) assessed the influence of P fertilisers on microbial activity using microbial substrate-induced respiration (SIR), and basal respiration techniques. Sarathchandra et al. (1988) examined seasonal changes and effects of fertiliser on some microbial characteristics of grazed pastoral soils, concentrating on microbial C and nitrogen (N), and microbial CO₂ production. Fraser et al. (1994) compared enzyme activities and microbial biomass in pastoral soils from a long-term (37yr) grazing trial varying in historical superphosphate (P and S) fertiliser inputs, and concluded that enzyme activity was highest in developed (P fertilised) pasture soils. Haynes and Williams (1999) contrasted microbial population size and enzyme activity by fluorescein diacetate (FDA) hydrolysis on stock camp and standard grazing areas in grazed hill soils, implementing similar methodologies. Most authors concluded that, generally, the quantity of the characteristic measured increased with increasing soil fertility status.

Bardgett and co-workers (Bardgett et al., 1993; Bardgett and Leemans, 1995; Bardgett et al., 1996; Bardgett et al., 1997) have extensively studied the effects of season, and fertiliser application, on soil biota of grazed hill pastures. These studies have included measuring the effects of the intensity of grazing by sheep (Bardgett et al., 1993; Bardgett et al., 1996) on microbial respiration, SIR, the nature of extractable phospholipid fatty acids (PLFA). Bardgett and Leemans (1995) also examined the effect of cessation of fertiliser, liming and grazing, on enzyme activity (dehydrogenase, urease, phosphatase) and soil respiration. In the latter study, the cessation of fertiliser application did not effect microbial respiration or enzyme activities, but significantly reduced microbial biomass C, and dehydrogenase activity. Seasonal effects on soil biota (Bardgett et al., 1997) were investigated using measurements of PLFA and CO₂ evolution. Maximum activities occurred in summer, and minimum in winter. Long-term (winter or annual) removal of grazing animals (sheep) resulted in significant reductions in microbial biomass, and microbial activities. In separate studies, Lovell et al. (1995) considered the effects of long-term fertiliser N addition, and field drainage on basal respiration, ATP levels, and enzyme activities (dehydrogenase, urease, phosphatase). These researchers showed that biomass ATP and microbial respiration were lower in soils receiving long-term application of N fertiliser, however, recent fertiliser inputs resulted in significant but

inconsistent changes in ATP content, enzyme activities, and respiration. Bardgett et al. (1999) have also compared microbial activities of grassland soils varying in fertility status, across seasons, using microbial respiration and PLFA measurements. The results indicated that fungi, dominant in low fertility soils, were replaced in their decomposer activity by bacteria in high fertility soils. Studies on the effect of soil moisture content and climate on soil microbes (Bottner, 1985; Kieft et al., 1987; Van Gestel et al., 1993; Bardgett et al., 1997) and concluded that:

1. drying and rewetting of soils enhanced C and N mineralisation, and that mineralisation flushes were partly biomass killed by drying, and partly non-living organic residues;
2. water potential increases were associated with the wetting of dry soil, and were suggested as being a major catalyst for soil C turnover;
3. the microbial biomass surviving rapid drying represented a dormant and protected fraction of the microbial population;
4. microbial biomass and activity showed pronounced summer maxima and winter minima. The abundance of active soil fungi, and bacteria was significantly lower where grazing stock were removed (long-term).

Various techniques have been used to indicate change in microbial population and function as soil conditions change. For example, some researchers have measured the influences of land use on microbial diversity through changes in soil organic resources (Degens and Harris, 1997; Degens et al., 1998). Sparling and Schipper (1999) used soil microbial measurements (microbial respiration, microbial biomass) to assess soil “quality” across land uses, such as extensive and intensive pasture, forest, arable cropping, and vegetable production soils. Grayston and co-workers (Grayston and Campbell, 1996; Grayston et al., 1998) used the BiologTM microtitre plate system to examine the functional diversity of soil microbes. The BiologTM microtitre plate system (described in Chapter 7, Section 7.2.3) involves measuring the growth (dehydrogenase activity; on each of 95 separate single carbon sources in microtitre wells) of a mixed inoculum of micro-organisms extracted from soil. These studies concentrated on the nature of microbial diversity in plant rhizospheres and root exudates on the dynamics of soil microbial populations (Grayston and Campbell, 1996; Hodge et al., 1996; Grayston et al., 1998; Hodge et al., 1998). Campbell et al. (1997) diverged from the use of carbon sources supplied in the BiologTM GN plate, which are ideal for Gram negative bacteria, to the development of a similar system using a select set of compounds more common to

plant root exudates. These authors showed that the substrate use patterns of microbial populations change with land use or fertility status.

These extensive research studies show that indicators of microbial activity in soil vary as a function of time and farming practice. This work lacks a definitive pattern of microbial activity, that could be used as an indicator to establish (rank or position) soil biological health or quality.

In this Chapter the use of BiologTM microtitre plates is examined to determine the carbon substrate use characteristics of microbial inocula from soils, ranging in both fertility status (historical fertiliser input), and climate (seasonal and annual rainfall) (Moir et al., 1997). A description of the BiologTM microtitre plates containing 95 different C substrates in separate microtitre wells is given earlier (Chapter 7, Section 7.2.3). Previously, only two research studies have used BiologTM plates to consider the influence of soil fertility status or climate on the functional diversity or types of micro-organisms present in pastoral soils (Campbell et al., 1997; Ghani et al., 1999). The study of Ghani et al. (1999) examined a wide range of pastoral management conditions (dairy cattle and sheep grazed pastures with unknown fertiliser histories). These researchers were unable to establish trends in substrate use patterns resulting from soil management change.

The terms ‘soil health’ and ‘soil quality’ are often used in the literature without definition and without reference to measures that can be used to indicate their status. My interpretation of the terms ‘soil health’ and ‘soil quality’ as used in the literature are that they broadly describe soil properties which influence the “life supporting capacity” of soil. These are terms which are often closely linked to, and are discussed along with, “sustainable land-use practices”, or the ability to produce primary crops from soil in a (long-term) sustainable manner that protects the ‘long-term life supporting capacity of the soil’.

In terms of soil biology, there has been some indication (Campbell et al., 1997; Bardgett et al., 1999) that the functional microbial diversity of soil microbes, and microbial population dynamics (e.g. bacterial: fungal ratios) may be useful measurements of soil quality. As yet, there is little evidence to support such claims.

The objective of the experiment reported in this Chapter was to use the BiologTM system to assess whether differences in pastoral land management create measurable changes in the functional diversity of resident (culturable) soil micro-organisms. The narrow range of sheep grazed hill soils represented in the trial sites (described in Chapter 3), with differing fertiliser histories and soil moisture regimes were selected for this study. At the time of soil sampling, all soils were in a “summer-dry” condition (field dry). The effect of rewetting the summer-dry soils on the BiologTM substrate use pattern was also studied.

8.2 METHODS AND MATERIALS

8.2.1 General Methods

Site descriptions (Chapter 3), soil treatment (incubation or field dry; Chapter 7, Section 7.2.3) and inoculation methodologies are fully described in Chapter 7, Section 7.2.3. The soils were either in field dry form, or had been moistened and incubated in the glasshouse for 28 days. Saline extracts of triplicate soil samples were prepared and used to inoculate BiologTM plates comprising 96 wells containing single carbon substrates. The plates were incubated for up to 12 days, and absorbance readings at 590 nm (OD) were taken at 24 hour intervals.

8.2.2 Data Processing

The patterns of OD readings produced from soil inocula in most wells were indicative of typical microbial growth; a lag phase lasting up to 2 days, end of exponential growth after 4-6 days, and the beginning of stationary phase at 6 days (cf. Figure 8.6). To minimise the data set, the data analysis described below uses OD readings taken at 2, 4 and 6 days. To normalise data to account for soil extract colour and microbial growth on soil derived carbon (discussed in Chapter 7), the control well OD was subtracted from the OD of all other 95 wells.

Simple ANOVA was then performed, comparing substrate OD with control well OD. Substrates which were greater (at a high level of significance; $P < 0.001$) than the control

well OD values were selected for further analysis and construction of substrate use “maps” (cf. Figures 8.2 and 8.3).

Numbers of substrates used

Using the full set of substrate data, the number of substrates used by each soil material after 2, 4 and 6 days of BiologTM plate inoculation was measured. Multiple comparison procedures (MCPs) are used to determine commonly used substrates, and extent of substrate use.

Discriminate and Cluster analysis

To add additional comparison, substrate use pattern data has been analysed using popular discriminate function and cluster analysis (Hitzl et al., 1997; Glimm et al., 1997; Hacket and Griffiths, 1997). These statistical methods were used to separate the microbial populations based on the types of, and extent to which, the individual carbon substrates were utilised.

8.3 RESULTS AND DISCUSSION

8.3.1 Number of Substrates Used by Each Soil

The number of substrates initially utilised (day 2) by saline-extracted soil inoculum ranged significantly across each field-dry soil (Figure 8.1 and 8.2A). This indicates that the initial substrate use capacity of the soil inoculum varied significantly between samples taken from field dry soils. Contrasting examples are soil 8 (using 58 of the 95 substrates), and soil 1 (using only 15 of the 95 substrates) at day 2.

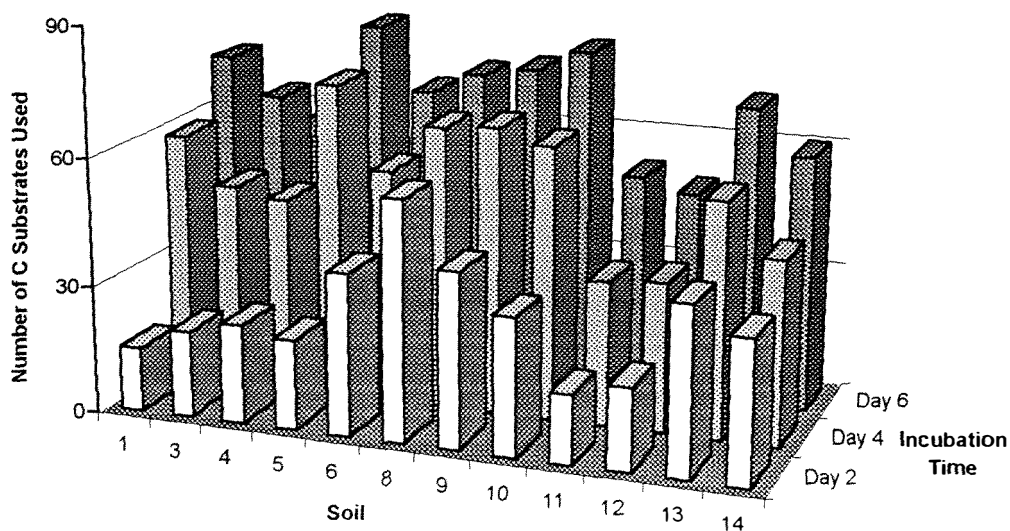


Figure 8.1 Number of Biolog™ C substrates used across all field dry soils after 2, 4 and 6 days of incubation.

After further incubation (at days 4 and 6 post-inoculation; Figure 8.1, 8.2B and 8.2C), the number of substrates utilised increased dramatically for each of the 12 soils. Most soils had returned a positive response ($\alpha = 1\%$) to 60 or more of the Biolog™ carbon substrates after 6 days of inoculation. However, numbers of substrates used were slightly lower on soils 11 and 12, from which inocula utilised 49 and 52 substrates respectively.

Two days after inoculation, inocula from glasshouse moistened and incubated soils had utilised more substrates than their field-dry counterparts (c.f. Figures 8.2A with 8.3A and 8.3A with 8.3B). The number of substrates used also varied less between soils at this time. An exception was soil 11, which utilised only 23 substrates. This may be explained, in part, by low CFUs in this soil (see Chapter 7, Section 7.3.3).

As with field-dry soils, the numbers of substrates used on moistened and incubated soils increased with time, and it was apparent that all soils could utilise most Biolog™ C substrates after 6 days of inoculation (c.f. Figures 8.3A, B and C and 8.4). The substrate use maps (Figures 8.2 and 8.3) graphically show which substrates were “significantly” or “not-significantly” used for each soil, and time interval. A positive result being an OD

significantly higher than the control OD. The maps (Figure 8.2A-C, 8.3A-C) clearly show that;

- a) the use of increasing numbers of substrates with time is a characteristic which is developed by the inocula of all soils, and
- b) that moistening and conditioning the soils through incubation increases the substrate use diversity of the soil inocula. These features of substrate use are discussed later.

The average numbers of substrates used by the inoculum from field dry soil material tended to be lowest after two days, subsequently increasing in number as the incubation increased in length to 6 days (Figure 8.1A). After 6 days some soils were capable of using up to 90 of the 95 substrates, whilst others (e.g. soils 11 and 12) could only use 60 or less (Figure 8.1A).

For field dry soils the pattern of increasing numbers of substrates used with time suggests that either substrate induced enzyme synthesis occurred in the well micro-cultures, or their was slow growth of a minority microbial species.



Figure 8.2 Graphical representation of “significant” (black cell) or “not-significant” (empty cell) substrate use responses across all field dry soils and all substrates after 2 (A), 4 (B) and 6 (C) days of incubation.



Figure 8.3 Graphical representation of “significant” (black cell) or “not-significant” (empty cell) substrate use responses across all glasshouse moistened and incubated soils and all substrates after 2 (A), 4 (B) and 6 (C) days of incubation.

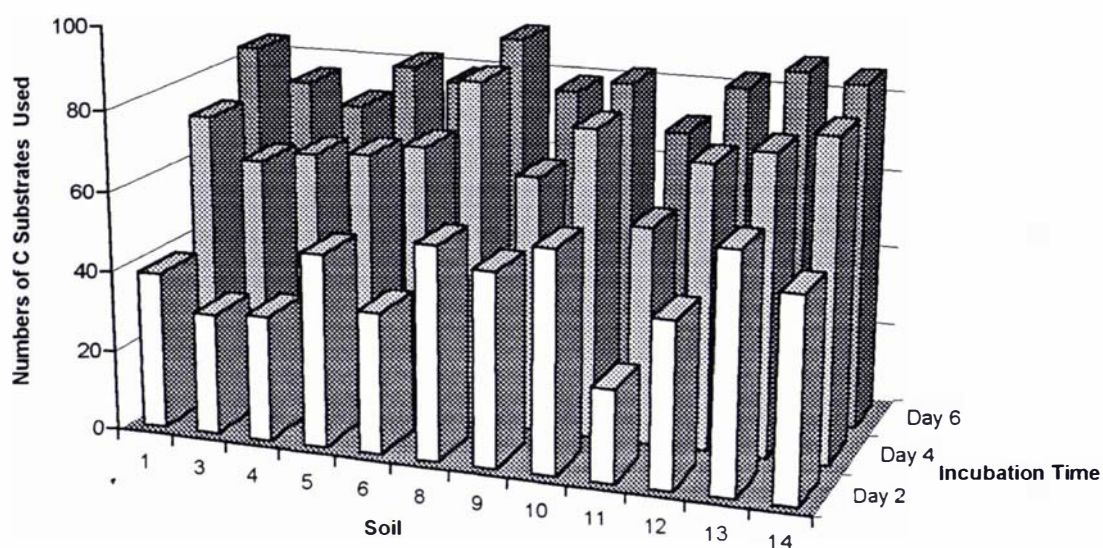


Figure 8.4 Number of Biolog™ C substrates used across all moistened and glasshouse incubated soils after 2, 4 and 6 days of incubation.

The large number of substrates used immediately at day 2 by inoculum from moistened and incubated soil indicates some preconditioning because more diverse substrate use occurs with wetting and incubation of soils. Others have shown that microbial populations increase to peak numbers and activities approximately 21 days after rewetting soils (Saggar et al., 1981; Kieft et al., 1987; Van Gestel et al., 1993; Ross et al., 1995a). Additionally, as microbial populations increase, substrate use diversity increases (Grayston, Pers. comm.).

Subtracting the numbers of substrates used at day 2 from the numbers of substrates used at day 6 gives a measure of the 'inducible' substrate use diversity in a soil sample (Figures 8.5A and 8.5B). In a field dry state, inocula from high fertility, coastal-dry soil 8 possesses the largest substrate use diversity after 2 days of incubation (Figure 8.1), but soils 1, 5 and 10 show the largest amount of 'induced' substrate use as the inoculum proceeds (Figure 8.5A). Inocula from glasshouse moistened soils show less induced substrate use than inocula from field dry soils. This is consistent with the hypothesis that moist incubation of soils either increases microbial diversity or exposes microbes to a greater range of substrates (transient decomposition products), which induces a wider range of hydrolytic enzymes or substrate permeases.

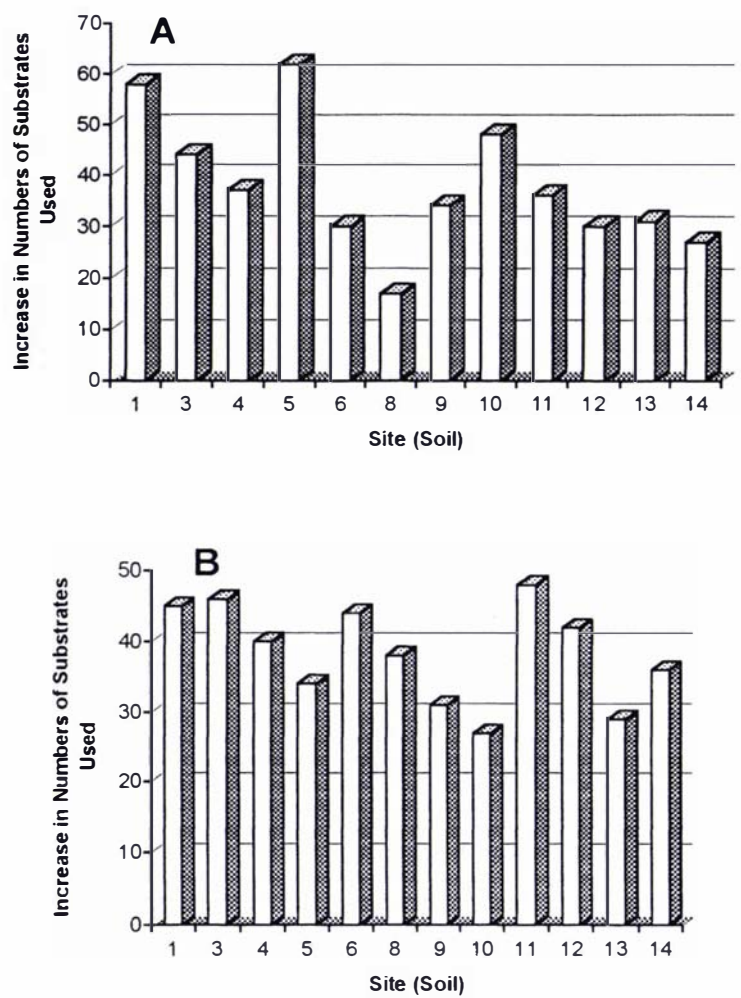


Figure 8.5 Numbers of ‘inducible’ (day 2 minus day 6) Biolog™ C substrates across all **A** field dry, and **B** moistened glasshouse incubated soils.

This is shown more clearly by the contrasting patterns of absorbance change, plotted for 4 of the substrates that show different C substrate use characteristics in Figures 8.2A-C. For example, cyclodextrin (substrate 2; Figure 8.6) is used rapidly, and probably completely, by inocula from all soils. Cyclodextrin is a high-energy compound, and absorbance readings of 1.4 to 1.8 are reached within 6 days. In contrast, growth on glucuronimide (substrate 63, Figure 8.7) is much more variable across soils, with some inoculum growing rapidly (soils 13 and 10), whilst other soils show little activity above the control (soil 11), and soils such as 8 and 12 show clear inducible substrate use over time.

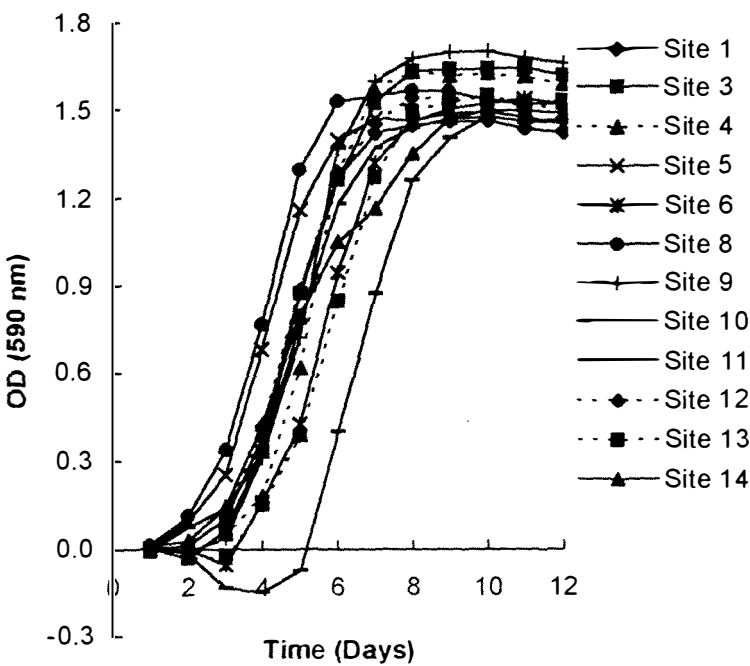


Figure 8.6 OD (microbial growth) over time across all field dry soils on Biolog™ C substrate cyclodextrin (substrate 2).

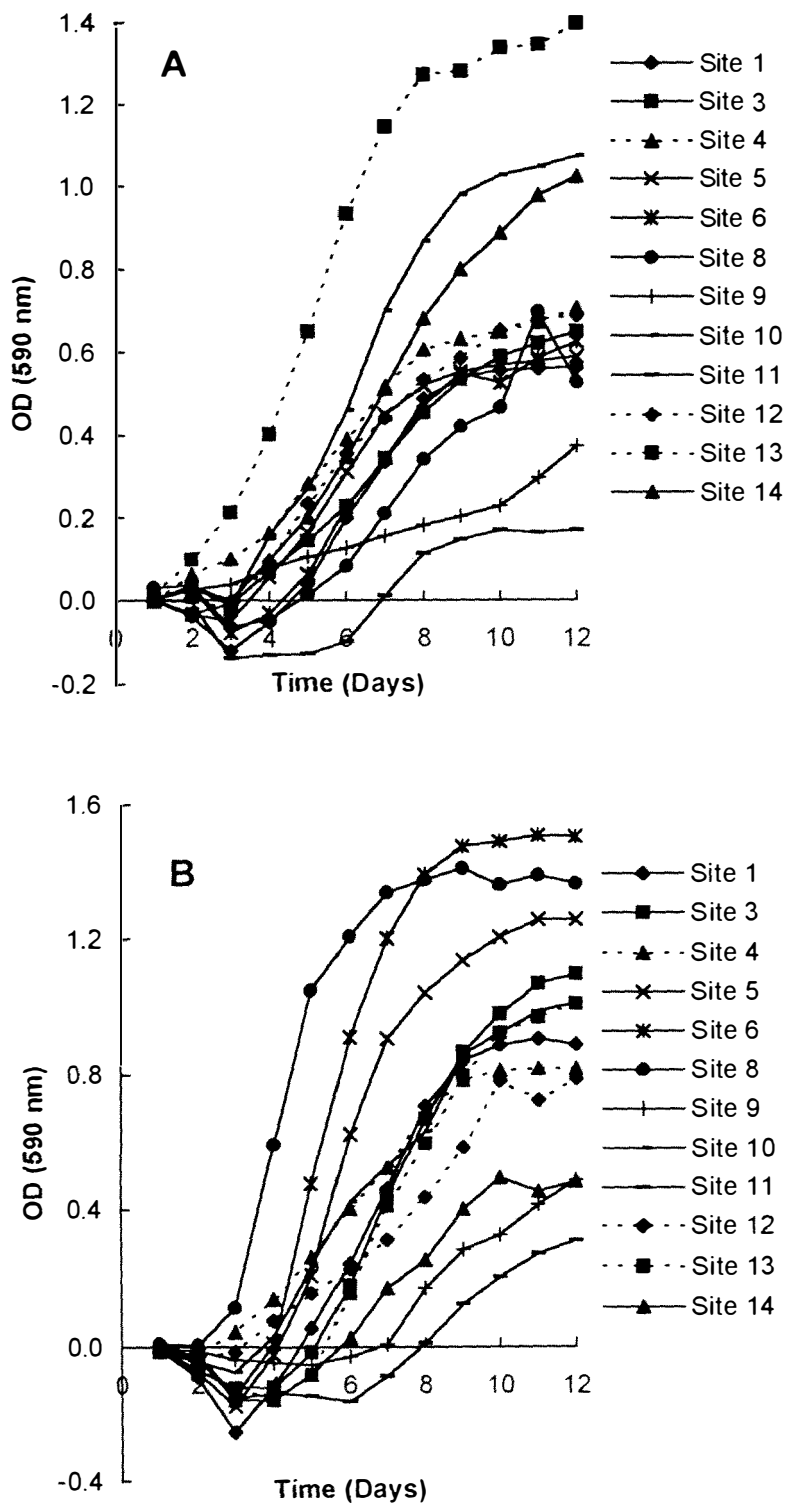


Figure 8.7 OD (microbial growth) over time across all field dry soils on Biolog™ C substrates **A**, glucuronimide (substrate 63), and **B**, phenylethylamine (substrate 89).

Energy poor (largely oxidised) compounds, such as acetic acid (Figure 8.8) generated little colour development (absorbance < 0.35) above the control, but a wide variation between soils. The presence of acetic acid produced no inducible substrate use, but did apparently repress growth of inoculum from soils 10, 11 and 12.

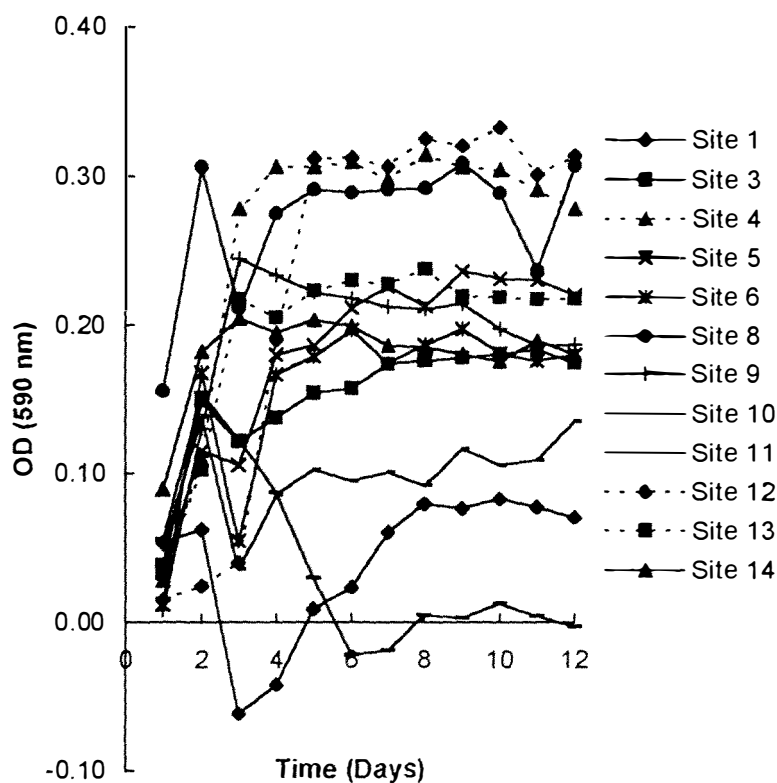


Figure 8.8 OD (microbial growth) over time across all field dry soils on Biolog™ C substrate acetic acid (substrate 37).

8.3.2 Numbers of Soils Using Each Substrate

The number of field-dry soils (soil inocula), and moist incubated soils significantly utilising individual Biolog™ C substrates after 2, 4 and 6 days of incubation are presented in Figures 8.9A-C, and 8.10A-C respectively.

During initial stages (Figure 8.9A), 18 Biolog™ substrates were utilised by 8 soils or more (see Appendix 8.2 for list), and by day 6 (Figure 8.9C), most substrates had been used significantly across most soils. However, there were 12 substrates which were used by fewer than 4 soils. For full data sets on the extent of substrate use, refer to tables in Appendix 8.2.

