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# **Root-soil-phosphate interactions in rice growing in aerobic soil**

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

**Stephen Neil Trolove  
2000**

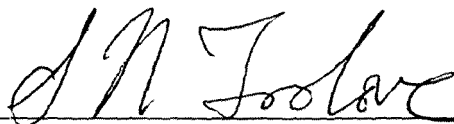
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**TO WHOM IT MAY CONCERN**

This is to state the research carried out for my PhD thesis entitled "Root-soil-phosphate interactions in rice growing in aerobic soil" in the Institute of Natural Resources, Massey University, Turitea Campus, New Zealand and in the Soil and Water Sciences Division of the International Rice Research Institute, Los Baños, Philippines, is all my own work.

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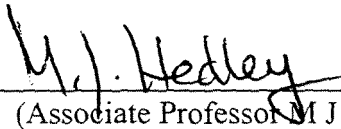
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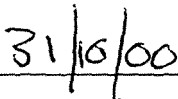
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This is to state the research carried out for the PhD thesis entitled "Root-soil-phosphate interactions in rice growing in aerobic soil" was done by Stephen Neil Trolove in the Institute of Natural Resources, Massey University, Turitea Campus, New Zealand and in the Soil and Water Sciences Division of the International Rice Research Institute, Los Baños, Philippines. The thesis material has not been used for any other degree.

**Supervisor:**

  
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(Associate Professor M J Hedley)

**Date:**

  
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## Abstract

Rice (*Oryza sativa* L.) is the staple food of subsistence farmers in the vast areas of Ultisols and Oxisols of the tropical and subtropical rainfed uplands and lowlands. Phosphorus (P) deficiency and soil acidity commonly constrain yields. Phosphorus fertiliser is considered an expensive input, and must therefore be used efficiently. The objective of this thesis was to investigate fertiliser strategies and plant mechanisms that could enhance the uptake efficiency of P by aerobically grown rice. The long-term aim of understanding rice P-uptake mechanisms is that such research will help in developing P-efficient rice varieties.

In acid soils, aluminium (Al) toxicity restricts root growth and therefore limits P uptake. A bioassay was developed as a basis to compare two techniques for assessing concentrations of phytotoxic Al. It was found that Al in soil solution extracted by centrifugation correlated better with rice root extension than Al extracted in 0.02 M CaCl<sub>2</sub>. Aluminium toxicity was found not to restrict root growth (hence P uptake) in the Philippines Ultisol (Cavinti soil) used in later experiments.

Experiments investigating the effect of different fertiliser management practices, showed that banding of fertiliser P, as opposed to incorporating P fertiliser throughout the soil, enhanced the availability of P to rice grown in the high P-fixing Cavinti soil. The practice of applying green manure with reactive phosphate rock (RPR) decreased the dissolution of RPR because mineralisation of green manure nitrogen increased the soil pH.

Aerobically grown rice exhibited a number of mechanisms that would enhance P uptake: rhizosphere acidification, localised proliferation of fine roots in P-rich zones, and association with mycorrhizae. Mathematical modelling indicated that upland rice must be able to release solubilising agents, e.g. organic anions, in order to explain the observed P uptake in banded, moderately fertilised soil. By extracting soil fertilised at different P rates with citrate solutions, it was found that more P was extracted, per mole of citrate added, from highly fertilised soil. This indicated that there would be a positive interaction between banding fertiliser P and solubilisation by organic anions. Initial extraction, storage and detection methods were unable to identify significant quantities of organic acids in the rhizosphere of aerobically grown rice. Better methods for extracting organic anions from soil were developed, and improved procedures for studying the mechanisms of plant induced changes in the rhizosphere are proposed.

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# Table of contents

Abstract.....	i
Acknowledgements.....	ii
Table of contents.....	iii
List of figures.....	ix
List of tables.....	xiv
List of symbols.....	xvi

## 1. INTRODUCTION. SOIL CONSTRAINTS TO RAINFED RICE PRODUCTION AND STRATEGIES TO OVERCOME THEM, WITH EMPHASIS ON PHOSPHATE SUPPLY..... 1