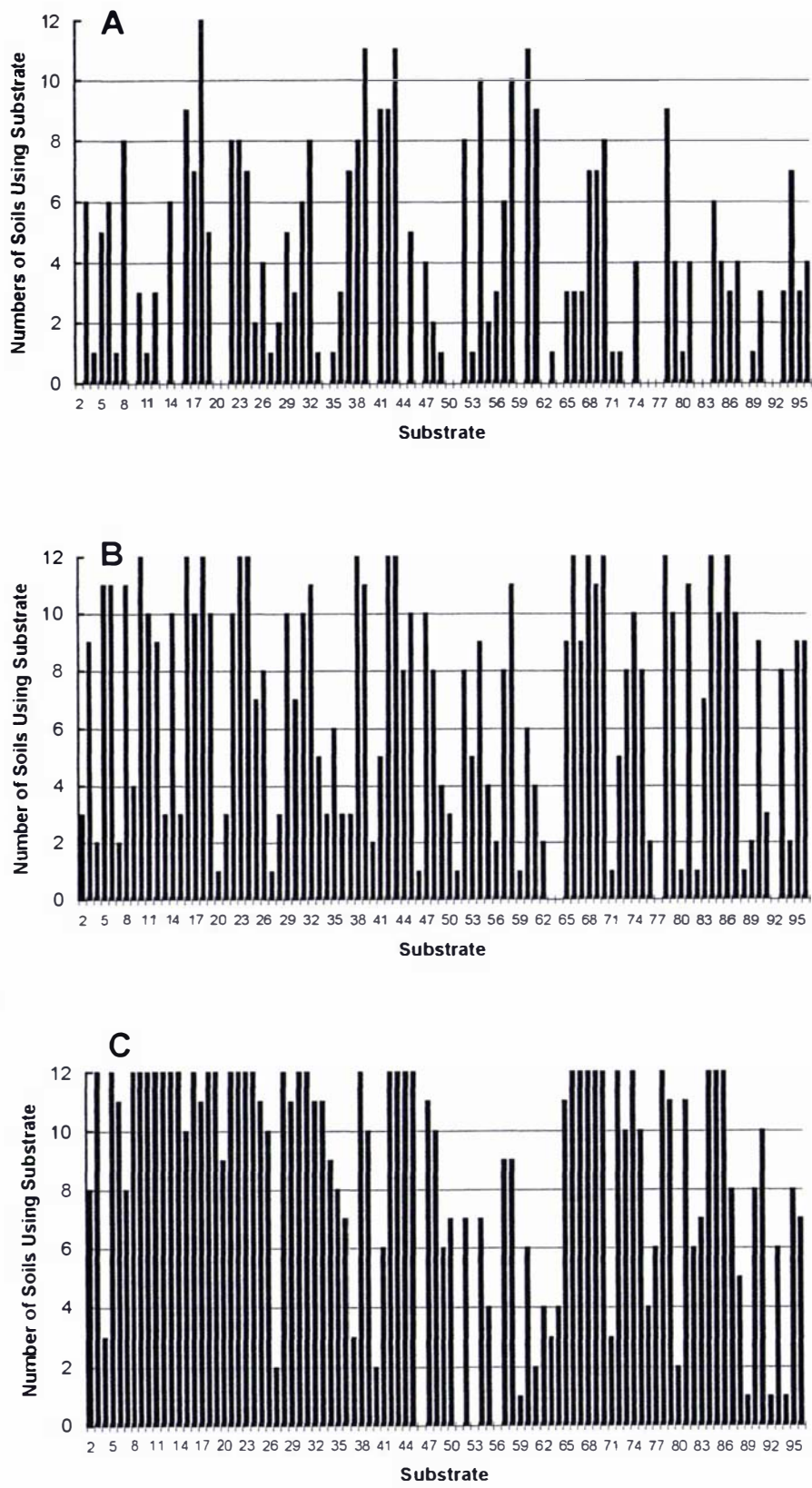


Figure 8.9 Number of field dry soils significantly using individual Biolog™ C substrates after A 2, B 4 and C 6 days of incubation.

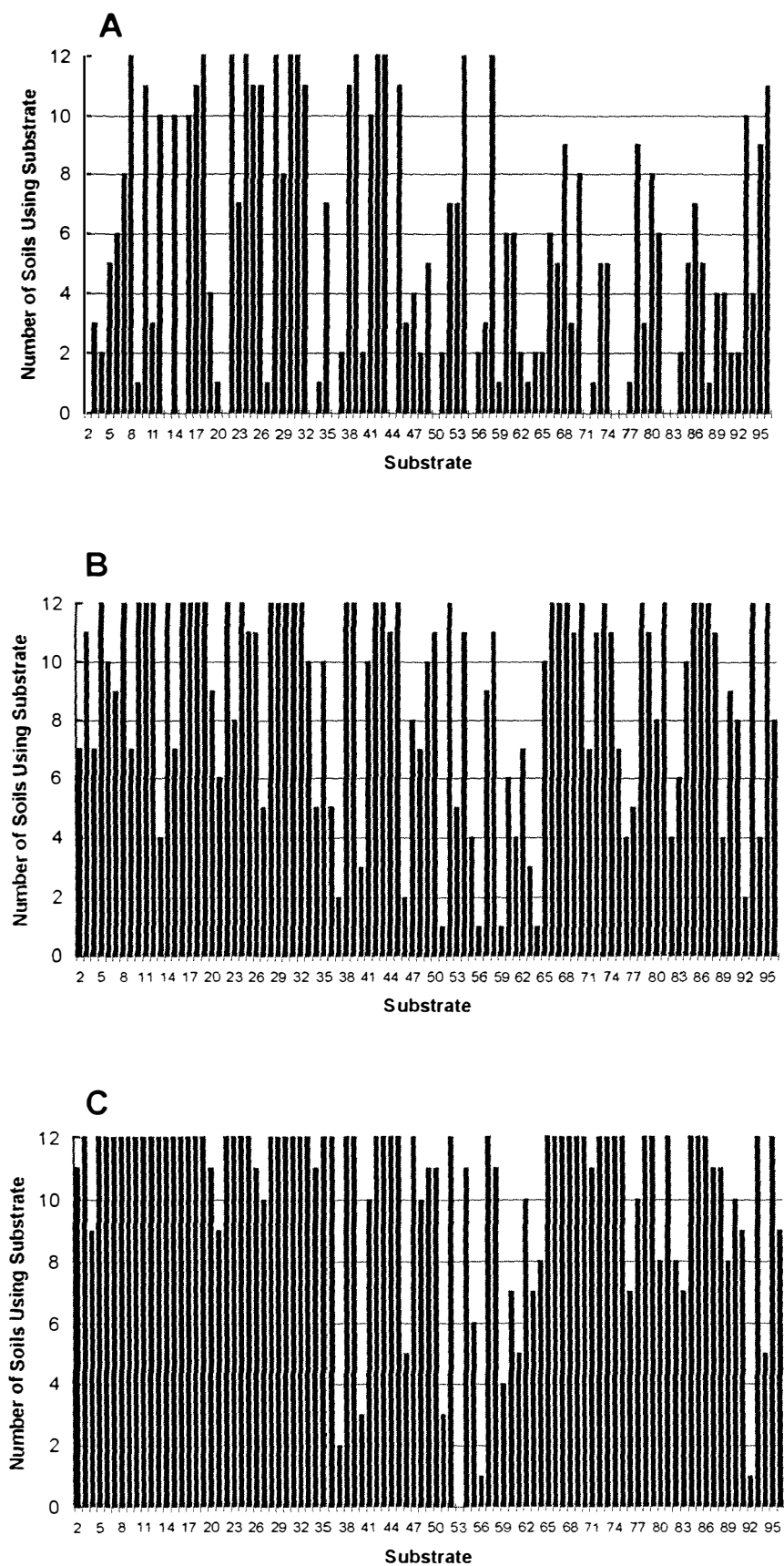


Figure 8.10 Number of moistened glasshouse incubated soils significantly using individual BiologTM C substrates after **A** 2, **B** 4 and **C** 6 days of incubation.

Substrate use patterns were analysed further using discriminate analysis and cluster analysis. Plots of the cluster analysis results are given in Appendix 8.3. The patterns of substrate use by each inocula were significantly clustered within replicates of the same soil. Inocula from lower fertility field dry soils (4, 6, 11, 12 and 13) appear to have different quadrant positions to the higher fertility sites (1, 3, 5, 8 and 10). As the incubation proceeded (2-6 days), the movement of site cluster groupings to different areas of the plot appeared to have no set pattern with respect to site fertility. The cluster pattern could also not be explained by groupings of soils by climate regime (1, 3, 4, 14 = very high rainfall; 5, 6, 8, 9 = low rainfall). Inocula from the moistened glasshouse soils exhibited very different plot positions c.f. the field dry inocula (Appendix 8.3).

Large differences in substrate use pattern for each soil cluster were observed after 2 days of incubation, but by day six, around 60% of the soils were exhibiting very similar substrate use preferences (overlapping clusters, or clusters in close proximity to one another). These results add strong evidence to the thesis of a wide range of inducible enzyme activity being common to most soils. Soil inocula appeared to use the BiologTM C substrates in very different ways during the first 2-4 days of incubation, but become increasingly similar beyond this time frame. Although the data in Appendix 8.3 suggest that culturable micro-organism populations in these soils can have quite different functional capabilities, the pattern of change in substrate use is extremely complex, and within the context of this investigation, could not be used as an indicator of soil 'health'. It appeared that the substrate use patterns are diverse, and vary widely across the test soils, but there is poor understanding of the factors driving this substrate use diversity.

8.3.3 Identifying "indicator component" substrate use patterns

For a single carbon substrate to play a role as a useful indicator of change in microbial functional diversity across the range of pasture soils selected in this study there must be significant differences in substrate use across soils. If significant substrate use was present after 2 days incubation, then this signals constitutive production of the necessary permease and metabolic enzymes for substrate use by the dominant micro-organisms in the culture. If significant substrate use does not occur at 2 days, but is present after 6 days, then this signals either the induction of permease and metabolic enzymes by the

dominant micro-organisms in culture, or that a minority of organisms in the culture at day 2 using the substrate are able to grow to a significant proportion of the population by day 6. It is assumed that loss of functional diversity (substrate use) is indicated only when significant substrate use has not occurred by six days. Likewise, if a substrate is not used by the field dry sampled soil but is used after rewetting the soils, then this is not loss of functional diversity. Useful indicators are substrates used by some soils and not by others. Therefore, substrates were placed into three categories which may aid the selection process of suitable indicators of land use:

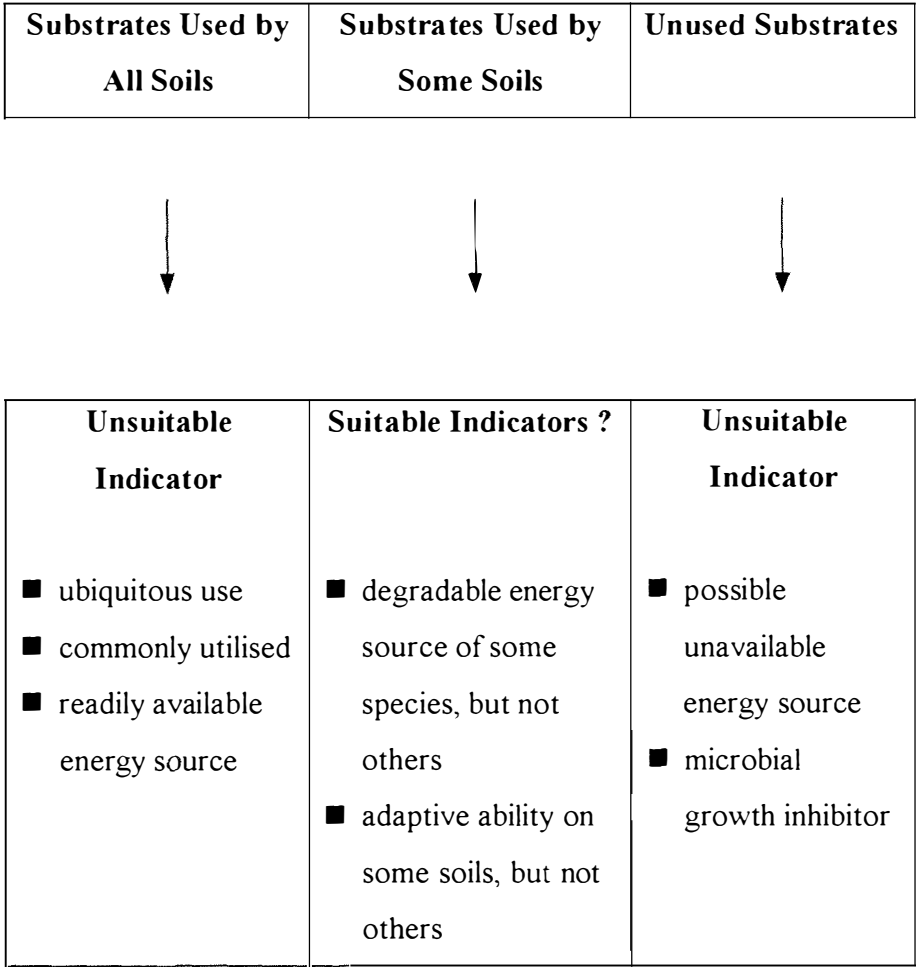


Table 8.1 Substrates falling into the category of “Suitable Indicators”

BiologTM Substrate Number	Substrate Compound
37	acetic acid
40	formic acid
46	α -hydroxybutyric acid
51	α -ketobutyric acid
56	propionic acid
59	sebacic acid
61	bromosuccinic acid
92	2,3-butanediol
94	D,L- α -glycerol phosphate

There are only nine substrates (Table 8.1) that meet the criteria of suitable indicators because moist incubation of the soils removes most substrates from the list of substrates used by 50 % or more of the soils by day 6 (Figure 8.10C). This leaves only 9 substrates that could be useful indicator substrates. Most of these nine substrates are low molecular weight, high oxidation state, low energy value compounds (e.g. formic, acetic, propionic and α hydroxybuteric acids), that are end products of decomposition and are substrates useful mostly to facultative methylotrophic bacteria (Hou et al., 1979; Fumihiko and Hiroyuki, 1994). Thus they are not indicative of wide ranging substrate use diversity, and as such, are not useful indicators of the microbial functional diversity of soils. Further research would be required to establish how stable this substrate use pattern is, and whether these indicator C substrates are of any relevance to field soil processes.

Examples of how inocula from two different soils (field dry soil 13, Figure 8.11A; and moistened glasshouse incubated soil 8, Figure 8.11B) utilise individual substrates differently are shown for two C substrates. Inoculum from soil 13 (Figure 8.11A) grow largely unhindered on both substrates (glucuronimide and D-serine). However, soil 8 (Figure 8.11B) has less than half the growth (OD) for glucuronimide than soil 13, even

when control OD, and utilisation of D-serine is high. This indicates that field dry soil 13 has a constitutive ability to utilise glucuronimide, whereas moistened glasshouse incubated soil 8 does not. Results such as this signal the need for further research, investigating the significance of individual C substrate utilisation by soil inocula.

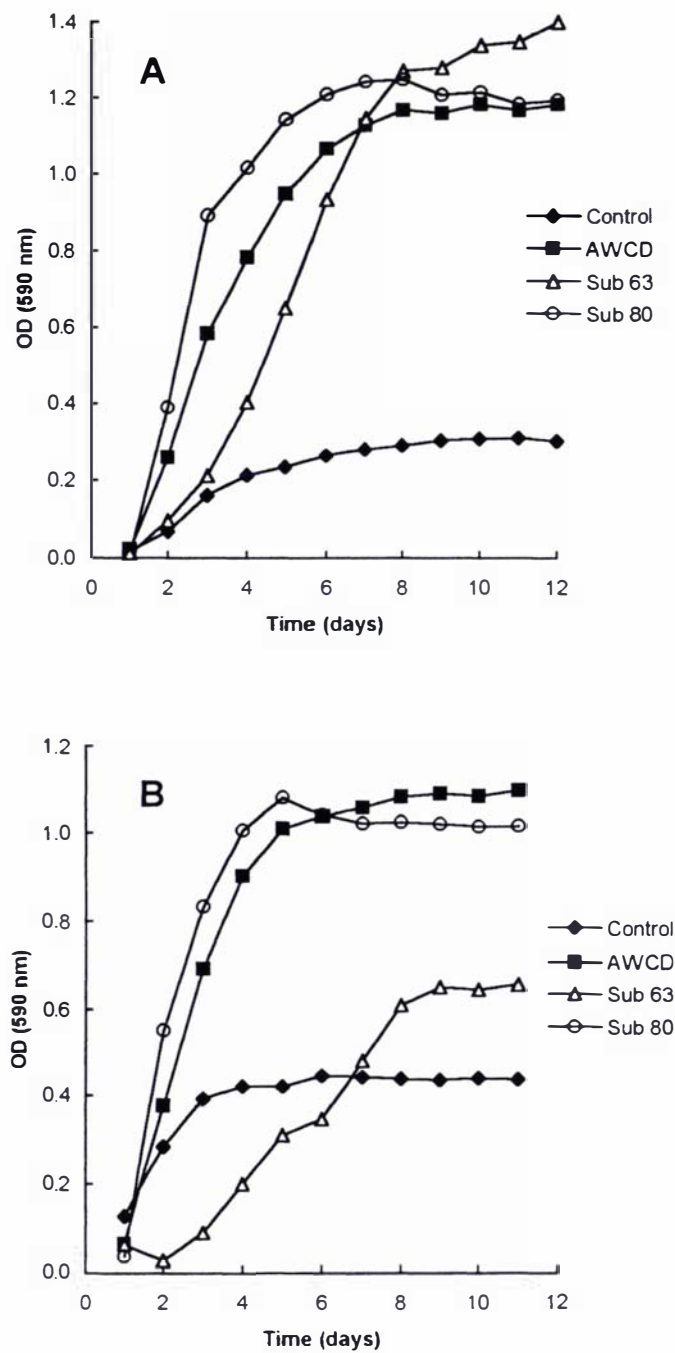


Figure 8.11 Soil inocula OD (growth) over time for control wells, average well colour development (AWCD), BiologTM C substrate 63 (glucuronimide), and BiologTM C substrate 80 (D-serine) for **A** field dry soil 13 and **B** moistened glasshouse incubated soil 8.

The results conclusively show that once soils have been remoistened and incubated there is a high percentage (approximately 70-90%) of single C substrates capable of being utilised by inocula from most soils. The substrate use diversity across the 12 pasture

soils is therefore more similar than different using BiologTM Gram negative GN type plates.

8.3.4 Correlation of numbers of substrates used with other soil characteristics

If a measure of substrate use diversity by soil micro-organisms is to be a useful soil quality index it could be expected to vary with changes in other soil characteristics, such as fertility status of pasture, dry matter yield of pasture, soil moisture regime, and mineralisation potential of soil organic matter. Relationships between the numbers of substrates used by each soil and soil fertility (Olsen P values; Figure 8.12), site annual pasture yield (Figure 8.13), soil moisture content at sampling (Figure 8.14) and soil mineralisable nitrogen (Figure 8.15) were examined. Weak linear relationships were found between numbers of substrates used and Olsen P ($R^2 = 0.2$), annual ($R^2 = 0.15$) and spring ($R^2 = 0.3$) pasture yields. However, numbers of substrates used were strongly related to CFUs (colony forming units, Chapter 7 Section 7.3.3) (day 2, $R^2 = 0.5$; day 4, $R^2 = 0.72$; day 6, $R^2 = 0.86$) in each soil inoculum (Figure 8.16), and to a lesser degree, soil mineralisable N (day 2, $R^2 = 0.23$; day 4, $R^2 = 0.46$; day 6, $R^2 = 0.59$; Figure 8.15). These latter relationships strengthened as incubation time increased. This result suggests that CFUs, or numbers of viable organisms, are an important determinant of frequency of C substrate use in the microtitre plate. This is to be expected, because as higher CFUs increase the probability that a line of organisms is present with the enzyme capability to use a substrate, or that a greater number of more facultative (adaptable to different substrates by enzyme induction) organisms are present. Soils with higher mineralisable N levels had higher substrate use frequency, perhaps due to higher N levels in the saline soil extract. This may be entering the extract as microbial tissue N, or as readily degradable forms of soil organic matter.

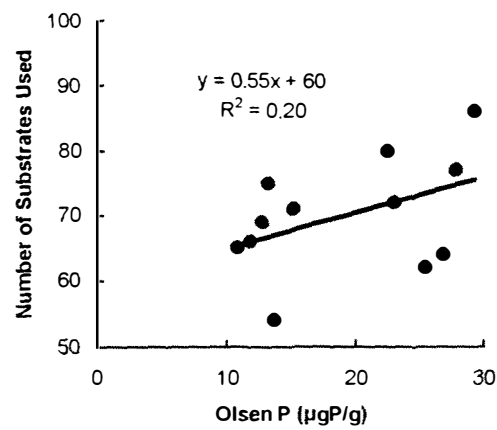


Figure 8.12 The relationship between Olsen P and number of Biolog™ C substrates utilised after 4 days incubation across all moistened glasshouse incubated soils.

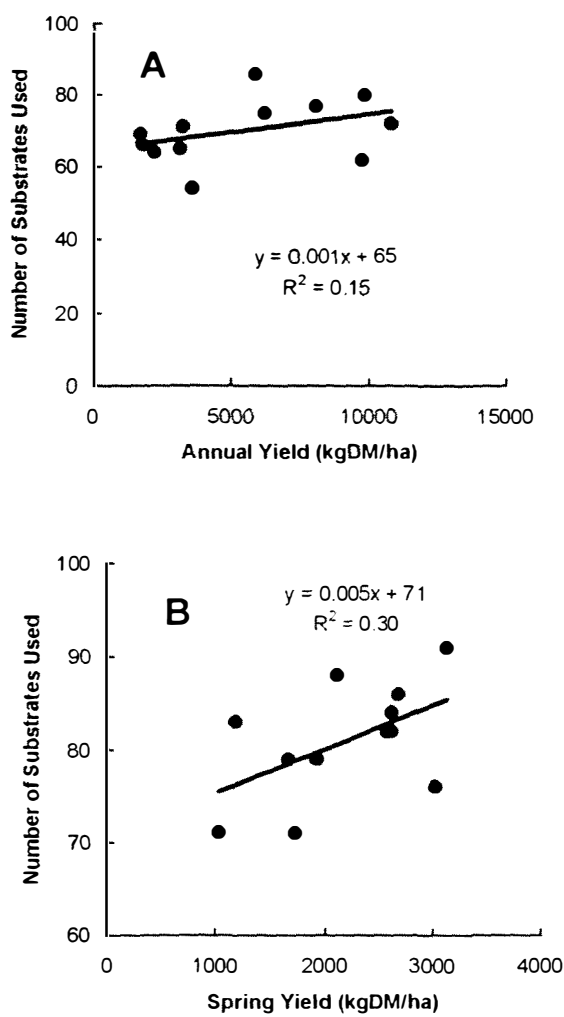


Figure 8.13 The relationship between annual pasture yield and **A** spring pasture yield, and **B** number of BiologTM C substrates utilised after 4 and 6 days incubation, respectively, across all moistened glasshouse incubated soils.

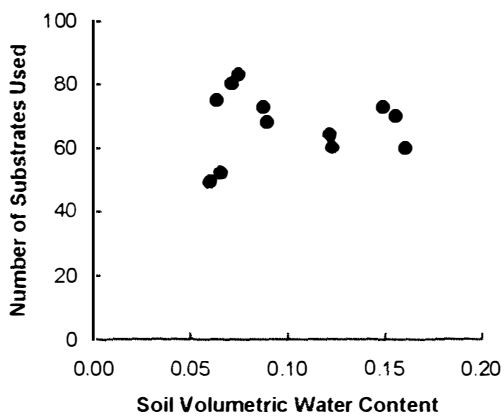


Figure 8.14 The relationship between soil moisture content at time of sampling and number of BiologTM C substrates utilised after 6 days incubation across all field-dry soils.

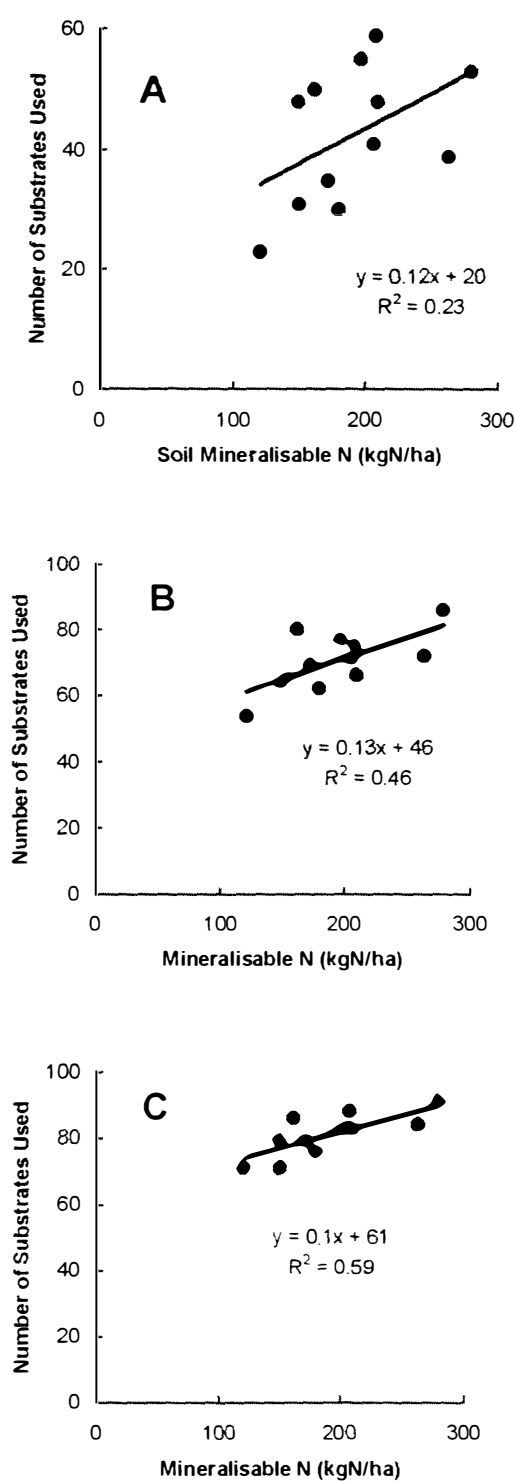


Figure 8.15 The relationship between Soil Mineralisable N and number of Biolog™ C substrates utilised after **A** 2, **B** 4 and **C** 6 days of incubation across all moistened glasshouse incubated soils.

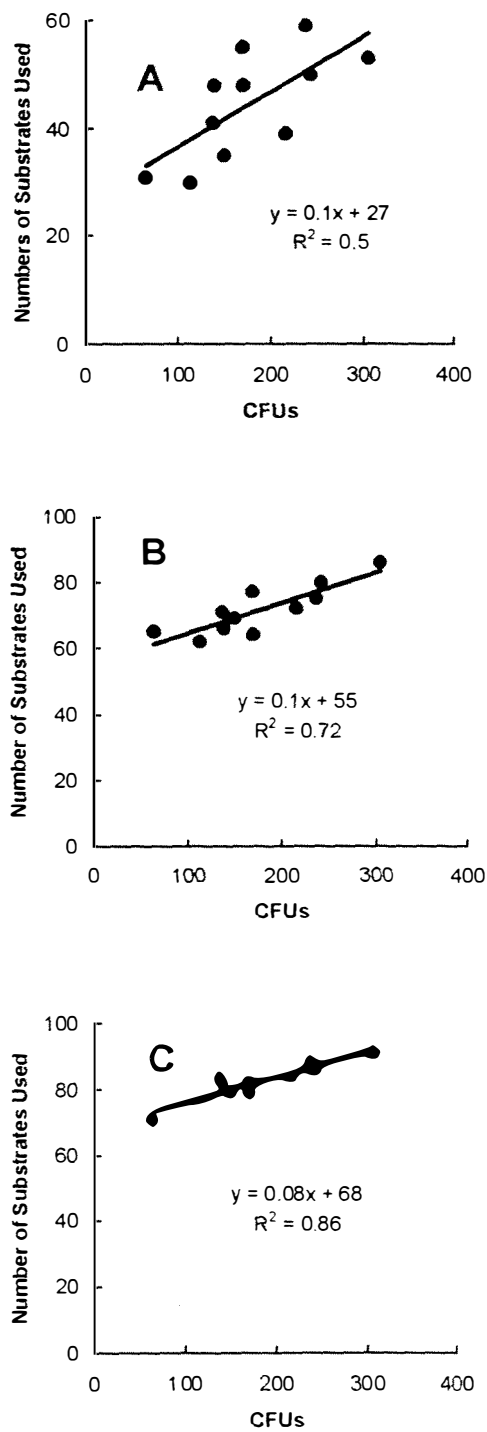


Figure 8.16 The relationship between CFUs and number of BiologTM C substrates utilised after **A** 2, **B** 4 and **C** 6 days of incubation across all moistened glasshouse incubated soils.

8.3.5 'Inducible' Substrate Use

Another more variable, and perhaps interesting character across soils is the substrate use obtained by subtracting the number of substrates used at 2 days from the greater quantity used after 6 days incubation of BiologTM plates. As discussed earlier in Section 8.3.1, this 'inducible' substrate use may occur because new enzyme synthesis is induced by the presence of an uncommon substrate or perhaps small numbers of microbes are present in the inocula that have the specific ability to use obscure C substrates, which would not normally occur in the natural soil environment. On supplying uncommon C substrates to these native soil populations, it is possible that less prolific microbial species could become more dominant in the BiologTM well.