1.1 THE NEED TO INCREASE PRODUCTION OF RICE.....	1
1.1.1 <i>The four main agro-ecosystems that produce the world's rice</i> .....	2
1.1.1.1 Irrigated.....	2
1.1.1.2 Rainfed lowland.....	3
1.1.1.3 Upland.....	3
1.1.1.4 Deepwater/Flood-prone.....	3
1.1.2 <i>The need to increase the production of rainfed lowland and upland rice</i> .....	4
1.2 MAIN FACTORS LIMITING THE YIELD OF RAINFED AND UPLAND RICE.....	5
1.3 SOIL-RELATED CONSTRAINTS TO RAINFED AND UPLAND RICE PRODUCTION.....	6
1.3.1 <i>Acidity related problems</i> .....	6
1.3.1.1 Soil acidity and processes of soil acidification.....	6
1.3.1.2 pH buffering reactions in acid soils.....	9
1.3.1.3 Acidity related toxicities.....	10
1.3.2 <i>Low available P and high P-fixation capacity</i> .....	12
1.3.2.1 Phosphorus availability in upland soils.....	12
1.3.2.2 Phosphorus availability in flooded soils.....	13
1.3.3 <i>Nitrogen deficiency</i> .....	14
1.3.4 <i>Deficiencies of other nutrients</i> .....	14
1.3.5 <i>Poor physical properties</i> .....	15
1.4 STRATEGIES TO OVERCOME SOIL CONSTRAINTS TO RICE PRODUCTION (EXCEPT P DEFICIENCY).....	16
1.4.1 <i>Fertiliser use</i> .....	16
1.4.1.1 Amelioration of acidity.....	16
1.4.1.2 Correction of N deficiency.....	18
1.4.1.3 Low basic cations, trace element deficiencies.....	19
1.4.1.4 Poor physical properties.....	19
1.4.2 <i>Organic amendments</i> .....	19
1.4.2.1 Acidity.....	19
1.4.2.2 Nitrogen deficiency.....	20
1.4.2.3 Deficiencies of S, basic cations, and trace elements.....	20
1.4.2.4 Poor physical properties.....	20
1.4.3 <i>Plant breeding strategies to overcome soil constraints</i> .....	20
1.4.3.1 Acidity and Al tolerance.....	20
1.4.3.2 Nitrogen deficiency.....	22
1.4.3.3 Improving uptake of nutrients (other than N and P).....	23
1.4.3.4 Breeding plants to tolerate poor soil physical properties.....	23
1.5 STRATEGIES TO OVERCOME P-RELATED CONSTRAINTS TO RICE PRODUCTION.....	24
1.5.1 <i>Fertiliser use</i> .....	24
1.5.1.1 Management strategies to improve the efficiency of P fertiliser use by rice.....	24
1.5.2 <i>Organic amendments</i> .....	27
1.5.3 <i>Breeding rice plants with an improved efficiency of P use</i> .....	28
1.5.3.1 Increasing internal P efficiency.....	28
1.5.3.2 Increasing external P efficiency.....	28
1.5.3.2.1 Phosphorus-efficient root structures.....	29
1.5.3.2.2 Manipulation of rhizosphere pH.....	29
1.5.3.2.3 Release of chelating agents.....	30
1.5.3.2.4 Release of phosphatase enzymes.....	38
1.5.3.2.5 Association with mycorrhizal fungi.....	39

1.5.3.3	Examples from rice breeding programs to date .....	40
1.6	THE PURPOSE OF THIS THESIS .....	41
<b>2.</b>	<b>ASSESSING THE EFFECT OF Al ON RICE ROOT GROWTH.....</b>	<b>43</b>
2.1	INTRODUCTION .....	43
2.2	OBJECTIVES .....	45
2.3	MATERIALS AND METHODS.....	46
2.3.1	<i>Experiment I: The influence of soil characteristics on extractable Al and root elongation....</i>	46
2.3.1.1	Soils .....	46
2.3.1.2	Preparation of root bioassay containers .....	46
2.3.1.3	Plants.....	47
2.3.2	<i>Experiment II: Evaluation of extraction methods and measuring techniques for determining the effect of Al on root elongation.....</i>	47
2.3.2.1	Experiment IIa: The effect of pH on rice root elongation and the evaluation of 0.02 M CaCl <sub>2</sub> -extractable Al to detect Al toxicity.....	47
2.3.2.2	Experiment IIb: A comparison of extraction procedures and measurement methods.....	48
2.3.3	<i>Experiment III: The effect of fertiliser amendment on soil Al concentration and root elongation.....</i>	49
2.3.4	<i>Experiment IV: Use of the bioassay to evaluate root growth in an Ultisol from Cavinti, Philippines.....</i>	51
2.3.5	Statistics .....	51
2.4	RESULTS AND DISCUSSION .....	51
2.4.1	<i>Experiment I: The influence of soil characteristics on root elongation.....</i>	51
2.4.2	<i>Experiment II: Evaluation of extraction methods and measuring techniques for determining the effect of Al on root elongation.....</i>	53
2.4.2.1	Experiment IIa: The effect of pH on rice root elongation and the evaluation of 0.02 M CaCl <sub>2</sub> -extractable Al to detect Al toxicity.....	53
2.4.2.2	Experiment IIb: A comparison of extraction procedures and measurement methods.....	58
2.4.3	<i>Experiment III: The effect of fertiliser amendment on soil Al concentration and root elongation.....</i>	65
2.4.4	<i>Experiment IV: Use of the bioassay to evaluate root growth in an Ultisol from Cavinti, Philippines.....</i>	68
2.4.5	General discussion.....	69
2.4.5.1	Evaluation of the bioassay technique.....	69
2.4.5.2	The use of high rates of N fertiliser at sowing.....	70
2.5	CONCLUSIONS.....	70
<b>3.</b>	<b>PHOSPHATE FERTILISER MANAGEMENT FOR RICE IN AEROBIC SOIL I. INTERACTIONS BETWEEN PHOSPHATE ROCK AND ORGANIC AMENDMENTS.....</b>	<b>72</b>
3.1	INTRODUCTION .....	72
3.2	OBJECTIVES .....	75
3.3	MATERIALS AND METHODS.....	75
3.3.1	Soil.....	75
3.3.2	Amendments.....	76
3.3.2.1	Phosphate rock.....	76
3.3.2.2	Organic manures.....	76
3.3.3	Treatments.....	76
3.3.4	Incubation study.....	77
3.3.5	Rice growth experiment.....	78
3.3.6	Soil analysis.....	80
3.3.6.1	Ammonium and nitrate.....	80
3.3.6.2	Soil pH and P fractionation.....	80
3.3.6.3	Microbial P.....	82
3.3.7	Statistics .....	82
3.4	RESULTS AND DISCUSSION.....	83
3.4.1	Incubation study.....	83
3.4.2	Rice growth experiment.....	84
3.4.2.1	Dry matter production and plant nitrogen status.....	84
3.4.2.2	The ability of organic amendments to supply adequate N and P for the growth of aerobic rice.....	85



3.4.3	<i>Liming or acidifying effects of the soil treatments</i> .....	87
3.4.3.1	Bulk soil pH.....	87
3.4.3.2	Rhizosphere pH .....	88
3.4.4	<i>Phosphorus fractionation</i> .....	88
3.4.4.1	NaCl/TEA-P plus Olsen P .....	89
3.4.4.2	1 M NaOH-Pf.....	90
3.4.4.3	1 M NaOH-Po.....	90
3.4.4.4	0.5 M H <sub>2</sub> SO <sub>4</sub> -P.....	93
3.4.4.5	Residual P.....	94
3.4.4.6	Recovery of P .....	95
3.4.5	<i>Soil microbial P</i> .....	95
3.4.6	<i>General discussion – the effect of green manure addition on RPR dissolution</i> .....	98
3.4.6.1	pH and Ca effect .....	98
3.4.6.2	Other factors that may affect RPR dissolution .....	98
3.4.6.3	Strategies for green manure and RPR use .....	99
3.5	CONCLUSIONS.....	100

#### **4. PHOSPHATE FERTILISER MANAGEMENT FOR RICE IN AEROBIC SOIL II. SOIL P ADSORPTION RELATIONS AND THE EFFECT OF FERTILISER BANDING..... 101**

4.1	INTRODUCTION.....	101
4.2	OBJECTIVES.....	103
4.3	METHOD.....	103
4.3.1	<i>Phosphate adsorption isotherm</i> .....	104
4.3.2	<i>The effect of increasing P addition on the P buffer power</i> .....	104
4.3.3	<i>Phosphate desorption by citrate</i> .....	105
4.4	RESULTS AND DISCUSSION .....	106
4.4.1	<i>Phosphate adsorption isotherm and the implications for fertiliser application</i> .....	106
4.4.2	<i>The effect of increasing P addition on the P buffer power</i> .....	108
4.4.3	<i>Phosphate desorption by citrate</i> .....	112
4.4.3.1	Solution pH.....	112
4.4.3.2	Distribution of citrate between the soil surface and solution .....	112
4.4.3.3	Amount of P, Fe, Mn or Al dissolved by citrate .....	113
4.4.3.4	Mechanism of dissolution of Al, Fe and P by citrate .....	114
4.4.3.5	The recovery of fertiliser P by citrate, and the implications for soil P solubilisation by citrate exuded from rice roots .....	117
4.4.3.6	Enhanced dissolution of P by an interaction between high soil P concentration and citrate addition.....	118
4.5	CONCLUSIONS.....	120

#### **5. STUDIES ON P UPTAKE MECHANISMS I. OBSERVED AND CALCULATED UPTAKE IN BANDED AND INCORPORATED THIN LAYER SYSTEMS..... 122**

5.1	INTRODUCTION.....	122
5.1.1	<i>P uptake efficiency mechanisms: moving from theory to practice</i> .....	122
5.1.2	<i>Means of identifying P efficiency – justification for the methodology used</i> .....	123
5.2	PRELIMINARY EXPERIMENT .....	124
5.2.1	● <i>Objective of the preliminary experiment</i> .....	124
5.2.2	<i>Method used for the preliminary experiment</i> .....	124
5.2.2.1	Soil.....	124
5.2.2.2	The thin layer system.....	125
5.2.2.3	Plants.....	125
5.2.3	<i>Results of the preliminary experiment</i> .....	127
5.2.3.1	Plant growth.....	127
5.2.4	<i>Conclusions of the preliminary experiment</i> .....	129
5.3	MAIN EXPERIMENT .....	130
5.3.1	● <i>Objectives</i> .....	130
5.3.2	<i>Materials and methods</i> .....	130
5.3.2.1	Preparation of thin layers .....	130
5.3.2.2	Plant growth system .....	130
5.3.2.3	Plant analysis .....	131
5.3.2.4	Soil analysis.....	132
5.3.2.5	Modelling P uptake .....	133

5.3.2.6	Statistics .....	140
5.4	RESULTS AND DISCUSSION.....	140
5.4.1	<i>Plant growth</i> .....	140
5.4.2	<i>Plant P concentration</i> .....	142
5.4.3	<i>Soil P concentration</i> .....	142
5.4.4	<i>Modelling P uptake in the absence of solubilisation effects (Model 1)</i> .....	143
5.4.4.1	Discussion of parameters used in the model.....	143
5.4.4.2	Comparing P uptake predicted by the model with observed P uptake.....	144
5.4.5	<i>Factors governing P uptake</i> .....	145
5.4.5.1	Water content.....	145
5.4.5.2	Mass flow.....	145
5.4.5.3	Root studies.....	146
5.4.5.4	Mycorrhiza.....	148
5.4.5.5	Release of organic anions (Model II).....	148
5.5	CONCLUSIONS.....	150
6.	<b>THE EXTRACTION, STORAGE, AND ANALYSIS OF ORGANIC ACIDS FROM HIGHLY WEATHERED SOILS.....</b>	<b>151</b>
6.1	EXTRACTION OF ORGANIC ACIDS .....	151
6.1.1	<i>Introduction</i> .....	151
6.1.2	<i>Objective</i> .....	153
6.1.3	<i>Method</i> .....	153
6.1.3.1	Effectiveness of different extractants.....	153
6.1.3.2	Effect of extractant concentration.....	154
6.1.3.3	Refining the procedure.....	154
6.1.4	<i>Results</i> .....	155
6.1.4.1	Comparing the effectiveness of different extractants.....	155
6.1.4.2	The effect of extractant concentration.....	157
6.1.4.3	Refining the procedure.....	158
6.1.5	<i>Discussion</i> .....	160
6.2	STORAGE OF ORGANIC ACIDS.....	162
6.2.1	<i>Introduction</i> .....	162
6.2.2	<i>Objectives</i> .....	163
6.2.3	<i>Method</i> .....	163
6.2.3.1	The effect of acidification and freezing.....	163
6.2.3.2	The effect of light.....	164
6.2.4	<i>Results</i> .....	165
6.2.4.1	Storage of organic acid samples.....	165
6.2.5	<i>Discussion</i> .....	167
6.2.5.1	Storage of organic acid samples.....	167
6.2.5.2	Storage: the effect of light.....	169
6.3	ANALYSIS: THE EFFECT OF DIFFERENT IONS ON THE MEASURED CONCENTRATION AND RETENTION TIME OF CITRATE .....	170
6.3.1	<i>Introduction</i> .....	170
6.3.2	<i>Objective</i> .....	171
6.3.3	<i>Method</i> .....	171
6.3.4	<i>Results</i> .....	171
6.3.5	<i>Discussion</i> .....	171
6.4	GENERAL DISCUSSION ON THE EXPERIMENTAL TECHNIQUE AS A MEANS FOR DETERMINING THE ROLE OF ROOT RELEASED ORGANIC ANIONS IN P UPTAKE .....	172
6.5	CONCLUSIONS.....	173
7.	<b>STUDIES ON P UPTAKE MECHANISMS II. FACTORS AFFECTING ROOT PENETRATION THROUGH FINE PORES.....</b>	<b>174</b>
7.0	INTRODUCTION .....	174
7.1	PART I: THE SECOND THIN LAYER EXPERIMENT .....	174
7.1.1	<i>Objectives</i> .....	174
7.1.2	<i>Materials and methods</i> .....	174
7.1.2.1	Soil.....	174