Therefore, in terms of a soil's microbial diversity, it is probable that an ability for a population to adapt (to substrate use) is as important as the soil microbial population having the substrate use as an initial capability. Adaptation factors, such as varying inducible enzyme activities, may play major roles in the apparent microbial diversity of a soil. Initial observations of the data in Figures 8.1 and 8.4, and also Figures 8.5A and 8.5B, suggest that the adaptive power of a soil inoculum (number of substrates used at 6 days - 2 days) also varies between soils. The question arises as to whether this is a true soil characteristic or a soil inoculum characteristic. This cannot be answered. It has already been noted (Chapter 7, Section 7.3.2.2) that the amount of soluble C extracted from each soil varied, and was a readily available source of C creating growth in the microtitre plate wells. It was possible that the variation in amount of soluble soil C added to the well with inoculum may influence (have a "priming" effect) on both initial and adaptive substrate use pattern, perhaps allowing organisms to grow, and giving time to adapt to the supplied substrate. No relationship could be found however between the amount of C supplied in the inoculum and the number of substrates used by that inoculum.

8.4 CONCLUSIONS

The BiologTM microtitre plate system, for use as an indicator of soil health, has been shown to have limited application to this range of pasture soils with differing fertiliser

histories. It appears that the nature of microbiological growth on these carbon substrates is inherently complex, given that mixed microbial populations are present in soil inoculum. The complexity of results has failed to indicate clear and simple interpretations or substrate use patterns which could be used as indicators (an index) of soil 'health'.

The twelve test soils used in this study, varying widely in fertility status, would seem to provide an ideal platform on which to test the suitability of this assay as a simple soil health indicator. However, it has not been possible to link functional diversity of inocula (substrate use patterns) from the twelve test hill country soils (which range widely in fertility status and climate regime) with soil chemical indices or pasture growth. Other workers (Degens and Harris, 1997), have developed alternative methodologies, by adding simple organic substrates to soil, followed by respiration measurements. The advantage of such a technique is that soil microbes need not be cultured, and therefore results may be expected to better reflect the "natural" environment of the microbial communities, without the dependence of organism culturability (and the short-comings of associated culturing assumptions). However, such a technique is also laboratory based, and depends upon disturbance of the soil sample from the natural field environment. This technique also depends upon loading of the sample with a synthetic single C substrate, similar to those used in the BiologTM assay. With present knowledge, it is difficult to conclude that the method of Degens and Harris offers major benefits over the BiologTM assay for the purpose of description of functional diversity of soil microbial communities.

However, for soil microbiology research purposes, the BiologTM assay technique has shown potential to separate the soils studied in this experiment, on the basis of microbial functionality. The differences in the constitutive and inducible abilities of soil inocula to utilise C substrates, demonstrated in this study, may in future yield valuable information to those studying not only soil microbiological functional diversity, but also the nature and degradation of soil C in such systems.

Future research could focus on the importance of individual substrate use by whole soil inocula. A microtitre plate would need to be designed specially for the purpose. Identification of 'indicator' C substrates, and full understanding of the relevance or

importance of such substrates to soil microbial populations (and their function), would be valuable information for those researching the dynamics of C in soil - pasture systems. The use of different C sources in the microtitre plate, such as the rhizosphere C compounds suggested by Campbell et al. (1997) may have some merit. However, the disadvantage of the approach suggested by Campbell et al. (1997) may be that too many of the supplied substrates could be readily degradable across wide-ranging soil microbial populations. If this were true, use of such substrates would diminish the ability of the technique to separate different microbial communities on the basis of functional diversity. An improved understanding of carbon substrate use patterns by soil micro-organisms is required before the BiologTM technique could be made relevant to soil quality assessment.

8.5 REFERENCES

- Bardgett, R.D., Frankland, J.C. and Whittaker, J.B., 1993. The effects of agricultural management on the soil biota of some upland grasslands. *Agriculture, Ecosystems and Environment*, 45: 25-45.
- Bardgett, R.D., Hobbs, P.J. and Frostegård, Å., 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biology and Fertility of Soils*, 22: 261-264.
- Bardgett, R.D. and Leemans, D.K., 1995. The short-term effects of cessation of fertiliser applications, liming, and grazing on microbial biomass and activity in a reseeded upland grassland soil. *Biology and Fertility of Soils*, 19: 148-154.
- Bardgett, R.D., Leemans, D.K., Cook, R. and Hobbs, P.J., 1997. Seasonality of the soil biota of grazed and ungrazed hill grasslands. *Soil Biology and Biochemistry*, 29(8): 1285-1294.
- Bardgett, R.D., Lovell, R.D., Hobbs, P.J. and Jarvis, S.C., 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. *Soil Biology and Biochemistry*, 31: 1021-1030.
- Bolan, N.S., Currie, L.D. and Baskaran, S., 1996. Assessment of the influence of phosphate fertilisers on the microbial activity of pasture soils. *Biology and Fertility of Soils*, 21: 284-292.

- Bottner, P., 1985. Response of microbial biomass to alternate moist and dry conditions in a soil incubated with ^{14}C - and ^{15}N -labeled plant material. *Soil Biology and Biochemistry*, 17(3): 329-337.
- Campbell, C.D., Grayston, S.J. and Hirst, D.J., 1997. Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. *Journal of Microbial Methods*, 30: 33-41.
- Degens, B.P. and Harris, J.A., 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. *Soil Biology and Biochemistry*, 29(9/10): 1309-1320.
- Degens, B.P., Schipper, L., Sparling, G. and Vojvodic-Vukovic, M., 1998. Changes in land use can influence microbial diversity through changes in soil organic resources, NZSS Conference. New Zealand Society of Soil Science, Gisborne, New Zealand, pp. 159-161.
- Doran, J. 1996. The international situation and criteria for indicators. In: *Soil Quality Indicators for Sustainable Agriculture in New Zealand: Proceedings of a Workshop*. (Eds. Cameron, K. C., Cornforth, I. S., McLaren, R. G., Beare, M. H., Basher, L. R., Metherell, A. K. & Kerr, L. E.). Lincoln Soil Quality Research Centre, Lincoln University, New Zealand, pp. 20-40.
- Fraser, P.M., Haynes, R.J. and Williams, P.H., 1994. Effects of pasture improvement and intense cultivation on microbial biomass, enzyme activities, and composition and size of earthworm populations. *Biology and Fertility of Soils*, 17: 185-190.
- Fumihiko, Y. and Hiroyuki, O., 1994. Ecology of *Methylobacterium* on the phylloplanes in *Pinus densiflora* forest. *Environment Control in Biology*, 32(2): 95-105.
- Ghani, A., Sarathchandra, U., Perrott, K. W., Singleton, P., Wardle, D., Dexter, M. and Ledgard, S. F., 1999. Are soil microbial and biochemical indicators sensitive to pastoral soil management? In: L.D. Currie (Editor), *Best Soil Management Practices for Production*. Fertiliser and Lime Research Centre, Massey University, New Zealand, pp. 105-115.
- Glimm, E., Heuer, H., Engelen, B., Smalla, K. and Backhaus, H., 1997. Statistical comparisons of community catabolic profiles. *Journal of Microbiological Methods*, 30: 71-80.
- Gordon, B., 1991. *Resource Management*. Brooker and Friend, Wellington, N.Z.

- Grayston, S.J. and Campbell, C.D., 1996. Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*). *Tree Physiology*, 16: 1031-1038.
- Grayston, S.J., Wang, S., Campbell, C.D. and Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry*, 30(3): 369-378.
- Hackett, C.A. and Griffiths, B.S., 1997. Statistical analysis of the time-course of Biolog substrate utilisation. *Journal of Microbial Methods*, 30: 63-69.
- Haynes, R.J. and Williams, P.H., 1999. Influence of stock camping behaviour on the soil microbiological and biochemical properties of grazed pastoral soils. *Biology and Fertility of Soils*, 28: 253-258.
- Hitzl, W., Henrich, M., Kessel, M. and Insam, H., 1997. Application of multivariate analysis of variance and related techniques in soil studies with substrate utilization tests. *Journal of Microbial Methods*, 30: 81-89.
- Hodge, A., Grayston, S.J. and Ord, B.G., 1996. A novel method for characterisation and quantification of plant root exudates. *Plant and Soil*, 184: 97-104.
- Hodge, A., Paterson, E., Grayston, S.J., Campbell, C.D., Ord, B.G. and Killham, K., 1998. Characterisation and microbial utilisation of exudate material from the rhizosphere of *Lolium perenne* grown under CO₂ enrichment. *Soil Biology and Biochemistry*, 30(8/9): 1033-1043.
- Hou, C.T., Patel, R., Laskin, A.I., Barnabe, N. and Marczak, I., 1979. Microbial oxidation of gaseous hydrocarbons: Production of methyl ketones from their corresponding secondary alcohols by methane-grown and methanol-grown microbes. *Applied and Environmental Microbiology*, 38(1): 135-142.
- Kieft, T.L., Soroker, E. and Firestone, K., 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biology and Biochemistry*, 19(2): 119-126.
- Lovell, R.D., Jarvis, S.C. and Bardgett, R.D., 1995. Soil microbial biomass and activity in long-term grassland: Effects of management changes. *Soil Biology and Biochemistry*, 27(7): 969-975.

- Moir, J.L., Hedley, M.J., Mackay, A.D. and Tillman, R.W., 1997. The effect of fertiliser history on nutrient accumulation and plant-available nutrient supply in legume-based pasture soils. In: J.G. Buchanan-Smith, L.D. Bailey and P. McCaughey (Editors), XVIII International Grassland Congress. International Grassland Association, Winnipeg, Canada, pp. 10, 68-69.
- Ross, D.J., Speir, T.W., Kettles, H.A. and Mackay, A.D., 1995a. Soil microbial biomass, C and N mineralisation and enzyme activities in a hill pasture: influence of season and slow release P and S fertiliser. *Soil Biology and Biochemistry*, 27(11): 1431-1443.
- Ross, D.J., Speir, T.W., Kettles, H.A., Tate, K.R. and Mackay, A.D., 1995b. Soil microbial biomass, C and N mineralisation, and enzyme activities in a hill pasture: influence of grazing management. *Australian Journal of Soil Research*, 33: 943-59.
- Saggar, S., Bettany, J.R. and Stewart, J.W.B., 1981. Sulphur transformations in relation to carbon and nitrogen in incubated soils. *Soil Biology and Biochemistry*, 13(6): 499-511.
- Sarathchandra, S.U., Perrott, K.W., Boase, M.R. and Waller, J.E., 1988. Seasonal changes and effects of fertiliser on some chemical, biochemical and microbiological characteristics of high-producing pastoral soil. *Biology and Fertility of Soils*, 6: 328-335.
- Sparling, G. and Schipper, L., 1999. Soil quality changes under intensive and extensive land use in New Zealand. In: L.D. Currie (Editor), *Best Soil Management Practices for Production*. Fertiliser and Lime Research Centre, Massey University, New Zealand, pp. 53-63.
- Van Gestel, M., Merckx, R. and Vlassak, K., 1993. Microbial biomass responses to soil drying and rewetting: the fate of fast and slow-growing microorganisms in soils from different climates. *Soil Biology and Biochemistry*, 25(1): 109-123.

CHAPTER 9

SUMMARY AND IMPLICATIONS FOR FUTURE RESEARCH

Quantitative information on nutrient cycling in grazed pasture systems is central to understanding the sustainability of pasture soil management. Phosphorus (P) and sulphur (S) fertiliser use efficiency in legume-based pastures has not been thoroughly examined in terms of associating pasture yield increases with long-term changes in soil fertility and soil quality. Current models used to predict pasture yield responses to soil fertility status use single indices of soil fertility status and do not incorporate the effect of climate on pasture growth. This thesis examined the influence of P and S fertiliser history and the interaction with climate on changes in pasture production, soil fertility status, and some indices of soil quality on Wairarapa hill pastures.

On the characterisation of soil fertility, 12 Wairarapa hill country soils were shown to have accumulated large quantities of P, and to a lesser extent, S and N as a direct result of traditional SSP applications. Despite 4 fold increases in pasture yield with fertiliser application, measures of mineralisable soil N changed approximately 4 fold (100-380 kgNha⁻¹). The Olsen P test values from these soils were strong indicators of the amounts of plant-available P, N and S in these soils. It was concluded that in P and S fertilised legume-based pastures the Olsen P test is a strong and dynamic indicator of general soil fertility.

Pasture growth response to strategic N fertiliser application was investigated in field trials conducted on the same Wairarapa hill country sites. Variation in site-to-site pasture growth response to applied N ranged from 0-31 kgDM kgN⁻¹ applied, but the size of the response could not be explained by simple single factors, representing soil P or N fertility indices, nor by climate regime. It was concluded that the ability of soils to supply plant-available nitrogen (plus P and S) was better examined in a glasshouse environment, where the complicating and variable effects of climate could be removed.

In the first of two plant-based glasshouse studies, soils that had received on average 250 kg SSP ha⁻¹yr⁻¹ for 20 years yielded up to 10 times more DM than low fertility soils (receiving low, < 100 kg SSP ha⁻¹yr⁻¹). Yields in the glasshouse were not correlated to field-measured seasonal, nor annual pasture DM production, confirming that field plant yield responses to soil nutrient availability were strongly modified by climate. Bringing these soils into a climate-controlled environment did not improve the relationship between Olsen P and DM yield demonstrated at field sites. Changes in herbage nutrient content as soil P history changed indicated that P availability in these soils was a major factor limiting plant growth, probably followed by N or S, which become limiting when P availability is adequate to high.

In the second glasshouse study (nutrient uptake to exhaustion), ryegrass yields were strongly correlated with various soil tests for N, S and P availability. Variation in S limited yield was strongly correlated with variations in hydrogen peroxide extractable HI reducible S (a new soil test). Large dry matter yields on small volumes of soil indicated that these soils have large pools of plant-available or mineralisable P and S, and, relative to plant demand, small pools of mineralisable N. This result suggests that without other nutrient or climate limitations, the rate of N cycling in these soil/pasture/animal systems is central to pasture growth, and that the size of the soil mineralisable N pool, although still related to pasture N supply, is of secondary importance. Results from both glasshouse experiments provided more strong evidence that the Olsen P soil test is a valuable soil fertility indicator of plant-available P, N and S on legume-based pastures with a history of superphosphate use.

To accommodate variations in climate at each site, daily soil water balance models were produced for each climate regime, and soil-limited evapotranspiration was calculated. Pasture growth was expressed per mm of soil-limited evapotranspiration. Growth rates per mm of evapotranspiration were strongly related to available P status at each site. A new model for the prediction of field pasture production, incorporating the effects of weather and soil fertility status, showed the potential to closely predict actual pasture yield, with the exception of growth after severe drought conditions. It is hoped that discrepancies between the modelled and measured production may lead to useful speculation and further research on the interacting effects of weather and fertility on pasture growth.

The suitability of the BiologTM GN microtitre plating system was assessed as an indicator of soil quality (microbiological functional diversity) across the 12 test hill soils. Saline extracts of the different fertility status pasture soils contained significant amounts of readily-available C, which influenced the substrate use patterns of the soil-inoculated BiologTM assays. This effect must be corrected for before substrate use patterns are interpreted.

After further testing of the BiologTM microtitre plate system, for use as an indicator of soil microbial functional diversity, it was concluded that mostly common substrates were used by all 12 soils, indicating reasonably similar microbial diversity. The small differences in observed substrate use patterns were not simply correlated to the fertiliser histories and soil fertility of these hill pasture soils. The nature of microbiological growth on the C substrates was inherently complex, and did not allow simple interpretations of substrate use patterns with respect to soil fertility change.

In this single landuse suite of soils, microbial functional diversity did not differ greatly between soils, and the ability of the BiologTM system to separate the soils, on the basis of microbial functionality could not be evaluated.

Future Research

In conclusion, a number of topics relevant to the sustainable management of soil fertility in hill pastures were examined in this thesis and a number of key areas have been identified as the focus for future research in grassland agriculture:

1. Further development and evaluation of the new soil S peroxide test, including assessment of the abilities of the soil test to predict S-limited pasture growth
2. The nitrogen economy of legume-based pastures remains a complex issue. Studies focusing on the seasonal rates of N recycling through the soil/plant/animal system at different soil P and S status may help our understanding of 'recurring' value of 1 unit of fixed N, and the economic value of P and S fertilised legume based pasture.

3. Improvement of the predictive power of the climate-driven, soil fertility dependent pasture production model (presented in Chapter 6) under drought conditions. This could involve studies on;

- (i) The affect of soil fertility status on plant rooting depth, and how this effects the readily-available water holding capacity of soil.
- (ii) The behaviour of pasture and soil following a prolonged dry spell e.g. plant survival and rejuvenation, plus a better understanding of soil hydrophobicity.
- (iii) If microbial functional diversity is to be developed as a soil quality index, the effect of soil drying and wetting cycles on soil microbial populations, and related mineralisation/immobilisation of nutrients requires further study.

4. The development of the carbon bioassay for soil extracts using microtitre plates (described in Chapter 7) could be extended and trialed as a soil quality index.

5. The development of reliable and simple indicators of soil biological 'quality' and 'health', for use by researchers, producers, and governing bodies alike. Such indicators may involve measures of the bio-availability and turn-over rate of soil carbon, in combination with more accurate description of dominant soil decomposer species (and how the structure of soil microbial populations varies with changes in soil management). The potential of using BiologTM microtitre plate type whole soil assays should be further investigated for this purpose.

APPENDIX 2

LITERATURE REVIEW (Moir, 1994)

2.1 SOILS, GEOLOGY AND CLIMATE OF WAIRARAPA HILL COUNTRY

2.1.1 Introduction

Excluding the alluvial Wairarapa basin, the landscape of the Wairarapa region is predominantly steep or easy rolling hill country. Large areas of hill country exist, both on the east coast, and bordering the steep Tararua and Rimutaka Ranges to the west.

Soils in this region are formed from a variety of rocks, mainly mudstone, sandstone, greywacke and limestone. Unlike much of the North Island, these soils are outside the dominant influence of volcanic ash.

Topography has a large influence on climate in the Wairarapa, and hence on soil formation patterns. The mean annual rainfall ranges from 800 mm (or less) in some coastal areas, to above 1800 mm in some areas near Mauriceville, and near the ranges to the west. Soil groups include central yellow-brown earths (YBE), yellow-grey earths (YGE), and yellow-grey earth/brown earth intergrades (Heine *et al.*, 1975).

Climatically this region has a wider range of conditions than other parts of the North Island. Mean annual rainfalls lie mostly between 850 - 1000 mm on the dry alluvial plains, and in coastal areas, and rise to more than 1800 mm in inland areas. On lowland areas east of the mountain ranges rainfall is unevenly distributed, and there is a strong tendency to dry periods in summer and autumn with droughts in lower rainfall areas. Dry westerly winds are common in the Wairarapa, and reduce the effectiveness of the rainfall. Occasionally, high intensity rains cause widespread slip erosion and flooding. The native vegetation was mainly broadleaf podocarp (and beech forest in the high rainfall and lower fertility of the western ranges), and coastal forest and bracken fern on the dry coastal hills.

Climate

New Zealand, lying as it does in mid Ocean and within temperate latitudes (temperate oceanic), has an overall temperature and insular climate, for the most part without extreme seasonal or daily fluctuations of temperature. It lies in the zone of prevailing westerly winds, and, although it is too narrow to have a very marked effect on the

general temperature of the air, its high relief has a profound effect upon airflow and upon the vertical distribution of temperature and moisture in the lower layers. The prevailing westerly winds are forced up and aside by the mountain ranges causing considerable diversity of rainfalls. In general the more directly a locality is exposed to the westerly wind and the higher it is above sea level the greater is the rainfall. Soil development is strongly influenced by seasonal variation in moisture regime. Day length fixes the time available for photosynthesis, and since it increases southward in summer it reduces the significance of the temperature contrast between the north and south of N.Z. in determining the annual moisture requirements of plants. In eastern districts hot dry föhn winds from the north-west frequent in spring and summer, and tend to extend the periods when soil moisture is deficient for plant growth.

Thus the Wairarapa region experiences a wide range of climatic conditions, strongly influenced by orographic conditions, and controlled to a large extent by the axial ranges. Annual rainfall varies considerably across the region, ranging from 800 mm on the dry alluvial plains to 1600 mm and above in the inland hill country. Most of the eastern coastal hill country has an annual rainfall below 1200 mm, and as a result of the rain shadow effect of the Ruahine and Tararua ranges it receives very little rain from the west. The eastern hills are exposed to persistently strong westerly winds, which deplete soil moisture reserves and retard pasture production. Wind is an agriculturally significant element of the Wairarapa climate. The coastal ranges cause a rain shadow effect in inland areas from S-SE rain, and have an important orographic effect in localising rainfall. The effectiveness of rainfall is reduced by strong westerly winds in spring and late autumn. Generally the climate is hot and dry in summer and cold and wet in winter. Summer temperatures frequently rise above 25°C in sheltered inland areas, and may exceed 32°C. Winter frosts are common.

Dry periods and drought in central Wairarapa often occur annually, and significantly reduce pasture growth in late spring, summer and autumn (Noble, 1985). It is unusual for the region to experience at least one period of low rainfall each year especially during summer months, and it is common for dry periods to last about 20 days. Easterly and southerly winds usually bring rain and colder temperatures, and are more frequent in winter. Westerly winds predominate during the spring and Autumn equinoxes, are occasionally of gale force and frequently strong enough to significantly affect plant growth through the rapid depletion of soil moisture. Northerly winds are generally hot and dry, desiccating pasture on north or north-westerly facing slopes, especially in the summer months. On exposed faces, the upper slopes and ridges tend to be more severely desiccated than the lower slopes. The more sheltered south-westerly scarps are subject to far less moisture variation.

Geology

In geological terms, the collision of the Pacific and Indian-Australian plates has dominated the formation of the regions landscapes. The region is strongly faulted with splinter faults of the main alpine fault causing the pronounced north-east/south-west trend of the hills and valleys. The north-east/south-west trend of the axial ranges, inland valleys and coastal hills can be thought of as parallel wrinkles or furrows caused by the collision of two of the great plates that make up the earths surface (Moore and Speden, 1984).

The higher axial (western) ranges consist of older greywacke and argillite rocks, and the plains have been built up from aggradational gravels deposited as fans, river terraces and floodplains through the erosion of these ranges during the late Quaternary. Most of the coastal hill country, however, consists of a complex mixture of softer rocks of Tertiary and late Cretaceous age - marine sandstones and siltstones, and bands of harder, upstanding limestone (Moore and Speden, 1984). Scattered throughout the pockets of more erodible Tertiary rocks - mudstone, greensand and conglomerates. All of these climatic and geological factors have predisposed the hill country of the region to widespread erosion.

The soils of the vast area of Wairarapa hill country have not been mapped in detail and are not well understood (Molloy, 1988). In the Wairarapa, steeplands predominate, and soils developed in siltstone are the most widespread. Here, periodically, the soil slips, exposing the underlying siltstone. Since forest clearance, over 40 percent of these hill soils have eroded and the youngest slips are still bare. After five years, pasture growth on the truncated soil is still only 20 percent of that on the undisturbed soil. Even after 20 years the soil is still recovering its former ability to store moisture and nutrients, for pasture production has increased to only 75 percent of the level of the undisturbed soil. Low rainfall and strong north-westerly winds lead to summer soil moisture deficits. Hillsides usually start drying out in October and pasture growth has ceased by February. Soils with a north-westerly aspect dry out earlier than those on the more sheltered, shadier south-easterly slopes.

However, there are significant areas of soils on mudstone which are fertile but exhibit problems of deep-seated mass movement similar to those in the East Cape-Gisborne area.

The Wairarapa is dominantly steep and moderately steep land produced by recent and

rapid dissection of soft Tertiary and Pleistocene sediments, and by slower dissection of older and harder greywacke rocks of the mountain ranges to the West (Gibbs, 1968). Generally speaking, heading west to east, soils are YBEs, alluvial recent soils, then limestone capped hills (melanic) draped with pallic soils. Eastern hill country could be YBE, YGE or intergrades. Light dustings of loess and or tephra could also be present.

Rock types in the Wairarapa are sedimentary, and form very complex patterns. Accumulation, consolidation or induration of sands, silts and muds have resulted in lithologies ranging from Quaternary alluvial and aeolian deposits, Tertiary mudstones, siltstones, sandstones and limestones, to Mesozonic argillites and greywackes. Tertiary sediments of sandstone, siltstone, mudstone or limestone comprise the majority of hill country, especially in eastern parts of the region (Gibbs, 1968). Part of the region is covered by loessial deposits which mantle, either wholly or in part, the underlying sediments. The five most extensive rock types, which together comprise over sixty percent of the region, are loess, jointed mudstone, greywacke, argillite and alluvium.

The eastern hills are composed of marine sediments of Tertiary age that increase in age and hardness eastward, and have been uplifted, folded and tilted. The predominant landform is of ridges and valleys that have been finely dissected by streams and rivers that have only small flood plains. The eastern area is prone to accelerated erosion. The western ranges comprise the Tararua and Rimutaka Ranges composed of Mesozoic greywacke rocks. Yellow-brown earths are commonly only in areas of higher rainfall i.e. above 1270 mm, but in the 1020 - 1270 mm zone, soils are more like yellow-grey earths if they have sandy parent materials and occur on rolling slopes.

Soils with parent materials containing limestone occur in this region and are notable for their strongly developed structure, moderately high content of cations, and brown colours. They have the highest natural fertility of all soils of the eastern hills and many sheep and cattle stud farms are located on them.

The soils of the western ranges are steepland soils, and are yellow-brown earths derived from greywacke or erosion products of greywacke.

2.1.2 Yellow-Grey Earths of the Wairarapa

The YGEs occur under a mean annual rainfall of 750-1000 mm in the Wairarapa, mid-Hawke's Bay and Manawatu regions. These soils tend to be silt loams which, except on the steeper hills, are inclined to have compacted subsoils and suffer from impeded drainage. Many of these soils are derived from river alluvium.

The YGEs of the Wairarapa are therefore confined to drier, mostly south-western (Martinborough) and coastal areas. It must be noted, however, that the YGEs of this region usually form under mean annual rainfalls of 900-1000 mm per annum, with slightly damper and cooler summers than similar soil types in mid-Hawke's Bay.

A typical Wairarapa YGE is the Kokotau silt loam. This soil is found on rolling land in extensive areas around Carterton, Martinborough and Masterton. It is a poorly drained soil formed with shallow deposits of loess which overlies fine sandstone and siltstone. In the past, pasture production on this soil type has been measured by Bircham and Crouchley (1976). Paddocks with a fertiliser history of 250-400 kg/ha/yr superphosphate were top dressed at a rate of 125 kg/ha/yr superphosphate for a five year period. Pasture production was maintained above 90% of maximum DM production at this rate of application.

In spite of their relatively long duration, these trials did not continue for a sufficiently long time to erase the residual effects of past fertiliser history. However, they suggest low maintenance requirements for sulphur (S) (During, 1984c). Pasture growth response to phosphate fertiliser on these soils is estimated to be similar to that on central YGE to YBE intergrades. During (1984c) recommends applying 175 kg/ha/yr superphosphate and 375 kg/ha/yr lime in order to maintain pasture production at 90% of maximum.

2.1.3 Yellow-Brown Earths of the Wairarapa

In the Wairarapa, YBEs are found extensively in the areas of Ekatahuna, Mauriceville, Pahiatua, Alfredton, Pongaroa and within the hill country of the ranges to the west. These soils are developed under an annual rainfall exceeding 1100 mm. They are formed from sediments ranging from claystones to conglomerates and from soft bentonitic mudstones to hard greywacke sandstones. In general, the YBEs have been more strongly weathered, have topsoils with higher organic matter contents, and are more free-draining, more friable, and are more phosphate deficient than the YGEs.

In this current study, trial areas were situated on four different yellow-brown earths. The Waimarama (Mauriceville, sites 1, 2 and 3) and Kourarau (Gladstone, site 10) soils are developed on calcareous siltstones and sandstones and are weakly to moderately leached. However, in this particular instance, the Kourarau soil at Gladstone has been classified as a central brown rendzina due presumably to high content of calcareous materials. The Matamau soil (Mauriceville, site 4) is usually classified as a YBE/YBL intergrade, but in this situation is a very strongly leached yellow-brown earth. This soil type is developed from pumiceous sandstones and mudstones. Lastly, the Kaikouta soil (site 14, near Tararua ranges) is a

strongly leached yellow-brown earth, derived from loess and sandstone (greywacke).

Initial P requirements on YBEs are high, but the residual effect of a capital fertiliser dressing is considerable (During, 1984d). This was shown by a rate of P mowing experiment where over a period of six years one initial application of 1920 kg/ha superphosphate led to pasture production approximately equal to that achieved by applying the same total amount evenly in six annual dressings of 320 kg/ha.