7.1.2.2	Plant growth.....	175
7.1.2.3	Measurements.....	175
7.1.3	<i>Results</i> .....	175
7.1.3.1	Growth and P uptake.....	175
7.1.3.2	Microscopic examination of roots.....	177
7.1.4	<i>Discussion</i> .....	178
7.2	PART II: INVESTIGATING WHY THE ROOT HAIRS PENETRATED THE NYLON MESH.....	179
7.2.1	<i>Objectives</i> .....	179
7.2.2	<i>Materials and methods</i> .....	179
7.2.3	<i>Results and discussion</i> .....	181
7.3	CONCLUSIONS.....	185
<b>8.</b>	<b>STUDIES ON P UPTAKE MECHANISMS III. THE EFFECT OF MYCORRHIZAL INFECTION.....</b>	<b>186</b>
8.1	INTRODUCTION.....	186
8.2	OBJECTIVE.....	186
8.3	MATERIALS AND METHODS.....	186
8.4	RESULTS.....	187
8.4.1	<i>Plant dry weight</i> .....	187
8.4.2	<i>Phosphorus uptake</i> .....	188
8.4.3	<i>Mycorrhizal infection</i> .....	190
8.5	DISCUSSION.....	193
8.5.1	<i>Explaining the observed P uptake</i> .....	193
8.5.2	<i>The effect of soil P concentration on the proportion of root length infected with VAM</i> .....	194
8.5.3	<i>The relationship between P deficiency, organic anion release, and VAM infection</i> .....	196
8.5.4	<i>Implications of this experiment on the importance of VAM in enhancing P uptake in rainfed rice</i> .....	197
8.5.5	<i>Why were mycorrhizae present in this experiment but not in the first thin layer experiment?</i> .....	198
8.6	CONCLUSIONS.....	199
<b>9.</b>	<b>STUDIES ON P UPTAKE MECHANISMS IV. ISSUES ASSOCIATED WITH GROWING PLANTS IN STERILISED SOIL.....</b>	<b>200</b>
9.1	INTRODUCTION.....	200
9.2	OBJECTIVES.....	201
9.3	MATERIALS AND METHODS.....	201
9.3.1	<i>Improvements made to the experimental set-up</i> .....	201
9.3.1.1	Preliminary experiment to check that irradiation had eliminated VAM.....	201
9.3.1.2	Main experiment.....	201
9.3.2	<i>Improvements made to the analytical techniques</i> .....	202
9.3.2.1	Soil measurements.....	202
9.3.2.2	Plant measurements.....	203
9.3.2.3	Modelling P uptake.....	203
9.3.2.4	Phosphate desorption isotherms for P5000 Cavinti soil at different pH.....	204
9.3.2.5	Model 3: P uptake with solubilisation by acidification.....	204
9.3.2.6	Statistics.....	205
9.4	RESULTS AND DISCUSSION.....	205
9.4.1.1	Effect of $\gamma$ irradiation.....	205
9.4.1.2	Plant dry weight.....	206
9.4.1.3	Soil pH.....	208
9.4.1.4	Phosphorus in solution.....	211
9.4.1.5	Plant P uptake.....	211
9.4.2	<i>Modelling P uptake</i> .....	213
9.4.2.1	Modelling P uptake in the absence of solubilisation (Model 1).....	213
9.4.2.2	Modelling P uptake to estimate the importance of $H^+$ release as a means of solubilising P (Model 3)..	216
9.4.3	<i>Uptake of Fe, Mn, and Al</i> .....	220
9.4.3.1	Manganese toxicity.....	221
9.4.4	<i>Organic acid release</i> .....	222

9.5 CONCLUSIONS .....	223
<b>10. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK.....</b>	<b>224</b>
10.1 SUMMARY AND CONCLUSIONS.....	224
10.2 FUTURE WORK.....	227
<b>REFERENCES.....</b>	<b>229</b>
<b>APPENDIX A</b> Stability constants for metal-ligand complexes in solution for Experiment 4.3.3 .....	<b>256</b>
<b>APPENDIX B</b> Composition of the modified Yoshida <i>et al.</i> (1976) nutrient solution.....	<b>257</b>
<b>APPENDIX C</b> Calculation of the phosphate-citrate interaction coefficient, $\lambda_c$ .....	<b>258</b>
<b>APPENDIX D</b> Estimation of the amount of citrate contained in bacteria in a rhizosphere extract.....	<b>261</b>

## List of figures

Figure 1.1. Relative distribution of the activities of $Al^{3+}$ and mononuclear hydroxy-Al species as a function of pH.....	11
Figure 1.2. Manganese transformations in <i>aerobic</i> soil.....	12
Figure 1.3. Schematic diagram showing the rate-limiting factors and variables determining phosphate rock ( $Ca_{10}(PO_4)_6F_2$ ) dissolution.....	26
Figure 2.1. Root elongation in samples of Egmont brown loam and various horizons of Wharekohe silt loam.....	52
Figure 2.2. pH buffer curve of Wharekohe silt loam subsoil (96–114cm depth).....	53
Figure 2.3 The effect of soil pH on 0.02 M $CaCl_2$ -extractable Al and Mn in Wharekohe subsoil (96–114 cm depth).....	54
Figure 2.4. The relationship between pH, 0.02 M $CaCl_2$ -extractable Al, and root length of rice seedlings after 42 h growth in Wharekohe silt loam subsoil (96–114 cm depth).....	55
Figure 2.5. The relationship between rice root length and Al extracted by 0.02 M $CaCl_2$ for Experiment IIa.....	58
Figure 2.6. The relationship between soil solution pH and root length for rice and lucerne seedlings grown in Wharekohe silt loam subsoil (96–114 cm depth).....	59
Figure 2.7. The effect of soil pH on rice root elongation and soil Al concentration (as recovered by centrifugation or extracted by 0.02 M $CaCl_2$ ) in Wharekohe silt loam subsoil (96–114 cm depth). The shaded rectangle indicates the range of critical concentrations for Al toxicity in rice, as given by the majority of studies in Table 2.4.....	60
Figure 2.8. The relationship between the concentration of Al extracted in 0.02 M $CaCl_2$ (as measured by PCV) and root length of rice grown in Wharekohe subsoil.....	61
Figure 2.9. The relationship between the concentration of Al in soil solution (as measured by PCV) and root length of rice grown in Wharekohe subsoil.....	62
Figure 2.10 The relationship between the $Al_{PCV}:Ca$ ratio in the soil solution and root length of rice grown in Wharekohe subsoil. The treatments with high fertiliser rates (high urea and high $Ca(NO_3)_2$ ) are shown separately.....	63
Figure 2.11. The relationship between Al measured by AAS by spectrophotometric analysis of Al reacted with PCV.....	64
Figure 2.12. Root length of 42-h-old rice seedlings grown in Wharekohe silt loam subsoil (36–53 cm depth) amended with different fertilisers.....	66
Figure 2.13. Root lengths of 42-h-old lucerne grown in Wharekohe silt loam subsoil (36–53 cm depth) amended with different fertilisers.....	68
Figure 3.1. The incubation system for measuring microbial respiration (after Jerez 1988).....	78
Figure 3.2. The experimental system showing a root study container (exploded view) on a moist sand bath.....	79
Figure 3.3. $CO_2$ production from soil and organic amendment mixtures in the presence or absence of P (Sechura RPR) incubated at 43% moisture.....	83

Figure 3.4. The effect of different organic matter amendments and RPR on shoot dry matter yields of upland rice after 25 d of growth.....	84
Figure 3.5. 0.02 M CaCl <sub>2</sub> -extractable soil NH <sub>4</sub> concentrations 18 d after sowing, and easily-extractable P (NaCl/TEA-P plus Olsen P) 25 d after sowing.....	85
Figure 3.6. Changes in soil pH with distance from the nylon mesh. (See Table 3.2 for treatment key). Data are means of two replicates.....	88
Figure 3.7. NaCl/TEA-extractable P and Olsen P values for Wharekohe silt loam amended with different organic materials and/or RPR.....	89
Figure 3.8. Inorganic P concentrations in the 1 M NaOH fraction of Wharekohe silt loam amended with different organic materials and/or RPR.....	90
Figure 3.9. Organic P concentrations in the 1 M NaOH fraction of Wharekohe silt loam amended with different organic materials and/or RPR.....	91
Figure 3.10. P concentrations in the 0.5 M H <sub>2</sub> SO <sub>4</sub> fraction of Wharekohe silt loam amended with different organic materials and/or RPR.....	94
Figure 3.11. Estimated dissolution of Sechura RPR after 13 wk incubation in Wharekohe silt loam amended with different organic materials.....	94
Figure 3.12. P concentrations in the residual fraction of Wharekohe silt loam amended with different organic materials and/or RPR.....	94
Figure 3.13. The total amount of P recovered from the soil (the summation of all six fractions) and that predicted by adding the concentration in the control soil, plus what was added as RPR and/or organic matter, for each treatment.....	95
Figure 3.14. A comparison of the amount of 0.5 M HCO <sub>3</sub> <sup>-</sup> -extractable P in soils treated (+) and not-treated (-) with chloroform.....	96
Figure 3.15. The increase in HCO <sub>3</sub> <sup>-</sup> -extractable P following chloroform fumigation in treatments with (+P) and without (-P) the addition of rock phosphate (averaged across amendment), and with different organic amendments (averaged across P treatment) (Con.=control, TL=tree lucerne, WS=wheat straw).....	97
Figure 4.1. P adsorption isotherm for 2 mm sieved Cavinti soil. P is added as MCP. A log-log line is fitted to the increasing portion of the curve. The critical concentration of P in solution for low P-requiring crops (Fox 1981) is shown as a dashed line.....	107
Figure 4.2. Freundlich (log-log) P desorption isotherms for Cavinti soil. The numbers in the legend are the rates of P applied to the soil, in µg P g <sup>-1</sup> soil. The volume of CaCl <sub>2</sub> solution used to desorb P increases from right to left. The data have been split into two plots for clarity.....	110
Figure 4.3. Log-log plots of P desorption isotherms for Cavinti soil. 'Linear' (log-log) regressions have been fitted for each soil P level.....	111
Figure 4.4. The effect of increasing soil MCP concentration on soil pH after 6 wk incubation. A linear regression has been fitted to describe the relationship above 2 mg P g <sup>-1</sup> soil.....	111
Figure 4.5. The percentage of citrate sorbed after a 16 h shake with Cavinti soil at two rates of P addition: 500 µg P g <sup>-1</sup> soil (P500) and 5000 µg P g <sup>-1</sup> soil (P5000).....	113