On a Mikimiki hill soil which had previously received 280 kg/ha superphosphate fairly regularly, with a Olsen P test of 10 (mgPdm⁻³ soil), Crouchley and Sinclair (1982) observed very small responses to rates higher than 280 kg/ha/yr superphosphate. In the same experiment these authors saw virtually no effect of omitting sulphur (P applied as MCP) application over a three year period. This soil's sulphate retention capacity was high for a YBE, which may have contributed to high extractable sulphate status of the unfertilised treatments. Historically, many farmers on soils developed from greywacke preferred basic slag as a fertiliser, which contains little S compared to superphosphate (During, 1984d).

During (1984d) recommends applying 230 kg/ha/yr superphosphate on these yellow-brown earths, lime at 300 kg/ha/yr (if required), potassium (if required) and molybdenum if a deficiency is apparent.

2.1.4 Wairarapa Yellow-Grey Earth to Yellow-Brown Earth Intergrades

In the Wairarapa, YGE/YBE intergrade soils can be found in large areas along the hilly east coast. These soils are associated with mean annual rainfalls ranging from 1000 to 1400 mm, usually in the area of 1150 mm.

Most of the YGE/YBE intergrades comprise soils on steep or hilly terrain, so that the effective rainfall is lower than that indicated above. The rejuvenation of soil profiles by sheet and other forms of erosion might be modifying factors in their development. Evidence suggests that many of these soils are only moderately weathered, containing some illite, hydrous micas, and much vermiculite (NZSB, 1968).

The majority of soils in this group, as well as containing exchangeable potassium (K), are expected to have non-exchangeable but plant-available potassium reserves. For this reason, the routine exchangeable K soil test may not be reliable, and the NaTBT test may fulfil a useful function (During, 1984c). Another feature of the YGE/YBE intergrades is that, in general, they have low phosphate retention, and also low sulphur retention, which may lead to higher sulphate leaching than on YBEs.

In this current study, three of the research areas are situated on YGE/YBE intergrade soils. The Atua soils (Gladstone, sites 11 and 12) are moderately leached YGE/YBE intergrades, and are silt loams over clay loams derived from siltstone (NZSB, 1968). They are extensive in Wairarapa hill country, and with light phosphate topdressing maintain excellent pastures for sheep and cattle breeding and fattening. Gibbs *et al.* (1968) showed that higher rates of superphosphate, 250 kg/ha/yr or above, are far more necessary on the Atua soils than on the Wanstead soils (discussed later in this section). In addition, over the common pH range for pasture (5.0-6.2), the pasture vigour for the Atua soils was much more sensitive to pH change than that on the Wanstead soils. This may have been due in part to a molybdenum deficiency on the Atua soil (not deficient on the Wanstead soil). In molybdenum-deficient areas the application of 50-100 g/ha every 4-5 years of sodium molybdate is advised if soil pH remains below 6.2 (During, 1984c).

The Wanstead soils, represented in this study by the Whareama sites 5, 6, 7, 8 and 9, are hill soils found on or very near the east coast. They are sticky clays, and are fertile but unstable. These clays are montmorillonitic, and are derived from bentonitic mudstone (NZSB, 1968). Again, these soils are phosphate deficient, and probably sulphur deficient. Gibbs *et al.* (1968) observed that on this soil type, there appeared to be little benefit from applying superphosphate at rates above 250 kg/ha/yr. According to Gibbs *et al.*, the greatest increase in pasture growth to the high P input as compared with medium P input, occurred at pH 5.0-5.5. This suggests that P requirements for maintenance are higher at low pH than high pH, an observation confirmed by more recent field trials in Wairarapa hill country (During, 1984c).

The final soil in this study (Site 14) is classified as being part of the Kohinui series (Pollok *et al.*, 1994). This soil is not found on sloping hill country, but is a soil present on the alluvial flats of the Wairarapa Plains, in this instance at the Massey University farm "Riverside". This is a stony soil associated with strongly leached intergrades between yellow-brown loams and yellow-brown earths. The soil consists of a relatively shallow silt cover over deep stony deposits, which cause excessive drainage. For this reason, and because of the uneven spread of rainfall experienced, this soil tends to be droughty in summer. Parent material is mixed alluvial, loessial and volcanic ash composition, which overlay the gravel beds (below 60-70cm). Phosphate retention is in the medium range (40-60%) for this soil type (Pollok *et al.*, 1994), possibly due to the presence of allophanic material.

Fertiliser requirements for YGE/YBE intergrades of the Wairarapa region are presented by During (1984c). He gives a figure of 140-160 kg/ha/yr superphosphate for sheep farms that are not potassium deficient, with 300-350 kg/ha/yr of lime. At this level of application, a relative dry matter production of 90% (i.e. 90% of maximum yield) could be expected on

well farmed properties carrying 11-13 ssu/ha.

For soils such as the Atua set, it is likely that maintenance requirements for phosphate are higher than those mentioned above, and molybdenum may also be a requirement.

However, one important point should be kept in mind when comparing the recommended "maintenance" levels stated by During (1984c) with present-day fertiliser requirements. Many of the fertiliser recommendations of During are based on studies of the 1960s and 70s. Since this time, the responsiveness of pasture production to fertiliser may have changed. Hence if fertiliser and lime have not been applied at maintenance rates, the optimum rates of fertiliser calculated in the 1950s, 60s and 70s may no longer be appropriate.

For example, during the rural economic downturn of the 1980s less fertiliser was applied for 5-10 years, but stock numbers were not decreased in proportion. This may lead to higher fertiliser requirements than those outlined in the 1970s. Likewise, if the maintenance fertiliser rates of the 1970s had been applied for the previous 20 years, but stocking rates had increased over this time period, we would expect the fertiliser requirements of today to be higher i.e. fertility levels have declined due to greater levels of stock transfer and nutrient loss. Even if the stocking rates and maintenance fertiliser applications of 20-30 years ago have been maintained consistently to present day, this gives no guarantee that the maintenance rates of 20 years ago will match the maintenance rates of today.

Another scenario may also exist, where both stocking rates and applied fertiliser have been increased over the past 20 years. In this situation soil fertility levels may have remained static, or increased over the past 20 years. This may have the effect of increasing plant-available nutrient reserves, subsequently reducing the maintenance fertiliser requirement when compared to that of 20 years ago.

2.2 FACTORS INFLUENCING THE FERTILITY STATUS OF WAIRARAPA SOILS

2.2.1 Sources of Phosphate in Soil Parent Rocks of the Wairarapa

Phosphate (P), like many nutrients required for plant growth, has to be derived from soil parent materials (primary and secondary P minerals) or added fertiliser. The quantities and sources of this phosphate can vary widely.

Until recently, there has been little published work on phosphate levels in New Zealand rocks, and the minerals they are sourced from. Eden and Parfitt (1992) studied this subject in detail, focusing on the hill country of the Wairarapa region. Much of what follows is a summary of their findings.

The aforementioned study reported on the acid-soluble phosphate levels of Wairarapa soils in order to provide information on the reserves of P. This information was thought to be helpful in identifying the parent materials which have the largest amounts of phosphate, and which may be available to plants under regimes of low phosphate fertiliser additions (Eden and Parfitt, 1992).

The general findings of this study were that the highest phosphate concentrations occur in mudstones. These contained 50% more phosphate than fine to medium sandstones, and at least ten times more phosphate than coarse sandstones. Also, most of the phosphate was found in the fine-medium silt fraction (20-2 mm), and in the clay fraction (<2mm).

This phosphate is associated with calcite or apatite mineral grains, which tend to have large reactive surfaces (Eden and Parfitt, 1992). These will dissolve rapidly in this soil environment, due mainly to acid leaching, and will release phosphate into the soil.

This process was noted by Walker and Syers (1976), where they observed that upper soil horizons may be depleted of phosphate as a result of weathering, plant uptake and leaching, and are often acidic as a result of the excess winter rainfall and the influence of indigenous forest. They also observed a relationship between particle size and phosphate content, and they found that sandstones had a lower rate of P loss than mudstones. They identified apatite as the major primary mineral, which had been previously noted in soils by the New Zealand Soil Bureau (Saunders, 1968).

Based on this information, areas where soils are formed on mudstone would be expected to have a high total soil P content. In the current study, this would include sites 5, 6, 7, 8 and 9 (Whareama), and site 10 (Gladstone). Sites 1-3 (Mauriceville) would also be expected to contain relatively high native P, due to the limestone parent material of the soil, which may contain apatite grains (calcium phosphate).

Eden and Parfitt (1992) drew a number of conclusions from their recent study of phosphate minerals in soils of the Wairarapa. They implied, from their results, that soils developed on mudstones are likely to be more productive than those on sandstones for pastoral farming under a regime of limited P input, particularly where deep-rooting grasses are planted (Eden and Parfitt, 1992). Deeper rooting plants would also have better access to the soil profile, and

hence to soil water. They also suggested that mudstones are more erodible than other soil types, and so it may be wise to plant steeper slopes in forestry.

It must be noted, however, that native supplies of soil phosphorus are considered to be inadequate for improved pastures on these soils (Saunders, 1968).

2.2.2 Evaluation of Fertility Status

At present, there is little published information on the current pasture growth response to fertiliser application on Wairarapa soils. However, one study was conducted in the early 1980s, investigating the response to phosphorus and sulphur fertilisers on Wairarapa hill country soils (Crouchley and Sinclair 1982). The purpose of these experiments was to determine whether current fertiliser practise was providing adequate P and S for pastures on these soils to maintain improved pasture species, and higher stocking rates.

Over a two and a half year period Crouchley and Sinclair looked at the effect of P and S additions to six Wairarapa hill soils. Phosphate was applied at three rates; 25, 50 and 100 kgP/ha as monocalcium phosphate (MCP), while sulphur was applied at two rates; 30 and 60 kgS/ha as gypsum. All trial sites had been top-dressed annually for at least 10 years prior to the experiments, with about 250 kg/ha superphosphate. Lime had also been applied on several occasions within this period.

The results of this study were that pasture on all soils responded to phosphate, but not always to sulphur (Crouchley and Sinclair, 1982). Twenty-five and 50 kgP/ha gave very similar responses, but often 100 kgP/ha was superior (Crouchley and Sinclair, 1982). Thirty and 60 kgS/ha gave similar responses.

Although all of these sites had received 250 kg/ha of superphosphate for at least 10 years, they were all still responsive to phosphate. This indicated that this rate had not been excessive but appeared to be adequate since 25 kgP/ha (equivalent to 250 kg superphosphate) was generally as effective as 50 kgP/ha in the subsequent trials (Crouchley and Sinclair, 1982). The higher response from the 100 kgP/ha treatment was unexplained.

The authors concluded that annual applications of 250 kg/ha superphosphate would provide adequate P and S for maintenance of improved pasture on Wairarapa hill-country soils. They also commented that applications should preferably be made in spring to avoid leaching of sulphate by winter rainfall (Crouchley and Sinclair, 1982).

In 1992, a detailed survey was conducted investigating fertiliser use (predominantly nitrogen

fertiliser) on sheep and beef farms of the East Coast (Morton et al., 1992). The survey, conducted by MAF, involved locating 78 farms which had used nitrogen fertiliser in some form over the past 4-5 years.

This survey revealed that most fertiliser nitrogen was applied to the survey farms as DAP, Urea, Cropmaster 20, and Ammonium Sulphate (in descending order). Feed demand was rated by farmers as being the most important factor influencing the timing and quantity of fertiliser nitrogen. In addition, the farmers considered the main benefits of fertiliser nitrogen to be the lower susceptibility to drought and greater feed supply during winter and early spring.

This survey also showed that the farmers who were using more nitrogen fertiliser were also applying more phosphorus, sulphur and lime. However, it appeared that where nitrogen fertiliser was being applied in only very small amounts, P and S was applied solely in the form of superphosphate. It should be noted that in this latter case, P and S inputs were only marginally lower than on farms applying large amounts of N, and in fact were considerably higher than those farms applying moderate levels of N fertiliser.

2.2.3 Nutrient Accumulation and Transformations Under Pastoral Grazing Systems

This section reviews literature on the accumulation and transformations of soil nutrients due to fertiliser application and climate, and how this may influence soil fertility status.

2.2.3.1 Phosphorus

P Nutrition of Pasture Species

Processes involved in pedogenesis cause phosphorus to be lost from the soil by leaching and erosion (Saunders, 1968). Since the only significant source of P for soils is the parent rock, all soils will gradually become P deficient over time. New Zealand soils are no exception, and it was identified as early as the late 1800's that pasture production in New Zealand was P limited. In order to compensate for this P loss, and higher demand for soil P, the farmer applied fertiliser (predominantly single superphosphate).

Phosphorus plays important roles in pasture; being a component of the photosynthetic process as a key element involved in the synthesis and transfer of chemical energy to plants (Hedley *et al.*, 1993), and as a requirement of legumes for nitrogen fixation. High levels of available P in soils are required in order to maintain the presence and N₂-fixing activity of the clovers in the pasture, and hence maintain the N input into the grass/legume sward (Haynes and Ludecke, 1981). Clovers in the pasture environment are said to be "nutritionally fragile", i.e. are less able to compete with grasses for growth-limiting nutrients, and therefore

continuing inputs of P and S are needed to maintain active clovers (Ball and Tillman, 1994). Since pasture production in New Zealand has been traditionally dependant on the clover component of the sward to supply soil nitrogen (rather than N fertilisers), the application of superphosphate has played a very important role.

Ball and Tillman (1994) reviewed the topic of efficiency of nutrient use by pastures in New Zealand. They argued that nutrients applied in fertiliser (especially P) were wasted in order to provide elevated soil nutrient levels for clover growth and N fixation. This was based on the assumption that excessive P uptake by grasses leads to greater animal transfer loss of P. The authors commented that Hart (1989) had found that P levels were about twice as high in root nodules than in young mature leaves of white clover, and that high levels of inorganic P had to be present in nodules for symbiotic N₂ fixation to occur. Using this information, Ball and Tillman then inferred that clovers may have nutritional P inefficiencies, and that providing elevated soil P levels (via fertiliser) for clover N₂ fixation would therefore be wasteful (Ball and Tillman, 1994). The authors then suggested that nutrients directed at clovers were instead being taken up in luxury quantities by grasses. Since grasses are the main component of the sward, it was suggested that nutrients (P, S) could be used more efficiently if new grass species could be bred which were largely incapable of luxury nutrient uptake (Ball and Tillman, 1994).

Soil P Status

The ability of a soil to supply phosphorus to the plant is related to the total amount of P present in the soil, and also to the forms in which it exists. The following review looks at the accumulation (from fertiliser application), and transformations of phosphorus in New Zealand based pastoral grazing systems. This accumulation of P, and its subsequent availability to pasture, will, to a certain extent, dictate the relative importance and efficiency of providing P fertiliser.

Soils under permanent pasture in New Zealand develop high organic matter contents because of the continual supply of plant debris and animal dung returning to the soil surface. Since organic compounds containing phosphorus are present in soil organic matter, it would be expected that such soils would have high levels of organic phosphorus, in part derived from applied phosphate fertiliser (Jackman, 1954a).

During the mid-1950s Jackman (1954a) investigated the relationship between the conversion of applied phosphorus into organic forms, and the subsequent availability of this phosphorus. He commented that soils carrying pastures responsive to additional phosphate often contain high levels of organic phosphorus, and that it was presumed that this form is only slowly

available to pasture plants (Jackman, 1954a). If this theory held true, then the change of fertiliser phosphorus into organic form is significant, since it implies a reduction in phosphorus availability.

Jackman examined the increases in total and organic phosphorus on a variety of soil types, where either superphosphate, animal manure or lime were applied. It was found that on soils with high organic phosphorus contents (e.g. Yellow-brown loams), applied phosphorus was largely converted into organic forms (Jackman, 1954a). In comparison, on soils with low natural organic phosphorus levels (e.g. Manawatu silt loam), phosphorus applied as fertiliser tended to remain in inorganic forms.

From these results Jackman suggested that pastures on soil groups such as yellow brown loam and yellow brown pumice soils, which commonly respond to topdressed phosphate, may be responding to a nutrient deficiency rather than the physical nature of the soil limiting plant growth. He further suggested that if the phosphate were not immobilised to the same extent, the level of fertility would be higher on these soils (Jackman, 1954a).

In a further study, Jackman (1954b) investigated the relationship between the organic phosphorus content of soil, and various soil characteristics. Results showed that the organic phosphorus was highly correlated to the organic carbon and phosphate-fixing capacity of the soils used.

He concluded from these findings that based on very high levels of organic carbon and phosphate fixing capacity, that allophane was probably responsible for the stabilisation of carbon and organic phosphorus in yellow-brown loams and pumice soils (Jackman, 1954a, and b; 1960). Other clay minerals were said to play a similar but less dominant role.

Many years later, Perrott and Mansell (1989) studied the effect of fertiliser phosphorus and liming on different soil phosphorus fractions. In this study the authors described the accumulation of inorganic and organic phosphate levels in New Zealand soils, which have built up as a result of pasture establishment and topdressing with phosphatic fertilisers (Perrott and Mansell, 1989).

This trial was conducted on a Kokotau silt loam near Masterton, where MCP was applied at 0, 20, 40, 60 and 80 kgP/ha, and lime applied at 0, 2.5 and 5 t/ha. Herbage was harvested on a regular basis from stock exclusion areas, and at the conclusion of the trial (5 years) a detailed fractionation of soil phosphorus was undertaken.

The results from this experiment showed that where fertiliser P was applied, P accumulated as

adsorbed inorganic P, and as occluded inorganic P (Perrott and Mansell, 1989). Also, fertiliser did not cause an accumulation of organic P, but it did increase the more labile forms and decrease the more stable forms of organic P. Changes observed in P fractions were attributed to the increase in nutrient cycling when fertiliser and/or lime was applied (Perrott and Mansell, 1989).

Perrott *et al.* (1989) also investigated the accumulation of phosphorus on yellow-brown pumice soils. On this occasion the author sampled a variety of YBP soils in the Taupo region, ranging from undeveloped land to high producing dairy farms. Again, a sequential P fractionation was used, this time to compare total soil P with microbial P and Olsen P.

It was shown that most of the inorganic P in these pumice soils originates from fertiliser application, as the inorganic P levels in samples from virgin sites were very low (Perrott *et al.*, 1989). Total soil P was used as a basis for comparing accumulation patterns i.e. fertiliser history. The more labile P fractions increased curvilinearly with total P, as did total organic P (Perrott *et al.*, 1989). All the other P fractions increased linearly with total P. The results suggested that at high levels of P application, when bioproductivity is no longer responsive to P, organic P ceases to accumulate with increased P addition, and the extra P accumulates as inorganic soil P forms.

Nguyen *et al.* (1989) re-emphasised that superphosphate application over several years can build up P and S reserves to a level where only a maintenance rate of superphosphate is required to offset losses from the soil-plant-animal systems. These authors demonstrated this using data accumulated over a 34 year period from a grazing experiment on a Lismore stony silt loam in mid Canterbury.

The grazing experiment mentioned was conducted at Winchmore Irrigation Research Station from 1952 to 1985. Detailed measurements of soil fertility and pasture production were made over the duration of the trials. This allowed Nguyen *et al.* (1989) to examine the influence of long-term superphosphate applications to irrigated pastures on dry matter production, and the accumulation of S and P reserves which may effect the residual value of superphosphate (Nguyen *et al.*, 1989). After detailed examination of this historical data, Nguyen and co-workers drew a number of conclusions. Firstly, they concluded that in the initial stage of pasture development, the absence of P or S fertiliser inputs can lead to a severe reduction in pasture production, and a major deterioration of pasture quality i.e. towards weed species. It was concluded that superphosphate applied at 21-24 kgP/ha/yr was sufficient to satisfy phosphate maintenance requirements, and excess sulphur requirements for irrigated pastures (Nguyen *et al.*, 1989). Superphosphate applied at above maintenance rates for six years did not safeguard against a yield reduction in the first season after discontinuation of fertiliser

(Nguyen *et al.*, 1989). However, superphosphate applications over several years built up soil P and S reserves that may contribute significantly to plant uptake. Under this situation, the residual P may be able to maintain pasture production and improved pasture species without any yield reduction for a longer period than that observed under short-term P applications (Nguyen *et al.*, 1989). These authors also suggested that because soil organic S, and organic and inorganic P can accumulate in pastoral soils, especially from superphosphate application, these reserves should be taken account of in conjunction with standard soil tests when assessing pasture requirements (Nguyen *et al.*, 1989).

Phosphorus and sulphur accumulation resulting from long-term superphosphate applications was studied by Lambert *et al.* (1988). The accumulation of these nutrients was monitored over an eleven year period at Ballantrae Hill Country Research Station. Research farmlets received applications of either 125 or 490 kg/ha/yr of superphosphate. Results showed that 50% of the extra P applied (above the lower rate of application) was present in the top 75mm of the soil at the trial completion (Lambert *et al.*, 1988). In contrast, these workers found that only 14% of the extra S applied accumulated in the topsoil. This rate of accumulation was said to be similar to the rate of accumulation of organic P (Lambert *et al.*, 1988).

Furthermore, the authors concluded that hill soils with a significant history of superphosphate application could be expected to have much larger available P than available S reserves (Lambert *et al.*, 1988). Also, when the direct effects of slope on superphosphate application rate were taken into account, it was found that slope did not affect the rate of P or S accumulation in the top 75mm of the soil (Lambert *et al.*, 1988).

Perrott and Sarathchandra (1989) studied phosphorus in the microbial biomass of New Zealand soils under established pasture. This is significant as soil microbes play a central role in the mineralisation and immobilisation of plant nutrients, and hence soil nutrient cycling. Soil samples were collected from sites on the major soil groups, and a extraction method was used to determine the P content of the microbial biomass.

The resulting data showed that the microbial P in the top 7.5cm of the soils ranged from 10.5 to 57.4 kgP/ha (mean 31.8 kgP/ha), which could amount to several times that in the standing crop of the above-ground pasture (Perrott and Sarathchandra, 1989). The extracting solutions used were found to extract a labile fraction of organic P from soils. Perrott and Sarathchandra (1989) concluded that the P status of New Zealand soils under pasture could be one factor influencing the amount of P in the soil microbial biomass.

A further study by these authors (Perrott *et al.*, 1990) looked at the subject of soil organic phosphorus in greater depth. An experiment was established on a yellow-brown loam near

Matamata, under grazed dairy pasture. Various seasonal and fertiliser effects were monitored over a two year period, including soil microbial properties, forms of soil P and organic matter in the soil.

It was found that topdressing with potassic superphosphate increased total inorganic phosphorus, NaHCO_3 -extractable inorganic phosphorus, but did not affect organic forms of phosphorus, microbial biomass phosphorus or organic debris (Perrott *et al.*, 1990). However, the amounts of phosphorus released from labile organic and microbial phosphorus during spring were large (29 kgP/ha) which could contribute substantially to plant P requirements (Perrott *et al.*, 1990).

Finally, the authors concluded that a number of seasonal mechanisms were responsible for changing the forms of biomass P in the soil at any one time. These included changes in fungal and bacterial populations during winter, mineralisation of labile inorganic P (P_i) in spring due to increased bacterial growth (promoted by plant growth), and the subsequent release of P from the microbial biomass as a result of bacterial " grazing " by protozoa (Perrott *et al.*, 1990).

In conclusion, all of the studies mentioned have shown an increase in total soil P with the application of P fertiliser. A summary of the observations made in this section can be seen in Table 2.1. Measurement of total soil P in this current study could therefore be useful in determining levels of historical P fertiliser application. The accumulation of soil P could be used to gauge the efficiency of historical fertiliser use when compared with current pasture yields.

Table 2.1 Summary : Observations of P Accumulation and Transformation in N.Z. Pastoral Grazing Systems.

Location	Treatment	P _T -	P _o -	P _i -	Reference
North Island	Tokomaru SL, +P	Yes	Yes	N/A	Jackman 1954a and b
	YBL Soils, +P	Yes	No	N/A	
Masterton	Kokotau SL, + 20-80 kgP+/ha	Yes	No	Yes	Perrott & Mansell 1989
Taupo	YBP Soils, Fertilised Undeveloped	Yes Low	Yes Low	Yes Low	Perrott <i>et al.</i> 1989
Canterbury	Lismore SL, 0	?	Yes	No	Nguyen <i>et al.</i> 1989
	17	Yes	Yes	Yes	
	34 kgP/ha/y	Yes	Yes	Yes	
Various Soils	Major Soil Groups (developed)	N/A	Yes	N/A	Perrott & Sarathchandra 1989
Matamata	YBL, +P	Yes	No	Yes	Perrott <i>et al.</i> 1990
Ballantrae	YBE, +P	Yes	Yes	Yes	Lambert <i>et al.</i> 1988
Various Soils	Observed - Organic Matter	No	Yes	No	Jackman 1964 a and b

P_T = total P, P_i = inorganic P, P_o = organic P, - = increased

Many of these studies reviewed above have shown that the proportion of organic P (P_o) and P_i fractions in the soil vary widely, depending on the degree of soil development (fertiliser history, etc), soil type, and climatic conditions. In light of this review, techniques involving fractionating P forms in soils would appear to provide a useful index of soil P status relative

to past fertiliser history and soil productivity. Greater increases in soil P_o relative to soil P_i would indicate that P application had stimulated the organic cycle i.e. more plant growth, more soil humus, more P_o .

In addition, it appears that Olsen P measurements could also be used as an index of fertiliser history, and could provide useful information for predicting pasture yield and the likely response to fertiliser P application.

These soil P measurements, plus others such as resin-extractable phosphate (Saggar *et al.*, 1992a), could therefore be used as a measure of plant P availability, and as an index of the efficiency of use of P applied as fertiliser in the hill country soils of the Wairarapa.

2.2.3.2 Sulphur

Sulphur (S), like phosphorus, is a key element for plant growth and plays an important role in forming plant proteins. Because the New Zealand farmer has historically applied superphosphate to overcome soil nutrient deficiencies, sulphur as a major component of superphosphate, was applied to pastures on a regular basis. As discussed in the previous section, such long-term applications of fertilisers can cause a subsequent build-up of soil / plant nutrient reserves.

Nguyen and Goh (1990) examined the accumulation of soil sulphur fractions in grazed pastures receiving long-term superphosphate applications. Once again, this study looked at the long-term trials conducted at the Winchmore research station since 1952 (see previous section, 2.2.3.1).

In this study grazed and irrigated pastures were fertilised annually at 0, 188 and 376 kg/ha superphosphate. This provided an annual S input of 0, 21 and 42 kgS/ha/yr respectively (Nguyen and Goh, 1990). Top soil samples were taken at the beginning of each growing season over a 35 year period, and were analysed for changes in different forms of S, organic nitrogen and total carbon.

The results obtained showed that over 95% of soil S was organic sulphur (S_o), with the remainder being adsorbed and readily-soluble S (Nguyen and Goh, 1990). Applications of superphosphate substantially enhanced the accumulation of S_o , but there was no further increase in S_o when S inputs were increased from 21 to 42 kgS/ha. This was attributed, in part, to high sulphate leaching, as the Lismore stony silt loam has a low capacity to adsorb S.

In addition, the accumulation of S_o reached a steady state after 25-27 years, whereas N and C reached this steady state some 3-4 and 9-12 years earlier, respectively (Nguyen and Goh, 1990). The authors suggested that S, N and C were being stabilised independently in organic matter. Therefore the accumulation of S_o was not directly related to the accumulation of N and C in soil organic matter (Nguyen and Goh, 1990).

Carbon bonded and HI-reducible made up the majority of S in the soil, with a greater proportion of carbon bonded S near the soil surface (Nguyen and Goh, 1990). This would be expected, as most plant litter, roots, dung and urine are deposited or situated near the soil surface. Microbial sulphur was not affected by superphosphate applications, and accounted for less than 3% of total soil S in all treatments (Nguyen and Goh, 1990).

In conclusion, these studies have generally shown an increase in total soil S with increased inputs of S fertiliser (superphosphate), mostly in the form of organic S (S_o). Therefore, in this study of Wairarapa soils it may be useful to measure total soil S, because this may reflect the past productivity of the soils as organic S has accumulated over time.

2.2.3.3 Carbon/Organic Matter and Nitrogen

In New Zealand, both climate and the soil environment combine to allow vigorous pasture growth. Consequently, organic matter tends to cycle and accumulate in pastoral based systems, and nutrient demand is high (Jackman, 1964a). The rate at which the organic matter accumulates and cycles will be highly dependant on climate and soil nutrient status i.e. relating to pasture yield. At the forefront of nutrient demand is soil nitrogen.

Although clovers are capable of adding very large amounts of symbiotic fixed N to the soil, many pastures suffer from chronic or near chronic N stress (Ball *et al.*, 1982). The main reason for this is poor distribution of the cycled nitrogen in dung and urine, which often makes nitrogen the key limiting nutrient in pastoral systems (During, 1984a). It can therefore be concluded that at times of rapid pasture growth, nitrogen stress may be considerable (Ball *et al.*, 1982). In this current study, herbage N and P analyses were made in order to identify these periods of N and P stress at the Wairarapa sites

The majority of soil N (95%) is present in soil organic matter and is unavailable to plants (McLaren and Cameron, 1990). Therefore the organic compounds in soil must first be broken down biologically in order to produce plant available N i.e. NO_3^- and NH_4^+ .