Figure 4.6. The amounts of P, Fe, Mn, and Al in solution for a given addition of citrate to P500 (left) and P5000 (right) Cavinti soil.....	115
Figure 4.7. The amount of P, Fe, Mn, or Al solubilised for a given addition of citrate to P500 (left) and P5000 (right) Cavinti soil.....	116
Figure 4.8. The relationship between the amount of Al+Fe solubilised and the amount of citrate in solution for P500 and P5000 Cavinti soil.....	117
Figure 4.9. The proportion of fertiliser P recovered into solution after shaking Cavinti soil for 16 h with different amounts of citrate. Both soils were fertilised with MCP: P500 soil at 500 $\mu\text{g P g}^{-1}$ soil and P5000 at 5000 $\mu\text{g P g}^{-1}$ soil.....	119
Figure 4.10. The amount of P solubilised by citrate per $\mu\text{mole of P}$ added to Cavinti soil plotted against the amount of citrate added. Note: the amount of P solubilised = moles of P in solution in the presence of citrate - moles of P in solution in the zero citrate treatment.....	120
Figure 5.1. The TL system developed for growing a planar layer of roots between two small volumes of soil.....	126
Figure 5.2. The experimental set-up used to grow rice in the TL systems.....	127
Figure 5.3. Plant dry weight after 4 wk growth in the TL systems with different additions of MCP. Also shown are the initial P concentrations in the soil solution estimated from the soil P adsorption isotherm (Figure 4.1).....	129
Figure 5.4. A comparison of two curves for predicting $b_p$ over the range of desorbable P for the P2000 soil.....	137
Figure 5.5. The effect of varying the soil:solution ratio on the solution P concentration and the change in soil P concentration.....	139
Figure 5.6. Root + shoot dry weight of four rice plants grown in TL systems. P fertiliser was either applied as a band occupying 10% of the soil volume or incorporated throughout the entire soil volume.....	141
Figure 5.7. Root:shoot ratio for rice grown in the TL systems.....	141
Figure 5.8. Observed and predicted P uptake for rice grown in the TL systems after 6 wk of growth.....	144
Figure 6.1. The efficiency of different acids in recovering citrate from Cavinti soil.....	156
Figure 6.2. The effect of increasing the concentration of $\text{H}_2\text{SO}_4$ on the recovery of citrate, and on the dilution factor needed to separate citrate from other compounds extracted from Cavinti soil.....	157
Figure 6.3. HPLC chromatograms of rhizosphere extracts soil spiked with 1.25 $\mu\text{g citric acid g}^{-1}$ soil. <b>a)</b> A $1/5$ dilution of the extract, which shows that the citric acid peak (retention time 6.5 min) could not be accurately quantified (indicated by the symbol 'V'), due to an interfering peak at 6.0 min. <b>b)</b> The same sample after a $1/12$ dilution, the 6.0 min peak has been diluted out and the peak can be quantified. ...	159
Figure 6.4. The effects of different storage procedures on the composition of a rhizosphere soil extract. (a) Sample acidified to pH 1.7, or acidified then readjusted to pH>4 with NaOH, compared to a fresh, non-pH-adjusted sample. (b) The non-acidified and acidified treatments from (a) stored for 1 d, compared to the fresh sample. (c) As for (b) but samples were stored for 15 wk.....	166
Figure 6.5. The effect of light, and acid addition, on the short-term storage of samples containing citrate.....	170