When the C:N ratio of plant material is low (i.e. narrow), N quantities will be surplus to microbe energy requirements, and hence plant available N may be released into the soil

(McLaren and Cameron, 1990). In contrast organic matter (OM) with a high C:N (i.e. wide) ratio will break down very slowly in the soil, unless N from other soil sources can be utilised. An appropriate C:N ratio for mature clover would be 20:1, while straw may be 80:1, and soil humus/microbes 10:1 or less (McLaren and Cameron, 1990). For net mineralisation to occur in agricultural soils, it is usually necessary for the C:N ratio of the organic matter to be less than 25:1 (McLaren and Cameron, 1990).

Jackman (1964a) investigated the accumulation of organic matter in pastoral soils in some detail. He reinforced the point that soil organic matter contains organic carbon (C_o), sulphur and organic phosphorus in relatively constant proportions (Jackman, 1964a). Further, Jackman suggested that since these forms are unavailable to plants, increasing amounts of OM must lead to immobilisation of these nutrients, and a consequent reduction in the supply of inorganic forms of N, S and P (Jackman, 1964a). In his study samples were collected from several New Zealand soil types in pasture, then analysed for C_o , S_o , P_o and organic nitrogen (N_o). The first finding of this study was that in general, organic matter had accumulated in the top 75mm, and consequently N, S and P were being immobilised (Jackman, 1964a). In contrast, net mineralisation was occurring in the 75-150 mm horizon. Also, in most of the soils studied, immobilisation of N, S and P exceeded mineralisation (Jackman, 1964a), i.e. soil available nutrient status was declining, constituting a maintenance fertiliser requirement. Further, it was observed that organic carbon, nitrogen and sulphur were present in constant proportions, but organic phosphorus was more variable (Jackman, 1964a). In a subsequent study of these soils, Jackman (1964b) looked at rates of OM mineralisation. He concluded that annual rates of immobilisation of N, S and P in organic forms may be important when compared with the amounts needed to maintain good pasture growth (Jackman, 1964b).

Haynes and Williams (1993) also examined the accumulation of soil organic matter, and organic sulphur, under pasture and the decline with cultivation. These authors used the soils and data set from the previously mentioned long-term grazing trial at Winchmore Research Station. In this study the amounts of C_o , N_o and S_o in the soil were compared between pasture trials receiving 0, 188 and 376 kg/ha/yr superphosphate treatments. These treatments were also compared with a wilderness site not used for agriculture, and an arable site which had been cultivated for eleven consecutive years. Results showed that levels of organic C, S and total N in the 0-4 cm soil layer followed the order of 376 = 188 > control ³ wilderness >> arable (Haynes and Williams, 1993). Compared with the control, accumulation of total N and organic S in the 188 and 376 treatments were proportionally greater than that of C, reflecting the inputs of sulphate S and increased clover growth and N_2 fixation in superphosphate-treated plots (Haynes and Williams, 1993). Mineralisation of S was also measured on these soils (0-4 cm) in an incubation study. The amounts of mineralised S followed the order of 376 > 188 > wilderness > arable > control (Haynes and Williams, 1993). Also, Sakadevan *et al.*

(1993a) found that the amounts of N and S mineralised were greater in soils from pastures which had received fertiliser continuously, compared with those hill pastures that had not received superphosphate in the previous seven years. In both the above studies, more mineralisation was associated with smaller soil carbon : nutrient ratios.

Perrott *et al.* (1992) investigated the effects of season and fertiliser inputs on the organic cycle in a hill country soil (Te Kuiti) under pasture. This included examining changes in soil phosphorus, as well as seasonal and superphosphate effects on soil microbial phosphorus and sulphur, Olsen P and sulphate S.

It was found that although pasture production declined, there was no effects of withholding superphosphate on the soil biological cycle i.e. on soil microbial P and S, total P_o and Olsen P (Perrott *et al.*, 1992). However, seasonal variations occurred indicating storage and release of phosphorus by the soil organic matter and microbial biomass (Perrott *et al.*, 1992).

In conclusion, it is apparent from this review that soil N and S will tend to accumulate with organic carbon in pastoral soils, and that this accumulation will be greater where fertiliser has been applied. Therefore, soil total N and C measurements could be useful in determining past soil productivity and fertiliser history, and in the case of N may indicate the level of historical N accumulation resulting from N fixed by pasture legumes.

2.3 NITROGEN FIXATION IN WAIRARAPA HILL COUNTRY

Over the past 60-70 years New Zealand farmers and agriculturists have developed pastoral systems based on grasses and clovers. This is unique when compared to other temperate countries, as New Zealand agriculture is almost completely dependant on clovers to supply large inputs of nitrogen required to sustain pasture production. Until relatively recent times, very little nitrogen fertiliser has been used.

However, this complete dependence on the legume component of the sward to supply nitrogen has limitations. For example, in areas with a high frequency of summer drought, clover N fixation and hence sward N supply may be confined to a short growing season, and therefore soil N may be severely limited on an annual basis. In order to determine the quantity and period of nitrogen fixation in the sward, it is important to measure rates of N fixation.

As with fertiliser response trials, studies measuring N fixation rates in the Wairarapa are virtually non-existent. However, a national series of trials were conducted by the MAF and the DSIR in the mid-late 1970s. These trials investigated the role of clovers in the N cycle of grazed pastures, and focused on nitrogen fixation. A total of nine sites were established,

ranging from Kaikohe in the far north, to Gore in the far south. At most of these sites, measurements continued for 2 or more years, beginning in 1974.

One of the sites included in this national series of trials was situated on the Wairarapa plains (Crouchley, 1979). This was the Masterton site, which was established on a Kokotau silt loam (see section 2.1.2). Superphosphate (@ 250 kg/ha/yr) had been applied on a regular basis at this site, which had also received lime. Over the duration of this study, a flock of sheep were rotationally grazed over the trial area, and dry matter yields and N fixation rates were measured.

The results from this study showed that the pasture yielded 9.4 -12.2 tDM/ha/yr, with N fixation, estimated by the acetylene reduction technique, varying from 90 to 241 kgN/ha/yr over two consecutive years (Crouchley, 1979). The author attributed the lower levels of N fixation and clover growth to soil moisture deficits, clover root cyst nematodes, and possibly clover flowering. On sub-plots receiving N fertiliser, there was a large growth response to a winter N fertiliser treatment over the first two years of the trial, but no response in the third winter, after a season of exceptionally high N fixing activity (Crouchley, 1979).

In general, the rate of N fixation reflected soil moisture patterns, with very high rates where summer rainfall occurred. The exception to this rule was in spring, where N fixation declined due to the increased activity of clover root cyst nematodes. Nitrogen fixation rates also decreased with the onset of clover flowering (Crouchley, 1979).

The author concluded that in the Wairarapa region soil moisture seemed to be a critical factor governing clover growth and N fixation rates (Crouchley, 1979).

2.4 SUMMARY

The climate regime (primarily rainfall) and nutrient reserves (soil nutrient status) will determine the productivity of a soil. Soil nutrient status may be a product of both the origin of soil parent materials, and the accumulation of soil nutrients from fertiliser applications. This review of literature has indicated that such variations in climate and fertiliser history impact greatly on soil nutrient reserves, particularly P, N and S.

Traditional fertiliser policy in the region has relied upon superphosphate, predominantly to create elevated soil P and S levels suited to improved legume growth, which in turn supplies nitrogen. In order to obtain an accurate description of soil fertility status, both nutrient reserves and nutrient plant-availability must be measured. The difference between soil P and S contents at fertilised and unfertilised sites should reflect the fertiliser history of the site,

whereas soil N accumulation in the same soil per unit of accumulated P and S may serve as a useful P and S fertiliser efficiency index.

The productivity of the soil under pasture will be determined by the pasture growth and/or animal production. Therefore, one measure of the efficiency of fertiliser use would be a comparison of measured soil fertility status with measured pasture yield.

Alternatively, because nitrogen availability appears to be the key factor limiting growth in legume-based pastures, a measure of soil fertility status compared to amounts of N fixed by the legume component of the sward would also be a useful measure of the effectiveness of traditional P and S fertiliser strategies.

2.5 REFERENCES

- Ball, P.R., Luscombe, P.C. and Grant, D.A., 1982. Nitrogen on hill country. In: P.B. Lynch (Editor), Nitrogen fertilisers in New Zealand agriculture. New Zealand Institute of Agricultural Science, Wellington, New Zealand, pp. 133-147.
- Ball, P.R. and Ryden, J.C., 1984. Nitrogen relationships in intensively managed temperate grasslands. *Plant and Soil*, 76: 23-33.
- Bircham, J.S. and Crouchley, G., 1976. Effects of superphosphate, lime, and stocking rate on pasture and animal production on the Wairarapa plains. 1. Pasture production and botanical composition. *New Zealand Journal of Experimental Agriculture*, 4(1): 57-63.
- Crouchley, G., 1979. Nitrogen fixation in pasture 8. Wairarapa plains dryland, Masterton. *New Zealand Journal of Experimental Agriculture*, 7(1): 31-33.
- Crouchley, G. and Sinclair, A.G., 1982. Response to sulphur and phosphorus fertilisers in improved pastures on some soils of the Wairarapa. *New Zealand Journal of Experimental Agriculture*, 10: 185-191.
- During, C., 1984. Fertilisers and Soils in New Zealand Farming. P.D. Hasselberg, Government Printer, Wellington, New Zealand.
- Eden, D.N. and Parfitt, R.L., 1992. Amounts and sources of phosphate in hill country rocks, South-eastern North Island, New Zealand. *Australian Journal of Soil Research*, 30: 357-370.
- Gibbs, H.S., Cowie, J.D. and Pullar, W.A., 1968. Soils of North Island. In: J. Luke (Editor), Soils of New Zealand (Part 1). NZSB Bulletin 26. A. R. Shearer, Wellington, New Zealand, pp. 48-63.

- Haynes, R.J. and Ludecke, A., 1981. Yield, root morphology and chemical composition of two pasture legumes as affected by lime and phosphorus application to a acid soil. *Plant and Soil*, 62: 241-254.
- Haynes, R.J. and Williams, P.H., 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Advances in Agronomy*, 49: 120-199.
- Hedley, M.J., Mortvedt, J.J., Bolan, N.S. and Syers, J.K., 1993. Phosphorus fertility management in agroecosystems, Scientific and Advisory Committee on Phosphorus Cycling (SCOPE).
- Heine, J.C., Gibbs, H.S. and Cowie, J.D., 1975. Interim report on soils of Wairarapa Valley, New Zealand. New Zealand Soil Bureau Report 40, DSIR, New Zealand, Wellington.
- Jackman, R.H., 1955a. Organic phosphorus in New Zealand soils under pasture 1. Conversion of applied phosphorus into organic forms. *Soil Science*, 79: 207-213.
- Jackman, R.H., 1955b. Organic phosphorus in New Zealand soils under pasture 2. Relation between organic phosphorus content and some soil characteristics. *Soil Science*, 79: 293-299.
- Jackman, R.H., 1960. Organic matter stability and nutrient availability in Taupo pumice. *New Zealand Journal of Agricultural Research*, 3: 6-23.
- Jackman, R.H., 1964a. Accumulation of organic matter in some New Zealand soils under permanent pasture 1. Patterns of change in organic carbon, nitrogen, sulphur and phosphorus. *New Zealand Journal of Agricultural Research*, 7: 445-471.
- Jackman, R.H., 1964b. Accumulation of organic matter in some New Zealand soils under permanent pasture 2. Rates of mineralisation of organic matter and the supply of available nutrients. *New Zealand Journal of Agricultural Research*, 7: 472-479.
- Lambert, M.G., Clark, D.A. and Costall, D.A., 1988. Phosphorus and sulphur accumulation after long-term superphosphate application to grazed hill pasture. In: R.E. White and L.D. Currie (Editors), *Towards the more efficient use of soil and fertiliser sulphur*. Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand, pp. 95-100.
- McLaren, R.G. and Cameron, K.C., 1990. *Soil Science*. Oxford University Press, Auckland, 304 pp.
- Molloy, L., 1988. *The Living Mantle: Soils in the New Zealand Landscape*. Mallinson Rendel, Wellington, 239 pp.
- Moore, P.R. and Speden, I.G., 1984. The Early Cretaceous (Albian) sequence of eastern Wairarapa, New Zealand. *NZGS Bulletin 97*. New Zealand Geological Survey, Wellington, 98 pp.
- Morton, J.D., Korte, C.J. and Smith, D., 1992. Preliminary report on a survey of nitrogen fertiliser practices on East Coast sheep and beef farms., Ministry of Agriculture and Fisheries, Lincoln, New Zealand.

- Nguyen, M.L. and Goh, K.M., 1990. Accumulation of soil sulphur fractions in grazed pastures receiving long-term superphosphate applications. *New Zealand Journal of Agricultural Research*, 33: 111-128.
- Nguyen, M.L., Rickard, D.S. and McBride, S.D., 1989. Pasture production and changes in phosphorus and sulphur status in irrigated pastures receiving long-term applications of superphosphate fertiliser. *New Zealand Journal of Agricultural Research*, 32: 245-262.
- Noble, K.E., 1985. Land use capability classification of the Southern Hawke's Bay - Wairarapa region. Ministry of Works and Development, Wellington, New Zealand, pp. 127.
- Perrott, K.W., 1992. Utilisation of inorganic and organic soil phosphorus in a hill country soil, New Zealand Grassland Association, pp. 65-69.
- Perrott, K.W., Maher, F.M. and Thorrold, B.S., 1989. Accumulation of phosphorus fractions in yellow-brown pumice soils with development. *New Zealand Journal of Agricultural Research*, 32: 53-62.
- Perrott, K.W. and Mansell, G.P., 1989. Effect of fertiliser phosphorus and liming on inorganic and organic soil phosphorus fractions. *New Zealand Journal of Agricultural Research*, 32: 63-70.
- Perrott, K.W. and Sarathchandra, S.U., 1989. Phosphorus in the microbial biomass of New Zealand soils under established pasture. *New Zealand Journal of Agricultural Research*, 32: 409-413.
- Perrott, K.W., Sarathchandra, S.U. and Waller, J.E., 1990. Seasonal storage and release of phosphorus and potassium by organic matter and the microbial biomass in a high producing pastoral soil. *Australian Journal of Soil Research*, 28: 593-608.
- Pollok, J.A., Neall, V.E. and De Rose, R.C., 1994. The soils and geology of Riverside farm. Publication No. 14, Massey University, Palmerston North.
- Saggar, S., Hedley, M.J. and White, R.E., 1992a. Development and evaluation of an improved soil test for phosphorus: 1. The influence of phosphorus fertiliser solubility and soil properties on the extractability of soil P. *Fertilizer Research*, 33: 81-91.
- Saggar, S., Hedley, M.J., White, R.E., Gregg, P.E.H., Perrott, K.W., Cornforth, I.S., 1992b. Development and evaluation of an improved soil test for phosphorus: 2. Comparison of the Olsen and mixed cation-anion exchange resin tests for predicting the yield of ryegrass grown in pots. *Fertilizer Research*, 33(2): 135-144.
- Saunders, W.M.H., 1968. Phosphorus. In: J. Luke (Editor), *Soils of New Zealand* (Part 2). NZSB Bulletin 26. A. R. Shearer, Wellington, New Zealand, pp. 95-102.
- Walker, T.W. and Syers, J.K., 1976. The fate of phosphorus during pedogenesis. *Geoderma*, 15: 1-9.

APPENDIX 4

4.1 THE ECONOMICS OF STRATEGIC N FERTILISER USE ON SUMMER-DRY N.I. HILL COUNTRY

4.1.1 Assumptions

- 30 kgN/ha applied as urea
- response = 20 kgDM/kg N applied
- therefore, extra feed on hand = 600 kgDM/ha
- All extra DM grown is consumed, and converted to stock liveweight gain (Scenario 1 only)
- Current prices for export lambs and beef are used (New Zealand Farmer Magazine, June 2000)
- LWG = liveweight gain
- lwt = liveweight
- cw = carcass weight
- MJME = Megajoules of metabolisable energy

Pasture dry matter conversion to stock liveweight gain (LWG), and carcass weight (cw):

1 kgDM = 11 MJME = 125g LWG (20 kg ewe lamb hogget, growing at 125g/d; 15.0 kg carcass weight [30 kg lwt] by early April), (Lincoln University Technical Manual, 1991).

OR

1 kgDM = 11 MJME = 200g LWG (150 kg growing beef cattle, with growing at 1.0 kg/d; 180 kg carcass weight [340 kg lwt] at 18-20 months old, (Lincoln University Technical Manual, 1991).

4.1.2 Economic Calculations

4.1.2 Costs

Urea = \$450/T applied x 65.2 kg/ha = \$30/ha (Ravensdown Fertiliser)

4.1.3 Returns (Gross):

Lamb = \$50 per 30 kg lamb (15.0 kg cw) = \$3.33 per kg cw, x 37.5 kg extra lamb carcass / ha = \$124.88 / ha

Beef = \$800 per 540 kg Steer (P2 Steer, 280 kg cw) = \$2.86 per kg cw, x 62.4 kg extra beef carcass / ha = \$178.46/ha

4.1.4 Returns (Net):

Lamb = \$94.88 / ha, or \$3.16 / kgN applied

Beef = \$148.46 / ha or \$4.95 / kgN applied

4.1.5 Breakeven Point:

4.1.5.1 Scenario 1: 100% utilisation of extra pasture DM

Lamb = 9 kg cw/ha = 18 kg LW = 144 kgDM/ha = 4.8:1 N Response to 30 kgN / ha

Beef = 10.5 kg cw/ha = 20.2 kg LW = 101 kg DM/ha = 3.4:1 N Response to 30 kgN/ha

4.1.5.2 Scenario 2: 70% utilisation of extra pasture DM

Lamb = 9 kg cw/ha = 18 kg LW = 206 kgDM/ha = 6.9:1 N Response to 30 kgN / ha

OR a net return of \$2.21/kgN (at 4.8:1 response)

Beef = 10.5 kg cw/ha = 20.2 kg LW = 144 kg DM/ha = 4.8:1 N Response to 30 kgN/ha

OR a net return of \$3.47/kgN (at 3.4:1 response)

4.1.5.3 Scenario 3: 50% utilisation of extra pasture DM

Lamb = 9 kg cw/ha = 18 kg LW = 288 kgDM/ha = 9.6:1 N Response to 30 kgN / ha

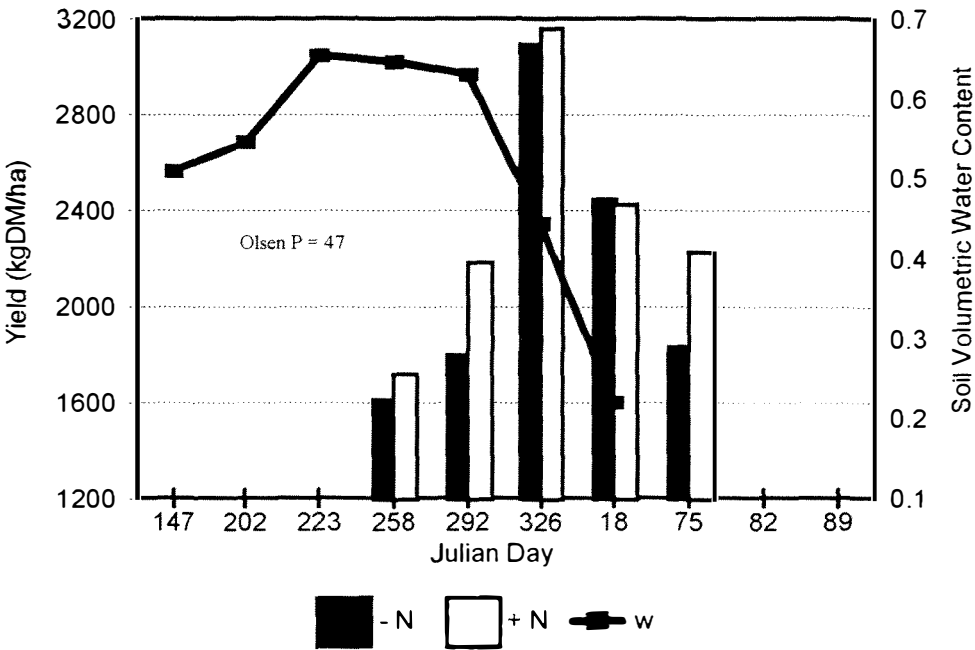
OR a net return of \$1.58/kgN (at 4.8:1 response)

Beef = 10.5 kg cw/ha = 20.2 kg LW = 202 kg DM/ha = 6.7:1 N Response to 30 kgN/ha

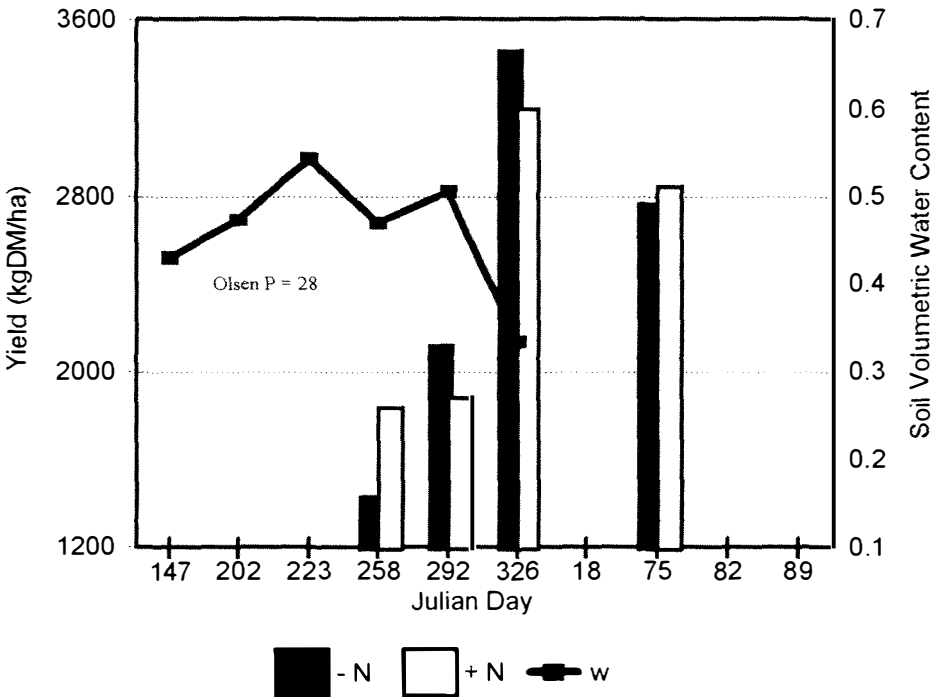
OR a net return of \$2.48/kgN (at 3.4:1 response)

4.2 MEASURED PASTURE YIELD RESPONSES TO STRATEGIC N FERTILISER

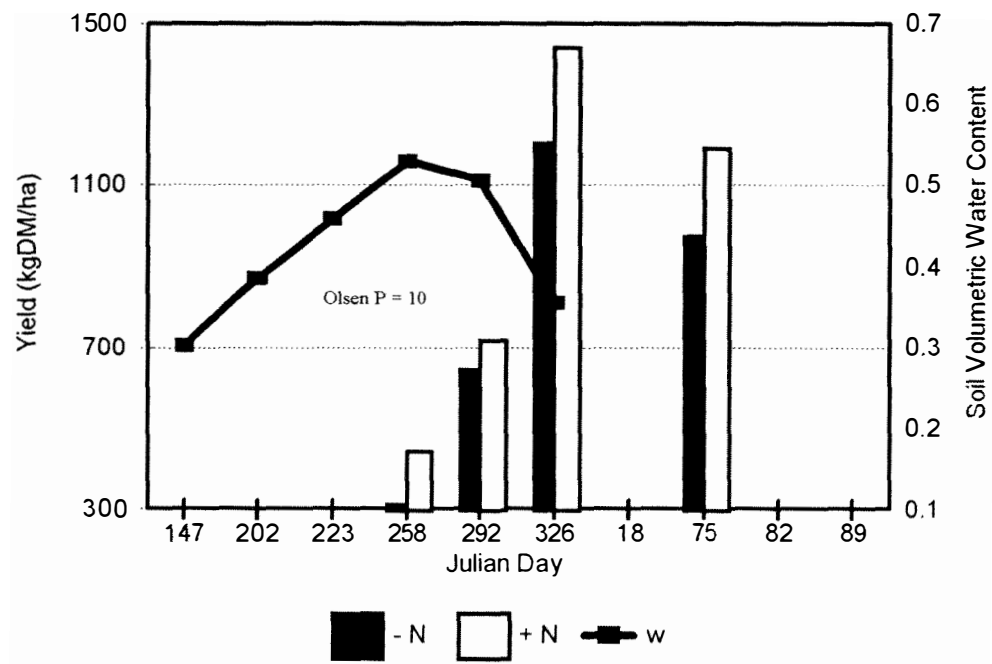
4.2.1 Site 1



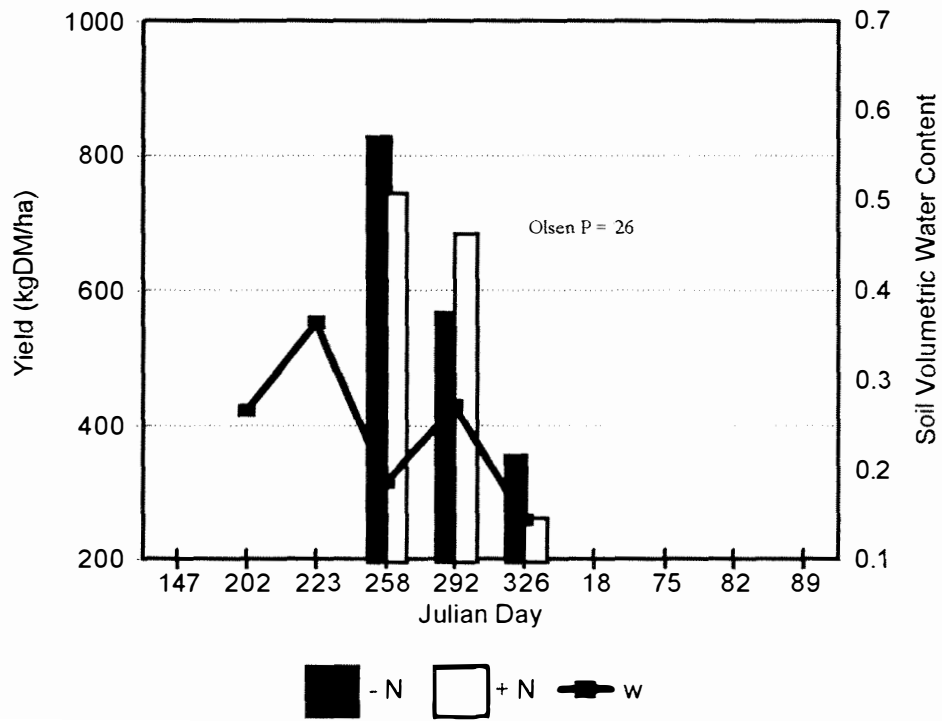
4.2.2 Site 3



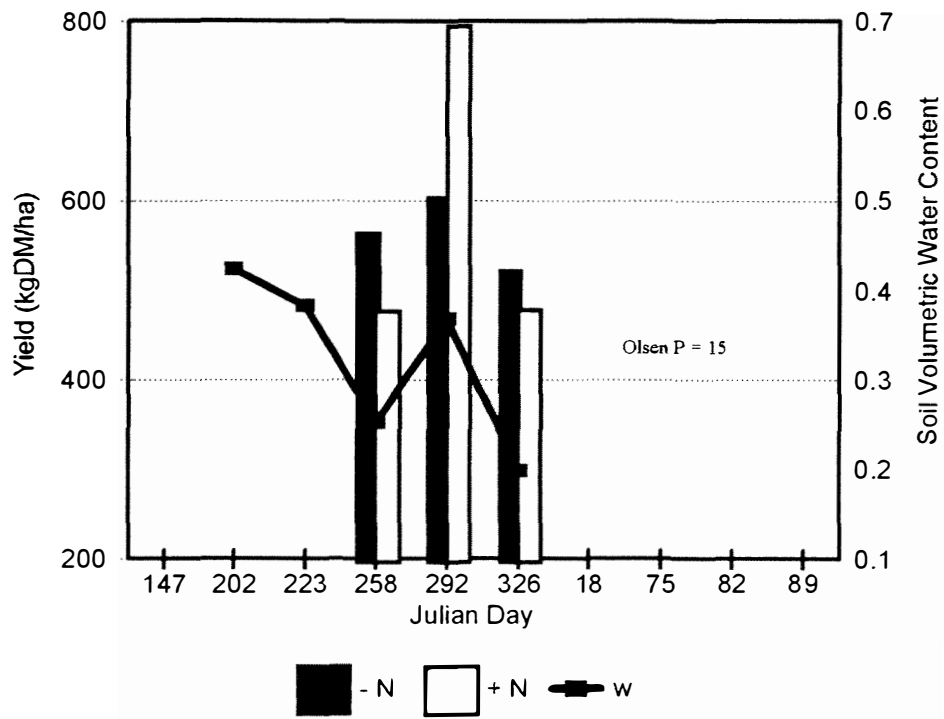
4.2.3 Site 4



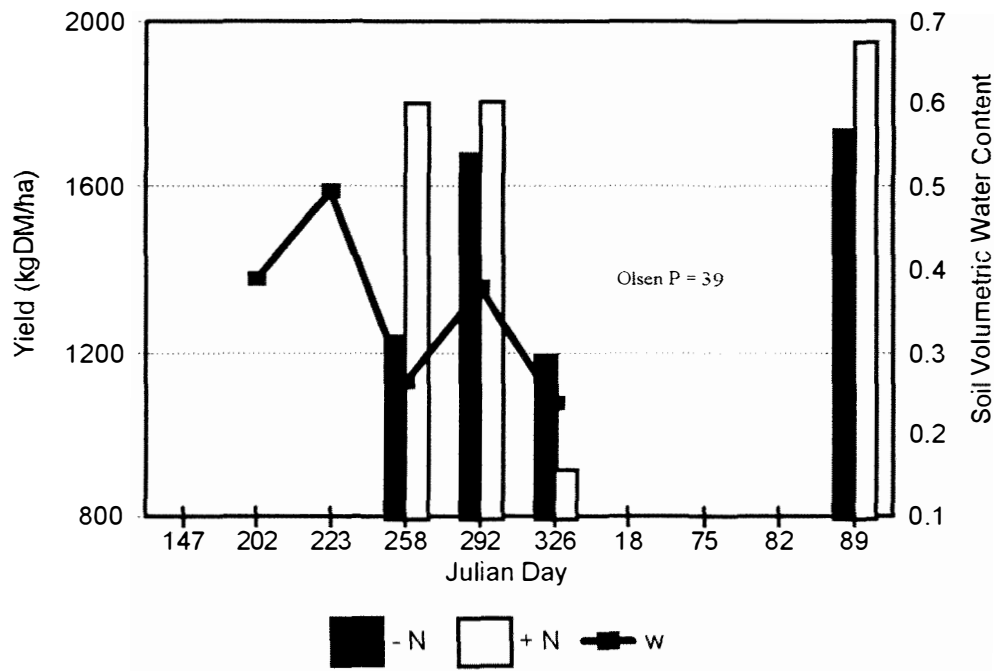
4.2.4 Site 5



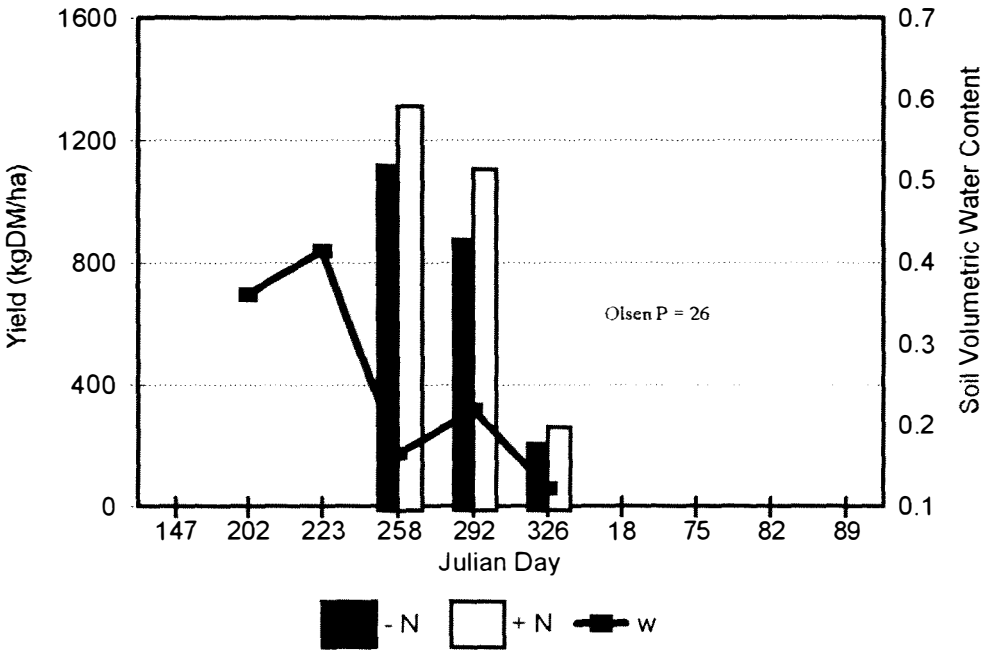
4.2.5 Site 6



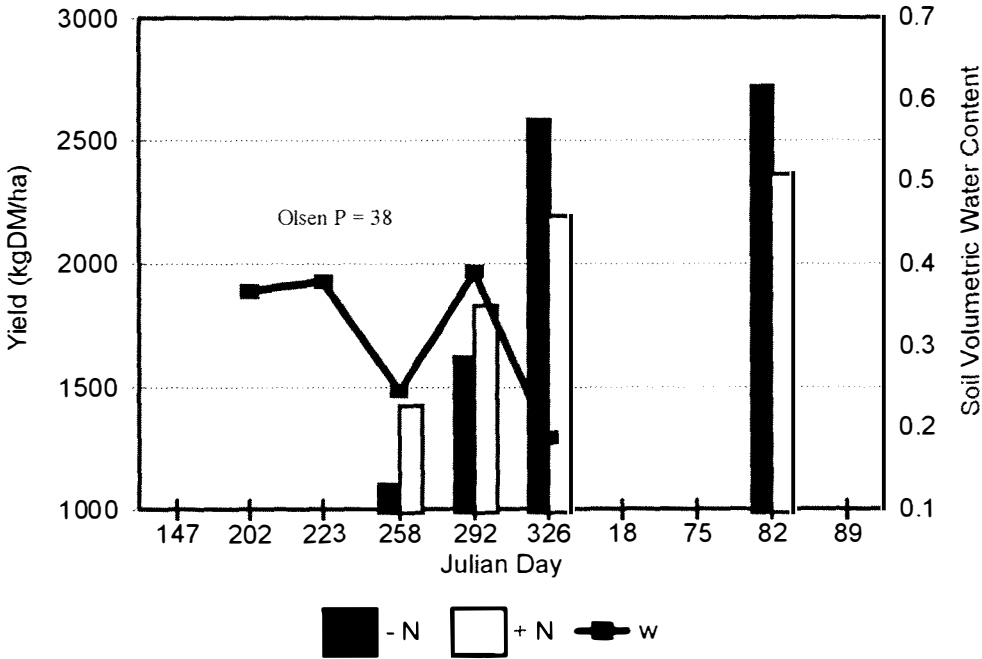
4.2.6 Site 8



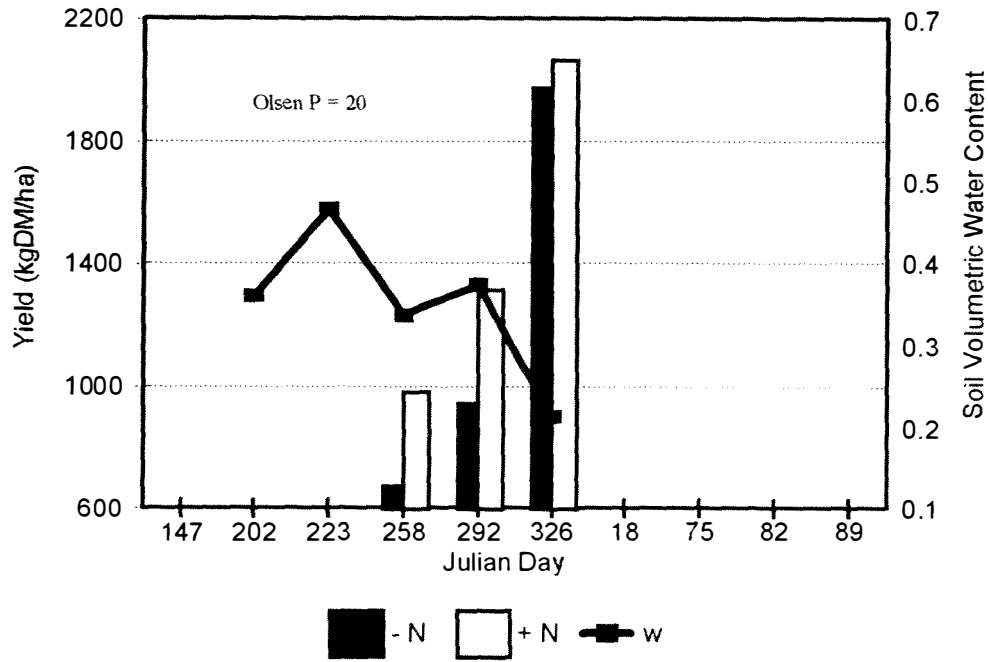
4.2.7 Site 9



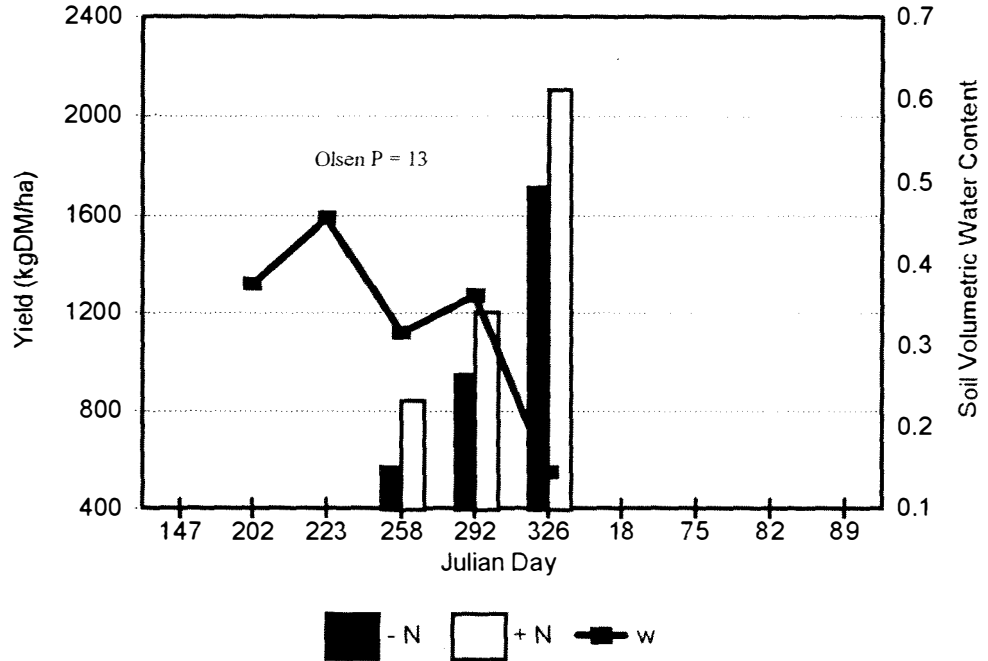
4.2.8 Site 10



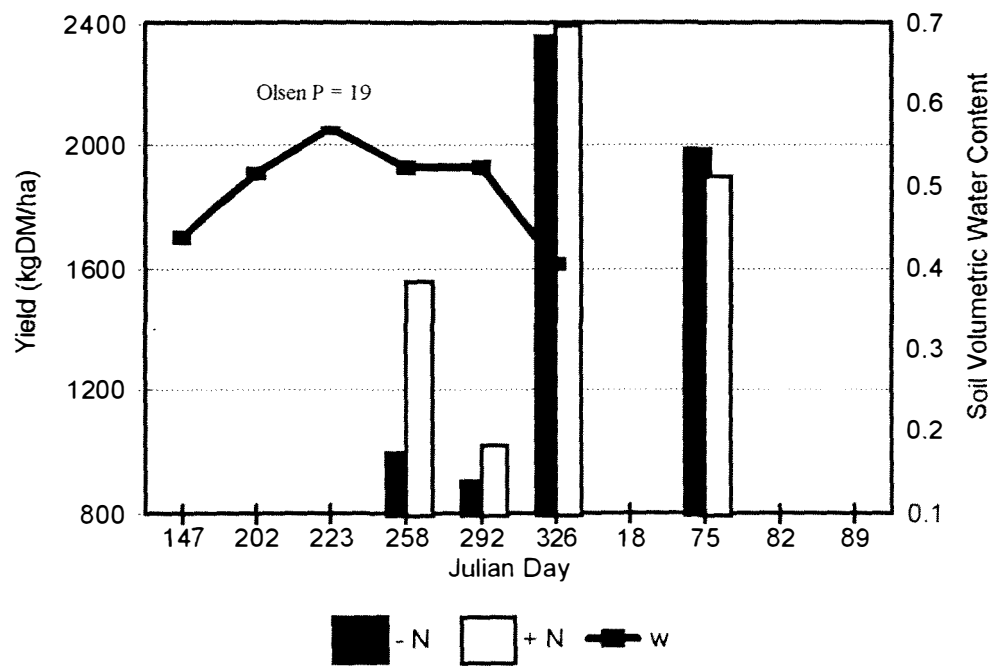
4.2.9 Site 11



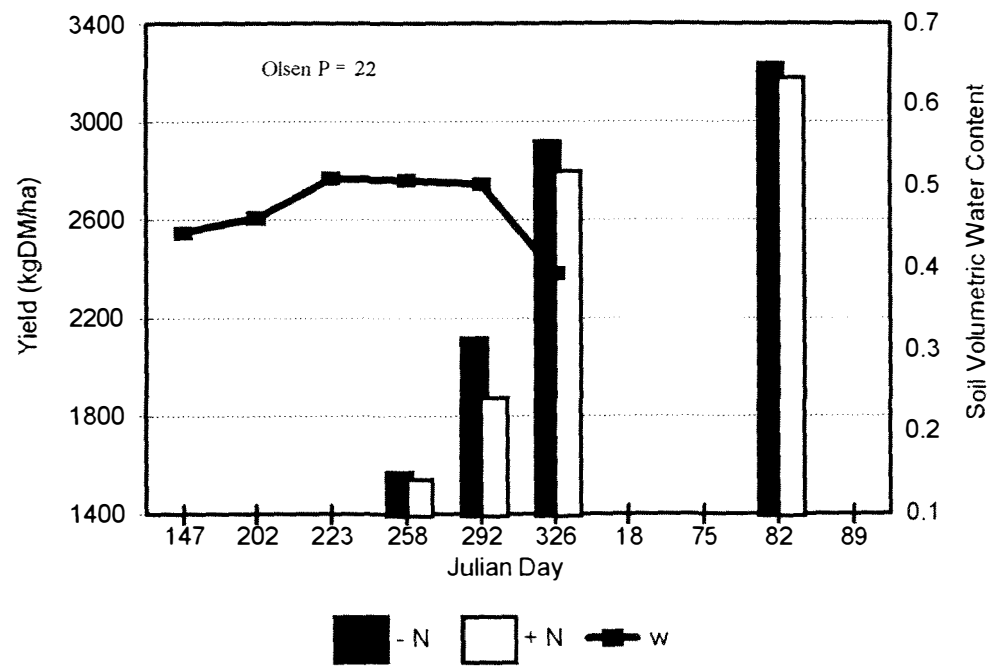
4.2.10 Site 12



4.2.11 Site 13



4.2.12 Site 14



APPENDIX 5

5.1 FIELD AND GLASSHOUSE SITE YIELD RANKINGS

Table 5.1^A

1. Yield comparison and relative yields

Yield Comparison and Site Ranking (kgDMha ⁻¹ or gDM pot ⁻¹)				
Site	GH Grass Yield [†]	GH Grass Yield	Field 1995 (spring)	Field 1995 (annual)
1	1.85	2614	3416	10790
3	1.11	1572	3555	9780
4	0.22	309	958	3135
5	2.01	2837	1395	1751
6	1.17	1652	1168	1690
8	1.54	2166	2919	5850
9	1.51	2123	1991	2196
10	2.05	2891	2728	8034
11	0.44	625	1618	3594
12	0.60	839	1519	3234
13	0.77	1082	1904	6246
14	1.91	2699	3691	9848
Maximum	2.02	2850	4100	11500
Relative Yields (95% Max)				
1	96.6	96.6	87.7	98.8
3	58.1	58.1	91.3	89.5
4	11.4	11.4	24.6	28.7
5	104.8	104.8	35.8	16.0
6	61.0	61.0	30.0	15.5
8	80.0	80.0	74.9	53.6
9	78.4	78.4	51.1	20.1
10	106.8	106.8	70.0	73.5
11	23.1	23.1	41.5	32.9
12	31.0	31.0	39.0	29.6
13	40.0	40.0	48.9	57.2
14	99.7	99.7	94.8	90.1

2. Sorted yield site rankings

Yield Comparison and Site Ranking (kgDMha ⁻¹ or gDM pot ^{-1†})				
Site	GH Grass Yield [†]	GH Grass Yield	Field 95 (spring)	Field 95 (annual)
10	2.05	2891	2728	8034
5	2.01	2837	1395	1751
14	1.91	2699	3691	9848
1	1.85	2614	3416	10790
8	1.54	2166	2919	5850
9	1.51	2123	1991	2196
6	1.17	1652	1168	1690
3	1.11	1572	3555	9780
13	0.77	1082	1904	6246
12	0.60	839	1519	3234
11	0.44	625	1618	3594
4	0.22	309	958	3135
Maximum	2.02	2850	4100	11500
Relative Yields (95% maximum)				
10	106.8	106.8	70.0	73.5
5	104.8	104.8	35.8	16.0
14	99.7	99.7	94.8	90.1
1	96.6	96.6	87.7	98.8
8	80.0	80.0	74.9	53.6
9	78.4	78.4	51.1	20.1
6	61.0	61.0	30.0	15.5
3	58.1	58.1	91.3	89.5
13	40.0	40.0	48.9	57.2
12	31.0	31.0	39.0	29.6
11	23.1	23.1	41.5	32.9
4	11.4	11.4	24.6	28.7

5.2 PLANT NUTRIENT UPTAKE (GLASSHOUSE)

Table 5.2^A Nitrogen (N) and phosphorus (P) concentration (%) and total elemental plant uptake (mg pot⁻¹) for ryegrass and white clover across all soils.

Site	Nitrogen							
	Concentration (%)				Plant Uptake (mg pot ⁻¹)			
	Ryegrass			Clover	Ryegrass			Clover
	Cut 1	Cut 2	Mean	Mean	Cut 1	Cut 2	Total	Total
1	3.65	1.68	2.67	3.40	42.4	11.6	54.0	77.9
3	3.92	1.82	2.87	2.61	22.4	9.9	32.2	24.7
4	2.04	1.91	1.98	2.94	2.6	1.8	4.4	7.8
5	3.77	1.81	2.79	2.56	42.0	16.2	58.3	60.2
6	3.51	1.76	2.64	2.44	24.6	8.3	32.8	38.7
8	3.24	1.75	2.50	2.53	30.6	10.3	41.0	39.3
9	4.42	2.07	3.25	2.30	29.5	17.3	46.8	31.5
10	3.47	1.78	2.63	2.39	36.2	18.0	54.2	36.8
11	1.87	1.95	1.91	2.68	3.9	4.6	8.5	18.5
12	2.03	1.93	1.98	2.91	7.9	4.0	11.9	16.9
13	3.91	1.97	2.94	2.53	15.7	7.2	22.9	11.5
14	4.58	2.87	3.73	3.77	30.2	36.0	66.2	46.5
Phosphorus								
1	0.282	0.461	0.372	0.286	3.28	3.18	6.45	6.57
3	0.171	0.276	0.224	0.159	0.98	1.50	2.48	1.51
4	0.136	0.256	0.196	0.134	0.17	0.24	0.41	0.36
5	0.212	0.334	0.273	0.218	2.37	3.08	5.45	5.12
6	0.142	0.294	0.218	0.168	0.99	1.39	2.38	2.67
8	0.235	0.481	0.358	0.250	2.22	2.83	5.05	3.87
9	0.192	0.255	0.224	0.161	1.28	2.14	3.42	2.21
10	0.261	0.495	0.378	0.260	2.72	4.98	7.71	4.00
11	0.142	0.193	0.168	0.124	0.29	0.46	0.75	0.86
12	0.139	0.310	0.225	0.159	0.54	0.64	1.18	0.92
13	0.179	0.255	0.217	0.160	0.72	0.93	1.65	0.73
14	0.193	0.205	0.199	0.200	1.28	2.57	3.84	2.47

Table 5.3^A Clover herbage concentrations (%) and total elemental plant uptake (mg pot⁻¹) of sulphur (S), potassium (K), calcium (Ca) and magnesium (Mg) as determined by ICP-AES.

Site	Sulphur		Potassium		Calcium		Magnesium	
	Conc	Uptake	Conc	Uptake	Conc	Uptake	Conc	Uptake
1	0.24	5.54	4.57	104.8	1.52	34.9	0.30	6.90
3	0.20	1.88	3.73	35.4	1.32	12.5	0.21	2.03
4	0.22	0.59	4.03	10.7	1.30	3.4	0.22	0.59
5	0.18	4.19	4.50	105.7	0.98	23.0	0.34	7.98
6	0.15	2.44	3.74	59.4	0.11	1.7	0.27	4.25
8	0.21	3.28	3.07	47.6	1.15	17.9	0.29	4.50
9	0.15	3.13	3.91	79.4	0.86	17.4	0.29	5.90
10	0.21	3.38	3.23	51.1	1.22	19.3	0.20	3.15
11	0.15	1.04	3.36	23.2	1.09	7.5	0.21	1.45
12	0.26	1.54	3.85	22.4	1.14	6.7	0.29	1.66
13	0.20	0.89	2.29	10.4	1.28	5.8	0.19	0.87
14	0.23	2.83	2.83	34.9	1.64	20.2	0.21	2.61

5.3 CLOVER P UPTAKE

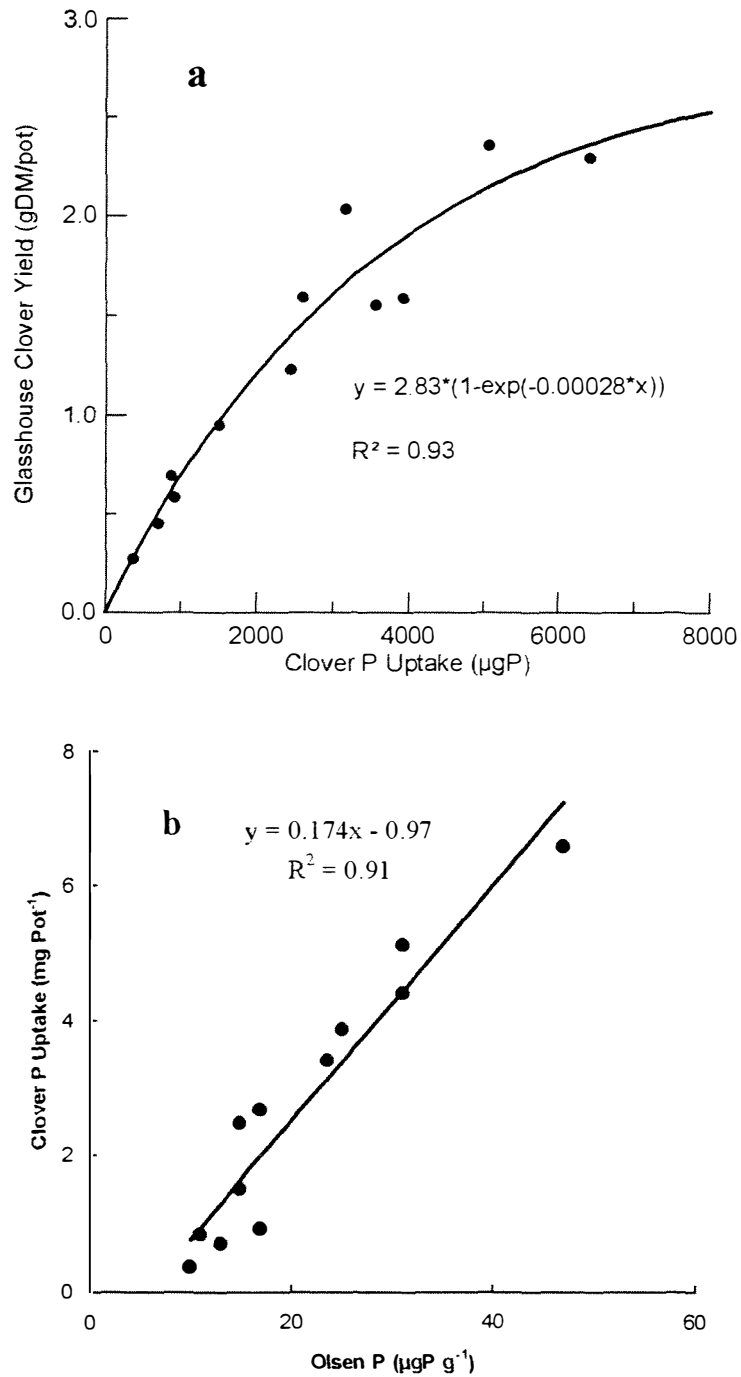


Figure 5.1^A The relationship between **a**, glasshouse clover yield and P uptake; and **b**, the relationship between Olsen P and clover P uptake.

5.4 NUTRIENT UPTAKE AND OLSEN P CORRELATIONS

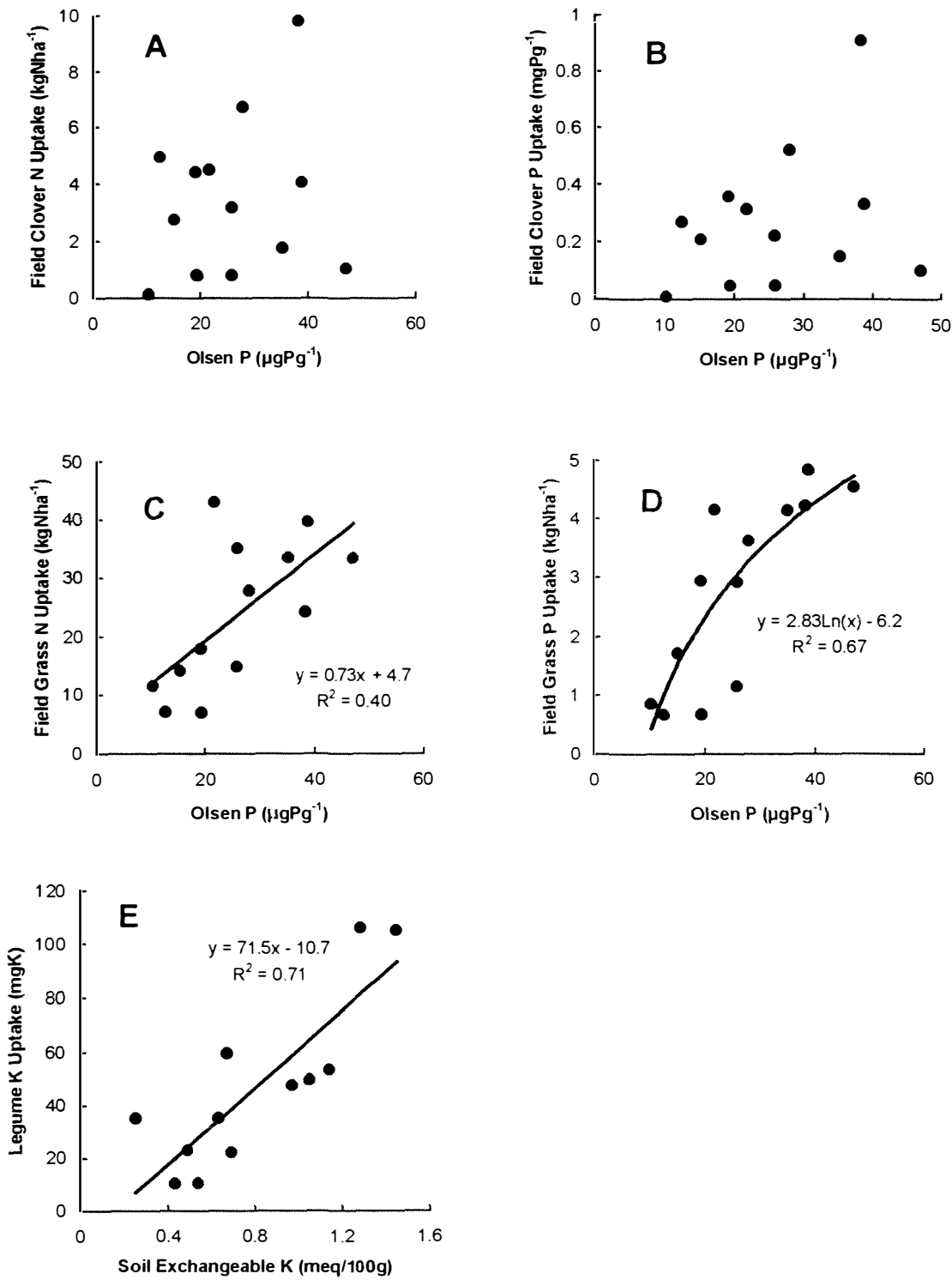


Figure 5.4^A The relationship between field Olsen P and **A**, field clover N uptake; **B**, field clover P uptake; **C**, field grass N uptake; **D**, field grass P uptake; and **E**, Soil Exchangeable K and legume K uptake across all sites.