Figure 7.1. Total plant dry weight of upland rice grown in the second thin layer experiment compared with those of the first experiment, 5 wk after transplanting. P fertiliser was incorporated throughout the thin layer soil, or mixed in a band occupying 12% of the thin layer soil.....	176
Figure 7.2. P uptake by rice plants grown in Experiment 1, where no roots penetrated the mesh (6 wk of growth), compared with Experiment 2, where roots penetrated the mesh (5 wk of growth).....	177
Figure 7.3. A fine root penetrating 24 $\mu\text{m}$ nylon mesh, viewed from above. The circular portion of the root at the top, slightly left of centre, is above the mesh, and the shaded portion bending towards the lower right, is below the mesh. The point of penetration (top, slightly left of centre) is in focus. Note that the mesh is not being forced apart by the root.....	178
Figure 7.4. Exploded view of the pressure plate apparatus used to control the moisture content for Experiment 7.2.....	180
Figure 7.5. Moisture content for each pot of the moist (M) (10 cm suction) and dry (D) (50 cm suction) treatments during Experiment 7.2.....	182
Figure 7.6. The relationship between the number of roots that penetrated the 24 $\mu\text{m}$ nylon mesh and the average water content during the 10 d of the different water regimes.....	183
Figure 7.7. Measurements made on roots that penetrated 24 $\mu\text{m}$ nylon mesh for parameters considered important for roots to penetrate fine pores.....	184
Figure 8.1. Total dry weight of upland rice grown in the first TL experiment (without VAM infection) compared with the third TL experiment (55–92% of the total root length infected with VAM).....	188
Figure 8.2. A comparison of P uptake from the first TL experiment (without VAM infection) against the third TL experiment (55–92% of the total root length infected with VAM).....	189
Figure 8.3. Root and shoot P concentration of rice grown with P fertiliser incorporated throughout the TL system (Inc) or banded within 10% of the soil volume (Band). For the banded treatment, a separate P concentration is given for roots taken from inside and outside the P-rich band. The optimum flag leaf % P is given for rice at flowering (Jakhro 1985).....	189
Figure 8.4. A rice root heavily infected with VAM. The blue-stained hyphae can be seen both inside and outside the root.....	190
Figure 8.5. A rice root, containing a large number of mycorrhizal vesicles (dark blue staining ellipses)....	191
Figure 8.6. A mycorrhizal vesicle.....	191
Figure 8.7. An arbuscule.....	192
Figure 8.8. A mycorrhizal spore.....	192
Figure 8.9. (a) The effect of P fertiliser rate on the proportion of root length infected with VAM. 0 = the unfertilised soil of the banded treatment, 200 = the incorporated treatment, 2000 = the band of the banded treatment. (b) The proportion of the total root length infected with VAM.....	193
Figure 9.1. Upland rice grown in sterilised ( $\gamma$ irradiation, 25 kGy) and non sterilised Cavinti soil 4 wk after germination.....	207
Figure 9.2. Close up of the leaves of upland rice grown in sterilised ( $\gamma$ irradiation, 25 kGy) Cavinti soil. The brown streaks are evidence of Mn toxicity.....	207



Figure 9.3. Dry weight of upland rice grown in sterilised soil in the fourth thin layer experiment. Phosphate fertiliser was either incorporated throughout the entire TL system (Inc.), or banded in 10% of the TL system (Band).....	208
Figure 9.4. Soil pH of moist soil and air-dried soil, 1:2.5 soil:CaCl <sub>2</sub> ratio, from the TL systems: P● = unfertilised soil outside the band, P500 = the incorporated treatment, and P5000 = the high P band..	209
Figure 9.5. pH buffer curve for P5000 Cavinti soil. Note: the native pH of P5000 Cavinti soil is 4.8. ....	210
Figure 9.6. P uptake per TL system when P fertiliser is incorporated throughout the soil (Inc.) or banded within 10% of the soil volume (Band). ....	212
Figure 9.7. Shoot P concentration for upland rice grown in incorporated or banded TL systems. The critical shoot P concentration below which P deficiency is likely (Tanaka and Yoshida 1970) is shown as a dashed line. ....	212
Figure 9.8. Observed and predicted P uptake by upland rice grown in the fourth thin layer experiment. The standard error in the observed uptake is approximately equal to the size of the symbols. ....	214
Figure 9.9. P desorption isotherms for the P5000 Cavinti soil in the presence of added H <sup>+</sup> . The pH (0.01 M CaCl <sub>2</sub> 1:2.5 soil:solution ratio) of P5000 Cavinti soil is 4.58.....	218
Figure 9.10. The predicted uptake of P by upland rice grown in TL systems with and without accounting for solubilisation by H <sup>+</sup> . ....	219
Figure 9.11. Acid release patterns from 2-wk-old upland rice grown on sand with N supplied as either (a) NH <sub>4</sub> NO <sub>3</sub> or (b) Ca(NO <sub>3</sub> ) <sub>2</sub> . The sand is stained with bromocresol purple, which is yellow below pH 4. All nutrients were supplied by P-free Yoshida nutrient solution (Yoshida <i>et al.</i> 1976), which was adjusted to pH 5.0. Acid release declined greatly when NO <sub>3</sub> <sup>-</sup> was the sole N source, although acid release was still observed from the root tips. ....	220
Figure A.1. P desorption isotherms for P5000 Cavinti soil. The numbers beside each data point are the final solution citrate concentrations (mM) after 16 h of shaking. The points are means, n=2. The value of λ <sub>c</sub> (see text) is calculated for a solution P concentration of 0.165 mM, shown by the dotted line. ....	260