5.5

HERBAGE NUTRIENT RATIOS

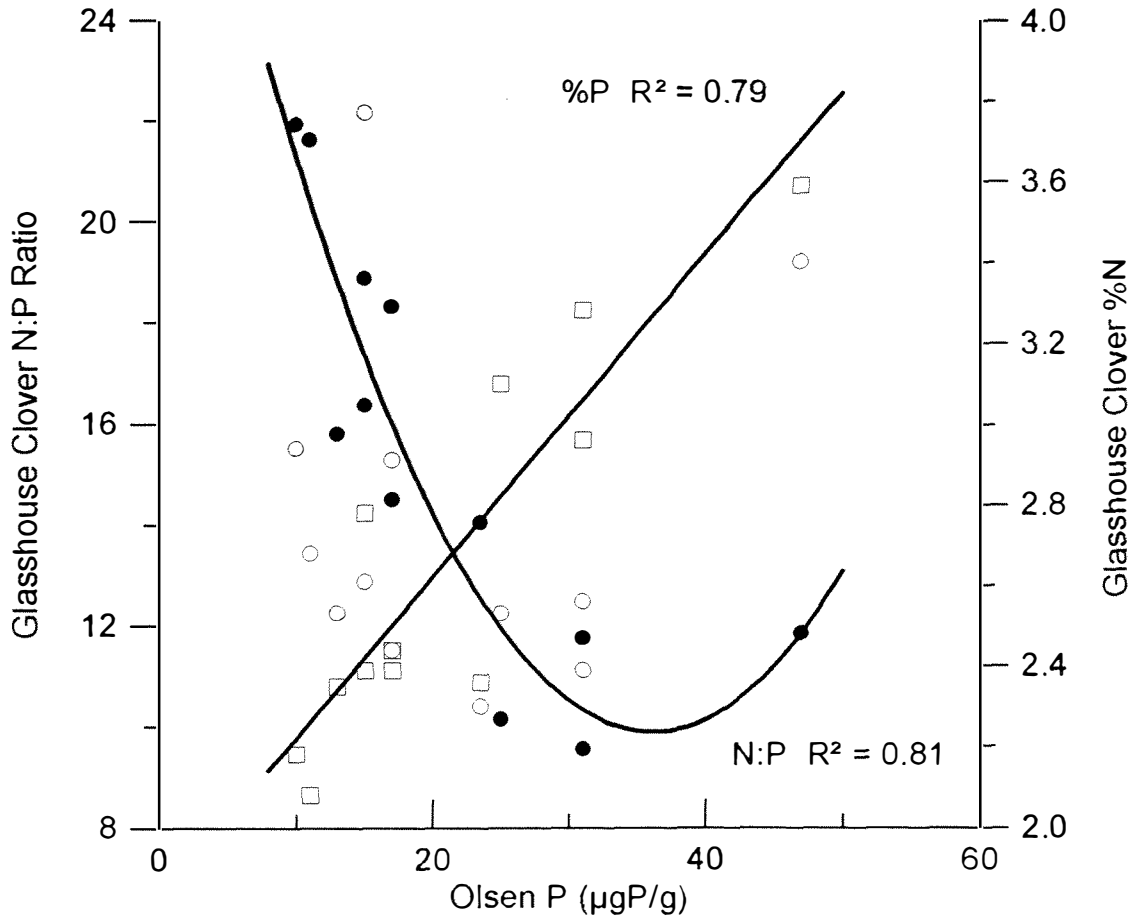


Figure 5.5^A The relationship between Olsen P, clover N concentration (%N), and clover N:P ratio in the glasshouse.

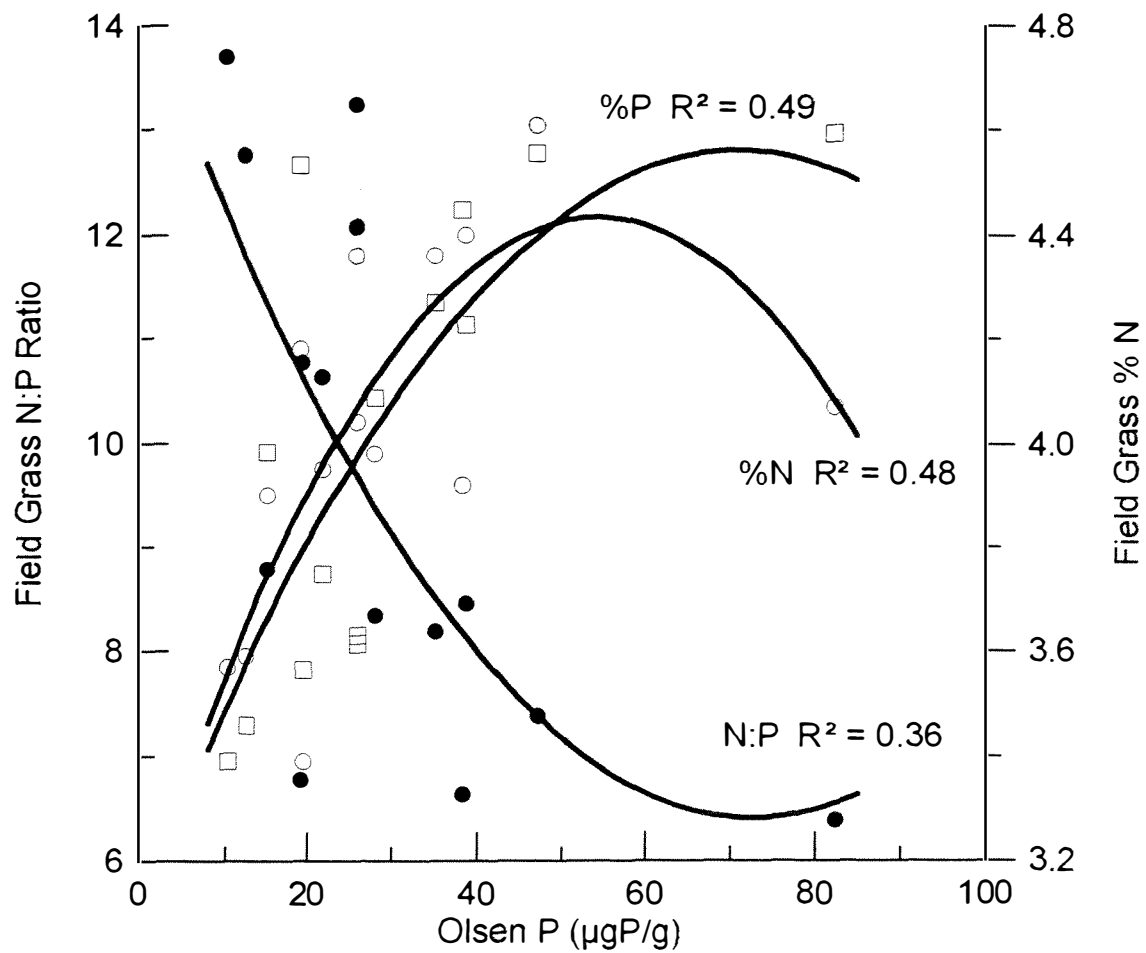


Figure 5.6^A The relationship between Olsen P, grass N concentration (%N), grass P concentration (%P), and grass N:P ratio in the field.

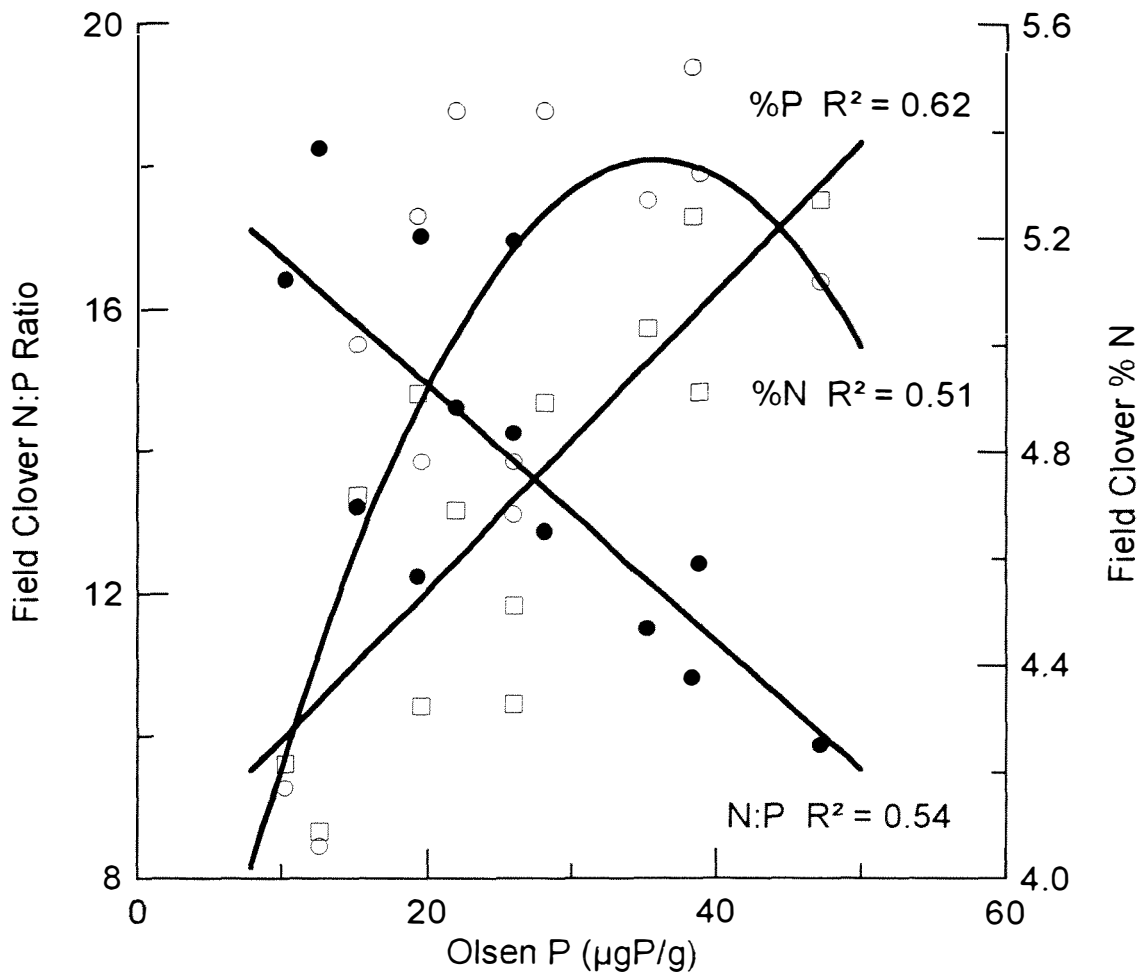


Figure 5.7^A The relationship between Olsen P, clover N concentration (%N), clover P concentration (%P), and clover N:P ratio in the field.

APPENDIX 7

7.1 REGRESSION ANALYSIS

Table 7.1^A Regression analysis output - curves of best fit

Site	Curve Type	R ² Value	Line Equation
1	Sigmoidal Hill, 4 par	0.994	$y=0.064+[(0.387*x^{7.11})/(2.30^{7.11}+x^{7.11})]$
3	Exponential rise to max	0.984	$y=-0.076+0.332*(1-\exp(-0.556*x))$
4	Sigmoidal Hill, 4 par	0.992	$y=0.051+[(0.26*x^{1.96})/(4.93^{1.96}+x^{1.96})]$
5	Sigmoidal Hill, 4 par	0.992	$y=0.073+[(0.324*x^{6.43})/(2.32^{6.43}+x^{6.43})]$
6	Sigmoidal Hill, 4 par	0.992	$y=0.066+[(0.302*x^{6.16})/(2.71^{6.16}+x^{6.16})]$
8	Sigmoidal Hill, 4 par	0.971	$y=0.076+[(0.294*x^{3.66})/(2.88^{3.66}+x^{3.66})]$
9	Power 2 Par	0.993	$y=0.065*x^{0.51}$
10	Sigmoidal Hill, 4 par	0.980	$y=0.069+[(0.352*x^{4.31})/(2.63^{4.31}+x^{4.31})]$
11	Sigmoidal Hill, 4 par	0.978	$y=0.057+[(0.371*x^{3.7})/(3.12^{3.7}+x^{3.7})]$
12	Sigmoidal Hill, 4 par	0.970	$y=0.056+[(0.331*x^{2.06})/(3.47^{2.06}+x^{2.06})]$
13	Sigmoidal Hill, 4 par	0.991	$y=0.053+[(0.317*x^{2.65})/(3.14^{2.65}+x^{2.65})]$
14	Sigmoidal Hill, 4 par	0.973	$y=0.054+[(0.335*x^{2.75})/(3.02^{2.75}+x^{2.75})]$

Table 7.2^A Regression analysis output - curves of best fit

Site	Curve Type	R ² Value	Line Equation
1	Sigmoidal Hill, 4 par	0.987	$y=0.087+[(0.505*x^{4.56})/(2.05^{4.56}+x^{4.56})]$
3	Sigmoidal Hill, 4 par	0.995	$y=0.04+[(0.645*x^{3.33})/(1.77^{3.33}+x^{3.33})]$
4	Sigmoidal Hill, 4 par	0.996	$y=0.077+[(0.514*x^{4.1})/(2.89^{4.1}+x^{4.1})]$
5	Exponential rise to max	0.968	$y=-0.296+0.935*(1-\exp(-0.588*x))$
6	Sigmoidal Hill, 4 par	0.998	$y=0.057+[(0.642*x^{3.21})/(2.29^{3.21}+x^{3.21})]$
8	Sigmoidal Hill, 4 par	0.996	$y=0.191+[(0.336*x^{3.96})/(1.93^{3.96}+x^{3.96})]$
9	Sigmoidal Hill, 4 par	0.995	$y=0.023+[(0.535*x^{2.9})/(2^{2.9}+x^{2.9})]$
10	Sigmoidal Hill, 4 par	0.991	$y=0.074+[(0.364*x^{4.18})/(2.48^{4.18}+x^{4.18})]$
11	Exponential rise to max	0.990	$y=-0.408+1.06*(1-\exp(-0.673*x))$
12	Exponential rise to max	0.994	$y=-0.224+0.745*(1-\exp(-0.53*x))$
13	Sigmoidal Hill, 4 par	0.999	$y=0.09+[(0.51*x^9)/(2.23^9+x^9)]$
14	Sigmoidal Hill, 4 par	0.999	$y=0.072+[(0.444*x^{4.53})/(1.96^{4.53}+x^{4.53})]$

7.2 DETERMINATION OF EXTRACTABLE SOIL CARBON BY LECO COMBUSTION.

Table 7.3^A Total carbon contents of soil saline extracts for Field-Dry and Glasshouse-moistened soils, by methabsorbance of LECO combustion (oven-dry basis)

Site	Field-Dry Soils			Glasshouse-Moistened Soils		
	Total C µg/g	Total N µg/g	C:N Ratio	Total C µg/g	Total N µg/g	C:N Ratio
1	539.0	118.1	4.6	109.5	34.8	3.1
3	578.2	97.7	5.9	126.2	39.5	3.2
4	344.2	71.6	4.8	139.3	24.5	5.7
5	702.4	168.4	4.2	155.9	39.7	3.9
6	539.4	101.3	5.3	67.9	25.4	2.7
8	687.7	87.0	7.9	201.9	65.0	3.1
9	604.4	58.9	10.3	130.0	46.2	2.8
10	535.8	72.2	7.4	113.9	88.0	1.3
11	445.1	63.3	7.0	185.8	76.5	2.4
12	745.3	187.6	4.0	136.7	46.5	2.9
13	572.8	65.0	8.8	91.2	16.6	5.5
14	543.7	55.7	9.8	156.3	28.5	5.5

Figure 7.2^A Total carbon contents of soil saline extracts for Field-Dry soils, by method of LECO combustion (oven-dry basis)

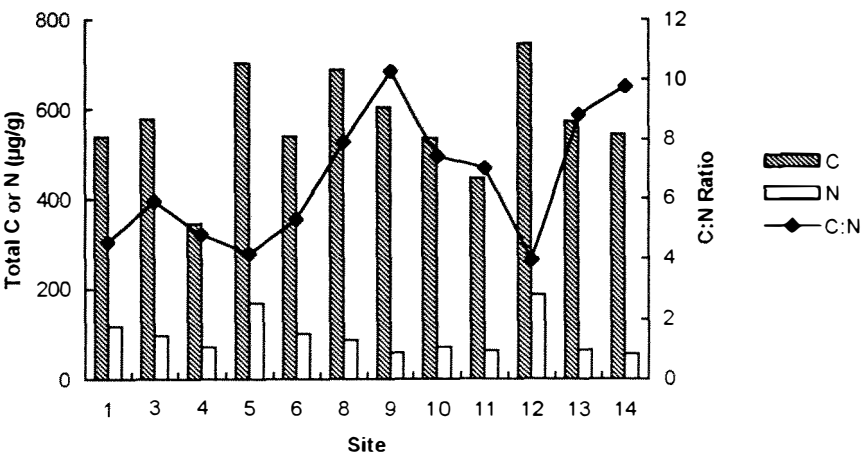
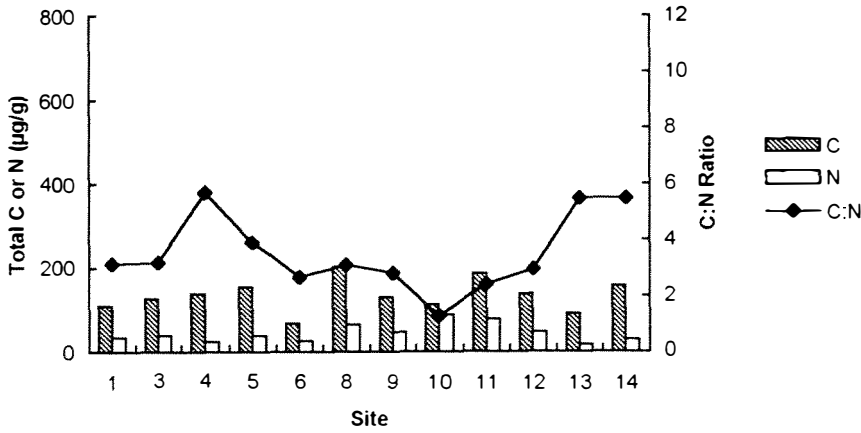


Figure 7.3^A Total carbon contents of soil saline extracts for Glasshouse-Moistened soils, by method of LECO combustion (oven-dry basis)



7.3 SOIL ANALYSES

Table 7.4^A Olsen P and mineralisable N (anaerobic incubation) across all soils used in BiologTM experiments.

Site	Field-Dry Soil		Moistened Glasshouse Incubated Soil	
	Olsen P (μgPg^{-1})	Mineralisable N (μgNg^{-1})	Olsen P (μgPg^{-1})	Mineralisable N (μgNg^{-1})
1	24.9	476.2	23.0	263
3	25.4	247.1	25.4	180
4	7.4	192.7	10.8	150
5	22.0	483.8	11.8	209
6	9.3	352.3	12.7	172
8	19.1	301.8	29.3	279
9	37.6	250.9	26.9	150
10	24.9	524.8	27.8	197
11	14.2	202.3	13.7	121
12	23.0	458.4	15.2	207
13	12.7	441.0	13.2	208
14	24.4	349.2	22.5	162

APPENDIX 8

8.1 BIOLOG™ C SUBSTRATE INFORMATION

Substrate No.	Name	Formula	Components	Notes
1	Water (blank)			
2	α -cyclodextrin	$C_{36}H_{60}O_{30}$		Starch gum - model substance for enzymatic reactions
3	dextrin	$C_6H_{10}O_5$		Starch gum
4	glycogen	$C_6H_{10}O_5$		Reserve carbohydrate (starch) of animals (also found in fungi and yeasts). In cell protoplasm - in liver and muscles.
5	tween 40		polyoxyethylene(20) sorbitan monopalmitate	
6	tween 80		polyoxyethylene(20) sorbitan mono-oleate	
7	N-acetyl-D-galactosamine	$C_8H_{15}NO_6$		
8	N-acetyl-D-glucosamine	$C_8H_{15}NO_6$		
9	adonitol	$C_5H_{12}O_5$	C 39.5% H 8% O 52.6%	
10	L-arabinose	$C_5H_{10}O_5$	C 40% H 6.7% O 53.3%	Pectin sugar (polysaccharide). Common in plants. Also in mycobacteria.
11	D-arabitol	$C_5H_{12}O_5$	C 39.5% H 8% O 52.6%	
12	cellobiose	$C_{12}H_{22}O_{11}$	C 42.1% H 6.5% O 51.4%	Unit of cellulose and lichenin. Does not occur free in nature.
13	i-erythritol	$C_4H_{10}O_4$	C 39.3% H 8.3% O 52.4%	Isoln from algae, lichens, grasses.
14	D-fructose	$C_6H_{12}O_6$	C 40% H 6.7% O 53.3%	Fruit sugar.
15	L-fucose	$C_6H_{12}O_5$	C 43.9% H 7.4% O 48.7%	
16	D-galactose	$C_6H_{12}O_6$	C 40% H 6.7% O 53.3%	Constituent of polysaccharides in many pectins, gums and mucilages.
17	gentiobiose	$C_{12}H_{22}O_{11}$	C 42.1% H 6.5% O 51.4%	
18	α -D-glucose	$C_6H_{12}O_6$	C 40% H 6.7% O 53.3%	Main source of energy for living organisms. Found free in plants, fruits and blood.
19	m-inositol	$C_6H_{12}O_6$	C 40% H 6.7% O 53.3%	Widely distributed in plants and animals. A growth factor for animals and microorganisms.
20	α -D-lactose	$C_{12}H_{22}O_{11}$	C 42.1% H 6.5% O 51.4%	Milk sugar.
21	lactulose	$C_{12}H_{22}O_{11}$	C 42.1% H 6.5% O 51.4%	
22	maltose	$C_{12}H_{22}O_{11} \cdot H_2O$	C 42.1% H 6.5% O 51.4%	Malt sugar from enzymatic degradation of starch.
23	D-mannitol	$C_6H_{14}O_6$	C 39.6% H 7.7% O 52.7%	Widespread in plants and plant exudates.
24	D-mannose	$C_6H_{12}O_6$	C 40% H 6.7% O 53.3%	
25	D-melibiose	$C_{12}H_{22}O_{11}$	C 42.1% H 6.5% O 51.4%	From raffinose, with fructose removed.
26	β -methyl-D-glucoside	$C_7H_{14}O_6$	C 43.3% H 7.3% O 49.4%	Glucose and methanol.
27	D-psicose	$C_6H_{12}O_6$	C 40% H 6.7% O 53.3%	A non-fermentable substance in cane molasses.
28	D-raffinose	$C_{18}H_{32}O_{16} \cdot 5H_2O$	C 42.9% H 6.4% O 50.8%	A trisaccharide built from one mol each of galactose, glucose and fructose
29	L-rhamnose	$C_6H_{12}O_5$	C 43.9% H 7.4% O	Found as part of glycosides in many

			48.7%	plants.
30	D-sorbitol	$C_6H_{14}O_6$	C 39.6% H 7.8% O 52.7%	Sugar found in berries, algae and seaweed. Many uses in food, medicine and industry.
31	sucrose	$C_{12}H_{22}O_{11}$	C 42.1% H 6.5% O 51.4%	Sugar from cane and sugar beet.
32	D-trehalose	$C_{12}H_{22}O_{11} \cdot 2H_2O$	C 42.1% H 6.5% O 51.4%	Occurs in fungi, some beetles.
33	turanose	$C_{12}H_{22}O_{11}$	C 42.1% H 6.5% O 51.4%	
34	xylitol	$C_5H_{12}O_5$		
35	methylpyruvate			
36	mono-methylsuccinate	$C_6H_{10}O_4$	C 49.3% H 6.9% O 43.8%	
37	acetic acid	$C_2H_4O_2$	C 40% H 6.7% O 53.3%	Organic solvent. Dissolves P,S compounds.
38	cis-aconitic acid	$C_6H_6O_6$	C 41.4% H 3.5% O 55.1%	Found in leaves and tubers of some plants - cane, sugar beet.
39	citric acid	$C_6H_8O_7 \cdot H_2O$	C 37.5% H 4.2% O 58.3%	Widely found in plants, animals and there fluids.
40	formic acid	CH_2O_2	C 25.1% H 4.4% O 69.5%	Strong reducing agent.
41	D-galactonic acid lactone			
42	D-galacturonic acid	$C_6H_{10}O_7$	C 37.1% H 5.2% O 57.7%	
43	D-gluconic acid	$C_6H_{12}O_7$	C 36.7% H 6.2% O 57.1%	
44	D-glucosaminic acid			
45	D-glucuronic acid	$C_6H_{10}O_7$	C 37.1% H 5.2% O 57.7%	Widely found in plants and animals.
46	α -hydroxybutyric acid	$C_4H_8O_3$	C 46.2% H 7.8% O 46.1%	
47	β -hydroxybutyric acid	$C_4H_8O_3$	C 46.2% H 7.8% O 46.1%	
48	γ -hydroxybutyric acid	$C_4H_8O_3$	C 46.2% H 7.8% O 46.1%	
49	p-hydroxyphenylacetic acid	$C_8H_8O_3$		
50	itaconic acid	$C_5H_6O_4$	C 46.1% H 4.7% O 49.2%	Product of aerobic fermentation of carbohydrates.
51	α -ketobutyric acid			
52	α -ketoglutaric acid	$C_5H_6O_5$	C 41.1% H 4.1% O 54.8%	Plays an important role in amino acid metabolism.
53	α -ketovaleric acid			
54	D,L-lactic acid	$C_3H_6O_3$	C 40 % H 6.7% O 53.3%	Fermentation product from lactic acid bacteria. Also found in some plants during germination.
55	malonic acid	$C_3H_4O_4$	C 34.6% H 3.9% O 61.5%	
56	propionic acid	$C_3H_6O_2$	C 48.6% H 8.2% O 43.2%	Acid in dairy products
57	quinic acid	$C_7H_{12}O_6$	C 43.5% H 6.3% O 50%	Found in some plants, and tree bark.
58	D-saccharic acid			
59	sebacic acid	$C_{10}H_{18}O_4$	C 59.4% H 9% O 31.6%	
60	succinic acid	$C_4H_6O_4$	C 40.7% H 5.1% O 54.2%	Occurs in fungi, lichens etc.
61	bromosuccinic acid	$C_4H_5BrO_4$	C 24.4% H 2.6% Br 40.6% O 32.5%	
62	succinamic acid			
63	glucuronamide			
64	alaninamide			
65	D-alanine	$C_3H_7NO_2$	C 40.4% H 7.9% N	

			15.7% O 35.9%	
66	L-alanine	$C_3H_7NO_2$	C 40.4% H 7.9% N 15.7% O 35.9%	An amino acid.
67	L-alanyl-glycine			
68	L-asparagine	$C_4H_8N_2O_3$	C 36.4% H 6.1% N 21.2% O 36.3%	A “non-essential” amino acid.
69	L-aspartic acid	$C_4H_7NO_4$	C 36.1% H 5.3% N 10.5% O 48.1%	A “non-essential” amino acid, occurs in plants and animals, especially cane and sugar beet.
70	L-glutamic acid	$C_5H_9NO_4$	C 40.8% H 6.2% N 9.5% O 43.5%	A “non-essential” amino acid.
71	glycyl-L-aspartic acid			
72	glycyl-L-glutamic acid			
73	L-histidine	$C_6H_9N_3O_2$	C 46.4% H 5.9% N 27.1% O 20.6%	An essential amino acid for animal growth.
74	hydroxy-L-proline	$C_5H_9NO_3$	C 45.8% H 6.9% N 10.7% O 36.6%	A “non-essential” amino acid.
75	L-leucine	$C_6H_{13}NO_2$	C 54.9% H 10% N 10.7% O 24.4%	An amino acid, essential component in human nutrition.
76	L-omithine			
77	L-phenylalanine	$C_9H_{11}NO_2$	C 65.4% H 6.7% N 8.5% O 19.4%	An amino acid, essential component in human nutrition.
78	L-proline	$C_5H_9NO_2$	C 52.1% H 7.9% N 12.2% O 27.8%	A “non-essential” amino acid.
79	L-pyroglutamic acid	$C_5H_7NO_3$	C 46.5% H 5.5% N 10.9% O 37.2%	Found in molasses
80	D-serine	$C_3H_7NO_3$	C 34.3% H 6.7% N 13.3% O 45.7%	A “non-essential” amino acid.
81	L-serine	$C_3H_7NO_3$	C 34.3% H 6.7% N 13.3% O 45.7%	Amino acid in silk fibers.
82	L-threonine	$C_4H_9NO_3$	C 40.3% H 7.6% N 11.8% O 40.3%	An amino acid, essential component in human nutrition.
83	D,L-carnitine	$C_7H_{15}NO_3$	C 52.2% H 9.4% N 8.7% O 29.8%	Constituent of muscle and liver.
84	γ -aminobutyric acid	$C_4H_9NO_2$	C 46.6% H 8.8% N 13.6% O 31%	
85	urocanic acid	$C_6H_6N_2O_2$	C 52.2% H 4.4% N 20.3% O 23.2%	
86	inosine	$C_{10}H_{12}N_4O_5$	C 44.8% H 4.5% N 20.9% O 29.8%	In meat and sugar beet.
87	uridine	$C_9H_{12}N_2O_6$	C 44.3% H 5% N 11.5% O 39.3%	
88	thymidine	$C_{10}H_{14}N_2O_5$	C 49.6% H 5.8% N 11.6% O 33%	
89	phenylethylamine	$C_8H_{11}N$		
90	putrescine			
91	2-aminoethanol			
92	2,3-butanediol	$C_4H_{10}O_2$		
93	glycerol	$C_3H_8O_3$	C 39.1% H 8.8% O 52.1%	Biprodukt from oils and fats.
94	D,L- α -glycerol phosphate			
95	glucose-1-phosphate	$C_6H_{13}O_9P$	C 27.7% H 5% O 55.4% P 11.9%	Found widely in plants and animals. Precursor for starch in plants, and also the first breakdown product of starch.
96	glucose-6-phosphate	$C_6H_{13}O_9P$	C 27.7% H 5% O 55.4% P 11.9%	Enzyme conversion product of (95) above.

8.2 C SUBSTRATE USE FREQUENCY

Table 8.1A Extent of Substrate Use

1. Field-Dry Soils

	Day 2	Day 4	Day 6
Upper Quartile	39, 31, 18, 8, 58, 43, 38, 32, 42, 38, 32, 42, 95, 68, 26, 96, 16, 14, 23, 24, 10, 52, 45, 60	16, 14, 18, 24, 10, 31, 38, 32, 78, 68, 69, 22, 8, 74, 23, 81, 84, 85, 29, 19, 17, 12, 79, 42	18, 14, 78, 16, 24, 10, 12, 3, 17, 19, 31, 23, 69, 6, 22, 11, 74, 32, 79, 38, 85, 30, 81, 8
Lower Quartile	2, 33, 63, 50, 88, 21, 15, 76, 77, 91, 62, 82, 75, 59, 9, 34, 13, 40, 92, 46, 64, 51, 89, 53	34, 55, 88, 4, 21, 27, 62, 71, 94, 61, 37, 77, 80, 56, 63, 82, 64, 59, 89, 40, 46, 51, 92, 53	4, 88, 83, 64, 71, 95, 96, 60, 41, 82, 55, 89, 59, 63, 94, 37, 61, 80, 51, 46, 56, 40, 53, 92

Soils (AWCD): D2; 13, 5, 14, 8, 12, 9, 10, 6, 3, 1, 11, 4
D4: 13, 9, 5, 3, 12, 8, 10, 14, 1, 6, 4, 11
D6; 13, 5, 9, 3, 12, 8, 10, 6, 4, 14, 1, 11

2. Moistened Glasshouse-Incubated Soils

	Day 2	Day 4	Day 6
Upper Quartile	31, 8, 18, 32, 14, 12, 30, 26, 24, 43, 42, 10, 16, 22, 28, 38, 25, 39, 96, 45, 17, 95, 23, 58	18, 14, 16, 78, 17, 8, 12, 10, 24, 30, 31, 38, 22, 32, 29, 68, 81, 74, 85, 26, 28, 69, 79, 86	18, 14, 78, 16, 17, 8, 6, 5, 7, 81, 79, 3, 24, 74, 10, 12, 22, 23, 69, 86, 15, 57, 33, 38
Lower Quartile	55, 50, 15, 71, 63, 21, 62, 83, 13, 77, 34, 82, 9, 91, 75, 76, 40, 51, 59, 64, 92, 46, 89, 53	60, 36, 94, 71, 34, 55, 75, 77, 61, 76, 13, 83, 89, 82, 63, 37, 59, 51, 40, 64, 56, 46, 92, 53	95, 96, 76, 48, 89, 80, 71, 59, 64, 41, 82, 55, 83, 63, 60, 51, 94, 61, 40, 46, 37, 56, 53, 92

Soils (AWCD): D2; 8, 1, 13, 14, 12, 4, 9, 6, 3, 10, 5, 11
D4; 8, 12, 14, 1, 13, 6, 9, 3, 10, 5, 11, 4
D6; 8, 12, 14, 13, 10, 1, 6, 9, 5, 11, 3, 4

8.3 CLUSTER ANALYSIS

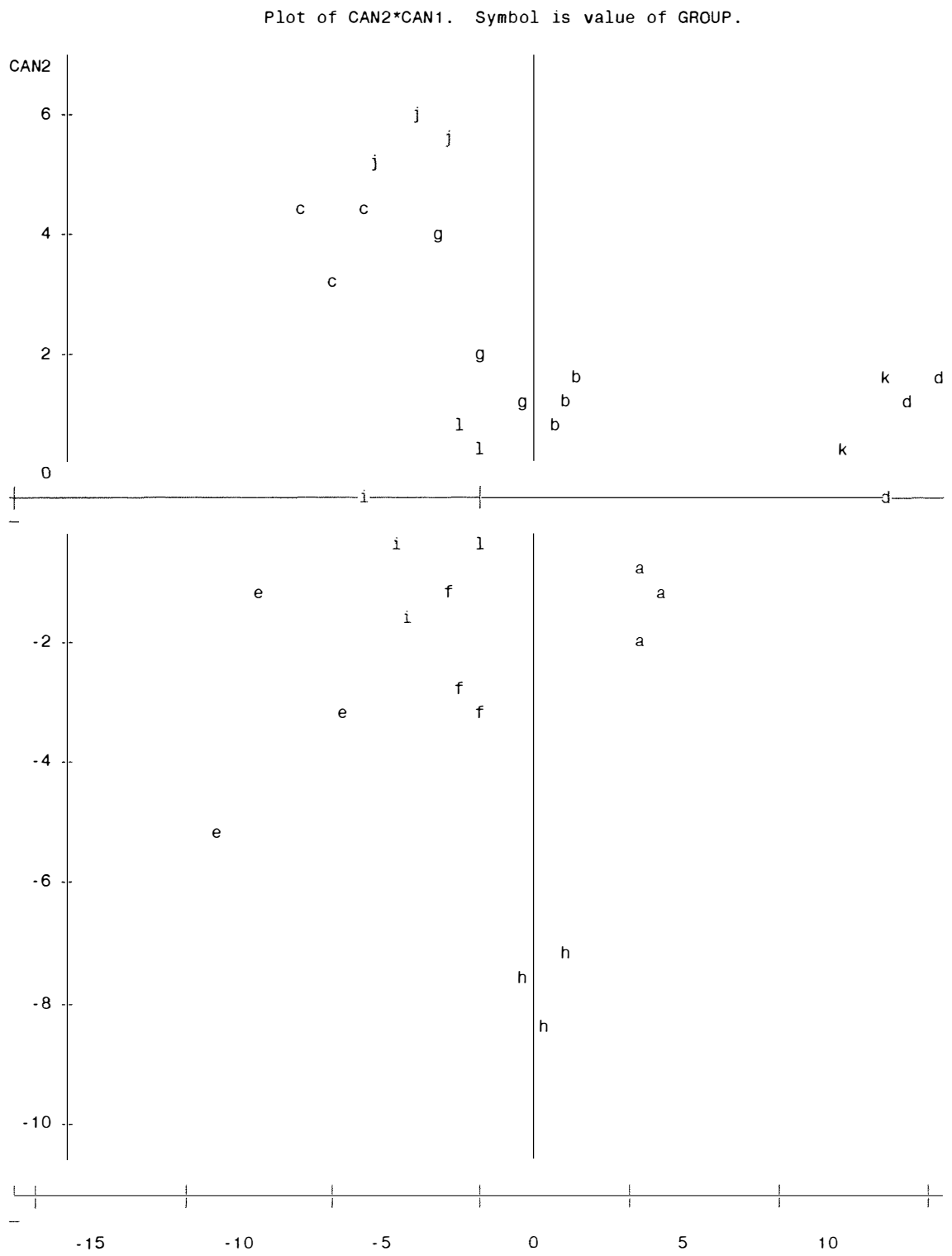


Figure 8.1^A Plot of CAN1*CAN2, for C substrate use at day 2 of incubation in the BiologTM plate, for all field-dry soil inoculum. See Figure 8.6^A for symbol key.

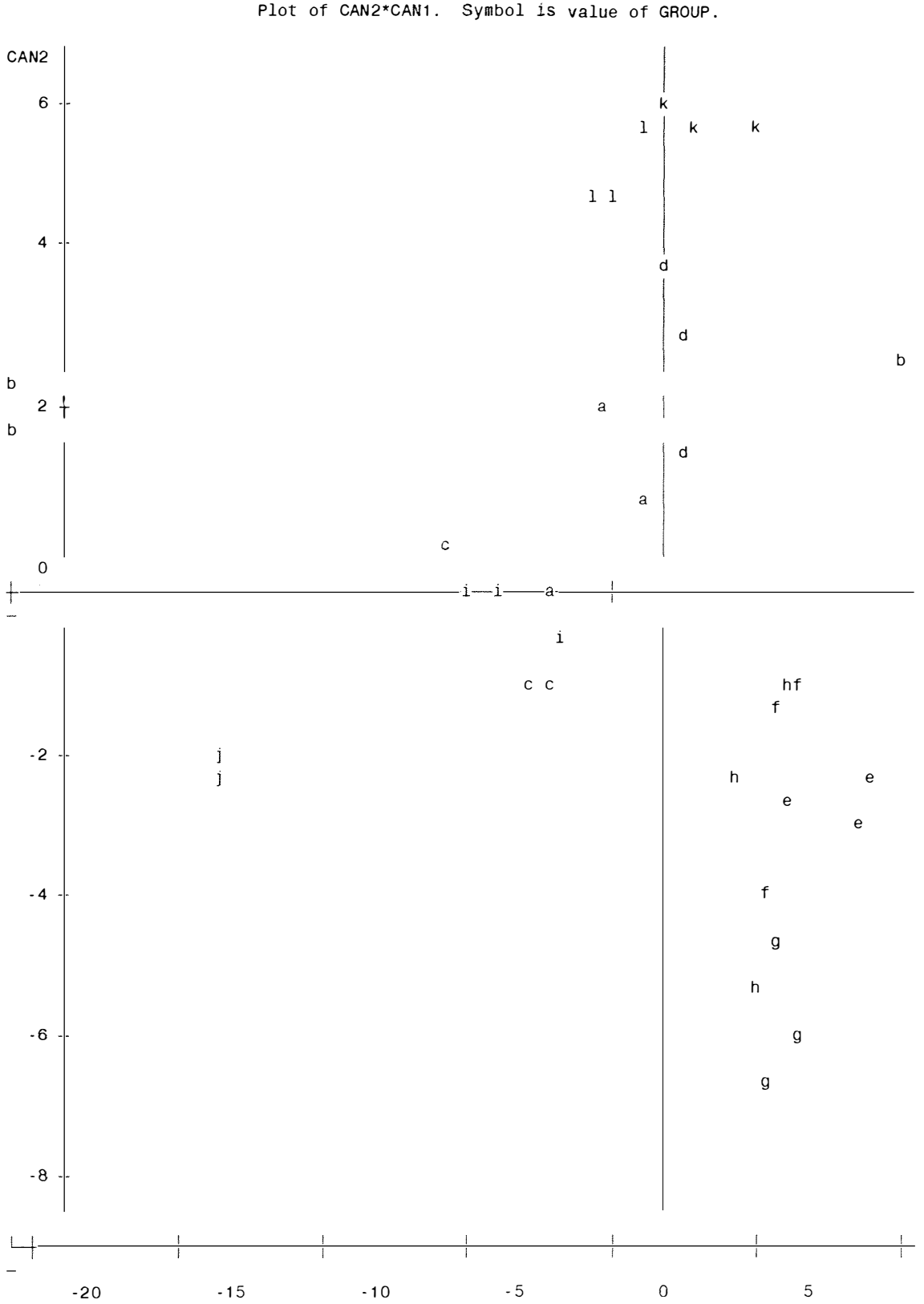


Figure 8.2^A Plot of CAN1*CAN2, for C substrate use at day 4 of incubation in the BiologTM plate, for all field-dry soil inoculum. See Figure 8.6^A for symbol key.

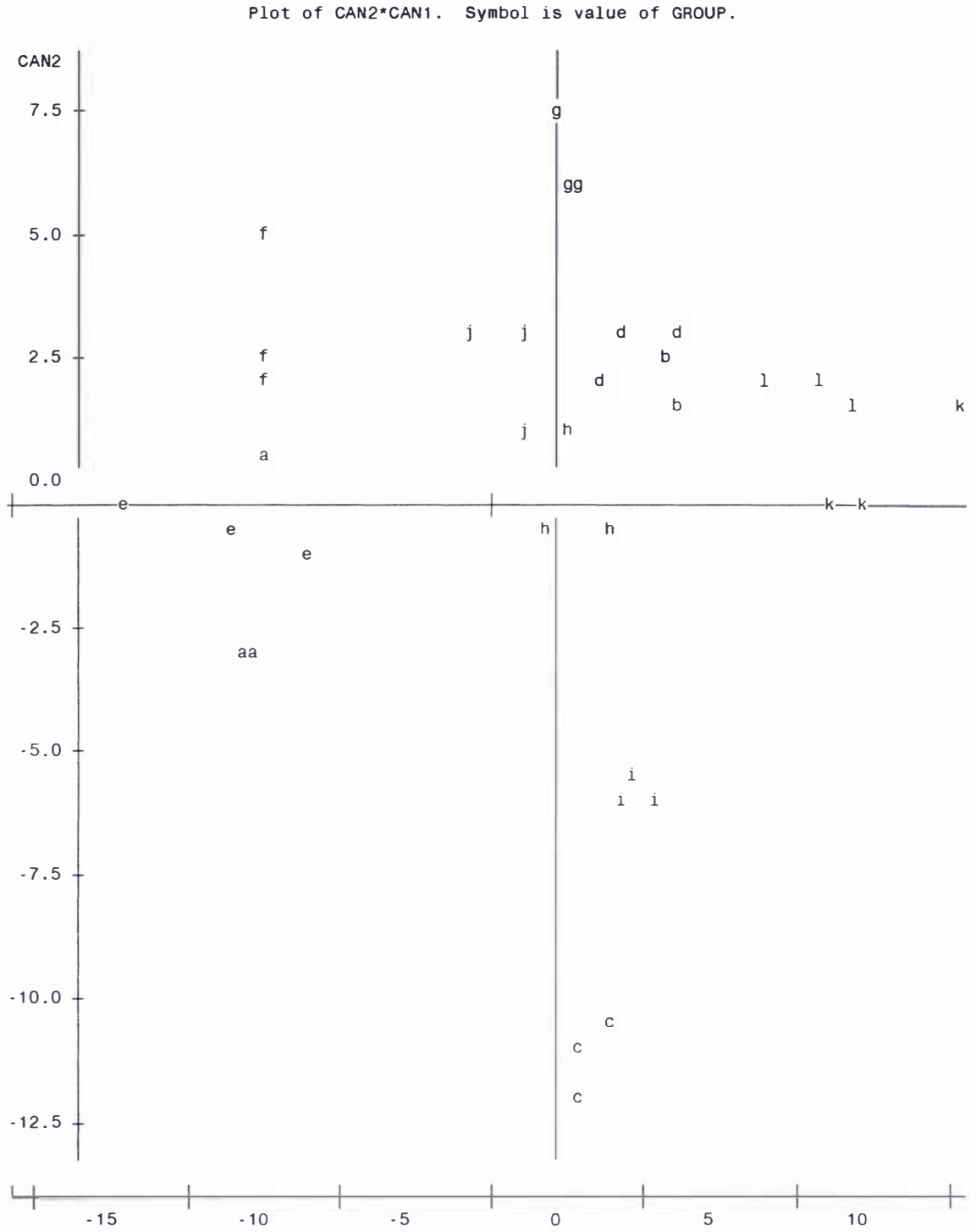


Figure 8.3^A Plot of CAN1*CAN2, for C substrate use at day 6 of incubation in the BiologTM plate, for all field-dry soil inoculum. See Figure 8.6^A for symbol key.

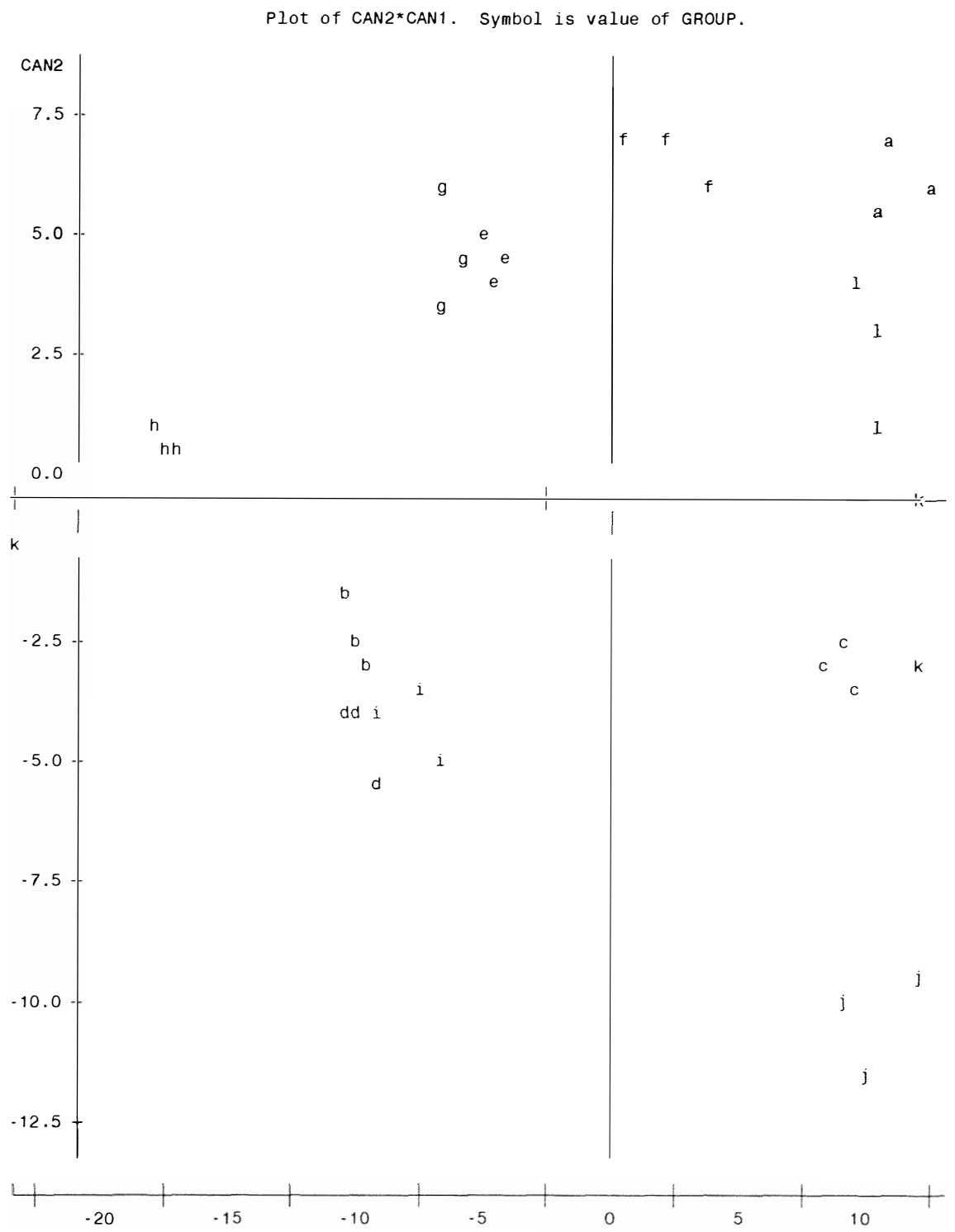


Figure 8.4^A Plot of CAN1*CAN2, for C substrate use at day 2 of incubation in the BiologTM plate, for all moistened glasshouse incubated soil inoculum. See Figure 8.6^A for symbol key.

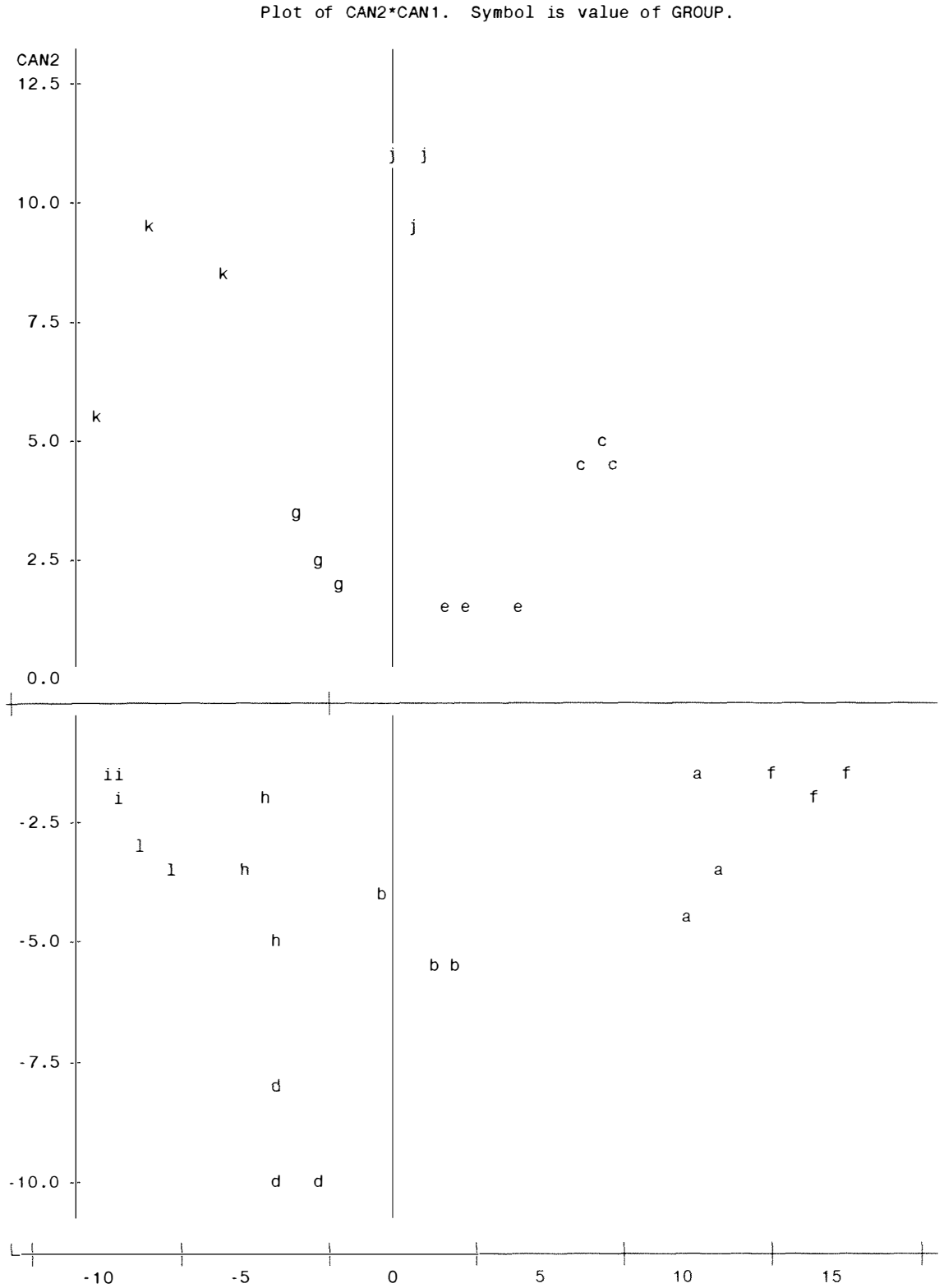


Figure 8.5^A Plot of CAN1*CAN2, for C substrate use at day 4 of incubation in the BiologTM plate, for all moistened glasshouse incubated soil inoculum. See Figure 8.6^A for symbol key.

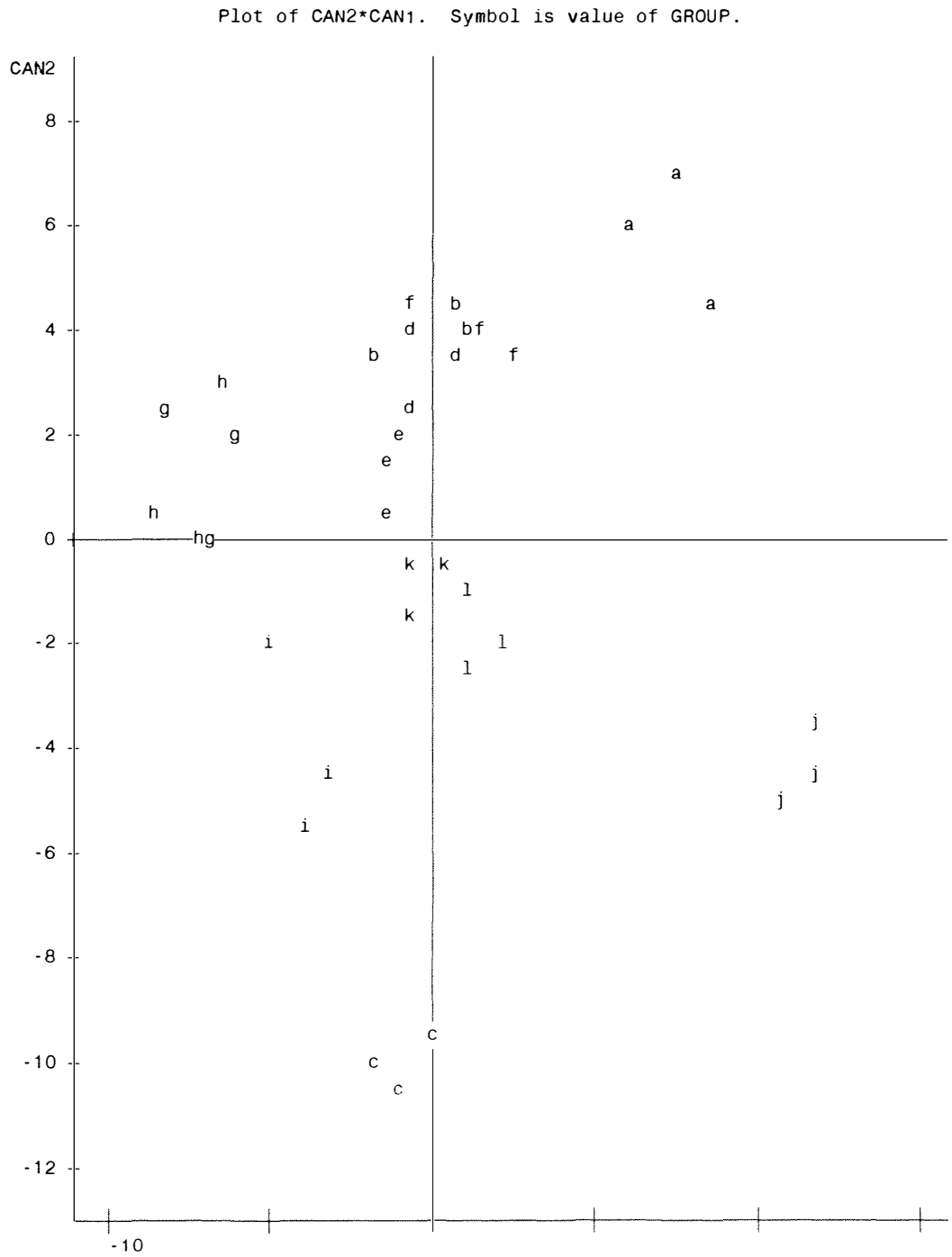


Figure 8.6^A Plot of CAN1*CAN2, for C substrate use at day 6 of incubation in the BiologTM plate, for all moistened glasshouse incubated soil inoculum.

Symbol Key: a = Site 1; b = Site 3; c = Site 4; d = Site 5; e = Site 6; f = Site 8; g = Site 9; h = Site 10; i = Site 11; j = Site 12; k = Site 13, and l = Site 14.