## List of tables

Table 1.1 Area occupied by the four main rice-producing agro-ecosystems and their yields in 1991. ....	2
Table 1.2 . Examples of processes affecting soil pH.....	7
Table 1.3. pH buffering systems in soils.....	10
Table 1.4. Yield data for barley varieties with differing Al tolerance in relation to plant-induced pH changes in nutrient solution.....	22
Table 1.5. Average composition of root exudates from four lowland rice cultivars. ....	37
Table 2.1. Important soil properties of Wharekohe silt loam horizons and Egmont brown loam.....	47
Table 2.2. Fertiliser treatments for Experiment III.....	50
Table 2.3. Soil solution chemical parameters that are important in explaining the root length observed in Experiments IIb, III and IV. Also given is the percentage difference in Al concentration measured using PCV compared to AAS.....	57
Table 2.4. Reported critical Al concentrations above which rice growth was affected in solution culture experiments.....	61
Table 3.1. Content of major nutrients in the organic amendments. ....	76
Table 3.2. The soil treatments.....	77
Table 3.3. A modified P fractionation procedure to measure soil and fertiliser P fractions.....	81
Table 3.4. Approximate calculations showing the fate of added C and P <sub>o</sub> from tree lucerne (TL) and wheat straw (WS) 65 d after addition to a Wharekohe silt loam topsoil:subsoil mix. ....	92
Table 4.1. Important characteristics of Cavinti soil.....	103
Table 4.2. Changes in soil solution P concentrations, Freundlich constants, and $b_p$ when different rates of P fertiliser are added to Cavinti soil.....	109
Table 5.1. The distribution of P between various pools in planted and unplanted Cavinti soil for the thin layer experiment, 6 wk after transplanting.....	142
Table 5.2. A comparison of total plant P uptake with total P depleted from the NaOH-P <sub>i</sub> fraction after 6 wk of plant growth.....	143
Table 5.3. Parameters and values used in Model 1 to predict P uptake from P200 and P2000 soil.....	143
Table 5.4. Root parameters of upland rice 2, 3 and 5 wk after transplanting, in the soils of the incorporated treatment (P200), the unfertilised soil outside the band (P0), and the P-fertilised band (P2000).....	147
Table 5.5. Additional parameter values required for Model 2 to explain P uptake from the banded treatment. Other parameters, which were the same as those used in Model 1, are given in Table 5.3.....	149
Table 6.1. Compounds extracted from Cavinti soil by KH <sub>2</sub> PO <sub>4</sub> , the area of the citric acid peak, and that of the nearest interfering compound, which eluted 0.3 min before citric acid.....	156
Table 6.2. Comparing different soil:solution ratios and concentrations of H <sub>2</sub> SO <sub>4</sub> for extracting citrate from the batch of soil used for the thin layer experiment in Chapter 9, n=2.....	160
Table 6.3. Dissociation constants (pK) of organic acids commonly found in soil (25°C).....	167

Table 6.4. Measured citric acid concentration (relative to standard 50 $\mu$ M citric acid) and retention time for solutions of different compositions, as determined by HPLC. ....	172
Table 9.1. Parameters used to model P uptake in the 4 <sup>th</sup> thin layer experiment. ....	213
Table 9.2 Values of additional parameters required by Model 3 to predict P uptake from the banded treatment as a result of H <sup>+</sup> release from the roots covering the P-fertilised band. ....	217
Table 9.3. Shoot Fe, Al, and Mn concentrations for upland rice grown in TL systems where P fertiliser was either incorporated throughout the soil (Inc.) or banded in 10% of the soil volume. ....	221
Table A.1 The stability constants for the possible metal-ligand complexes that may form in solution for Experiment 4.3.3. ....	256
Table A.2. Composition of the modified Yoshida <i>et al.</i> (1976) nutrient solution used for the thin layer experiments. ....	257

## List of symbols

Symbol	Definition	Units
<u>General symbols</u>		
$\rho$	soil bulk density	kg dm <sup>-3</sup>
$E_h$	equilibrium redox potential	V
$g$	the unit for relative centrifugal force (RCF) (in this thesis the maximum radius is used to calculate the RCF)	m s <sup>-2</sup>
$P_i$	inorganic phosphorus	–
$pK$	dissociation constant	–
$P_o$	organic phosphorus	–
<u>Symbols used in Model 1</u>		
$\alpha$	root absorbing power for P (The maximum value of $\alpha = F_{\max}/K_m$ )	dm s <sup>-1</sup>
$\theta$	volumetric soil water content	–
$A$	area of root-soil contact	dm <sup>2</sup>
$b_P$	soil buffer power for phosphorus, $d[P]/d[P_L]$	–
$D_{LP}$	diffusion coefficient of P in free solution	dm <sup>2</sup> s <sup>-1</sup>
$f$	diffusion impedance factor	–
$F_{\max}$	maximum influx that the roots can achieve	mol dm <sup>-2</sup> s <sup>-1</sup>
$K_m$	Michaelis-Menten constant ( $K_m = P$ concentration in solution when P uptake by roots is half of the maximum P uptake)	mol dm <sup>-3</sup>
$L$	width of thin layer	dm
$l_h$	width of the root hair zone	dm
$[P]$	concentration of phosphorus (P) in the whole soil	μmol dm <sup>-3</sup> soil
$[P_L]$	concentration of P in the soil solution	μmol dm <sup>-3</sup> solution
$[P_L]_0$	the concentration of P in the soil solution at $x = l_h$ ,	μmol dm <sup>-3</sup> solution
$t$	time	s
$x$	distance	dm

Symbol	Definition	Units
<u>Additional symbols used in Model 2 (solubilisation by citrate (C))</u>		
$b_C$	soil C buffer power, $(\partial[C]/\partial[C_L])_P$	–
$b_{P^*}$	soil P <sup>*</sup> buffer power, $(\partial[P]/\partial[P_L^*])_C$	–
[C]	concentration of C in the whole soil	$\mu\text{mol dm}^{-3}$ soil
[C <sub>L</sub> ]	concentration of C in the soil solution	$\mu\text{mol dm}^{-3}$ solution
$D_{LC}$	diffusion coefficient of C in free solution	$\text{dm}^2 \text{s}^{-1}$
$D_{LP^*}$	diffusion coefficient of P <sup>*</sup> in free solution	$\text{dm}^2 \text{s}^{-1}$
$F_C$	flux of C across root plane	$\text{mol dm}^{-2} \text{s}^{-1}$
$k_C$	rate constant for C decomposition	$\text{s}^{-1}$
[P <sub>L</sub> <sup>*</sup> ]	concentration of P species (ortho P and P complexed with C) in the soil solution	$\mu\text{mol dm}^{-3}$ solution
$\lambda_C$	P-C interaction coefficient, $(\partial[P_L^*]/\partial[C_L])_P$	–
<u>Additional symbols used in Model 3 (solubilisation by the hydrogen ion (H<sup>+</sup>))</u>		
$b_H$	H <sup>+</sup> ion buffer power, $(\partial[H^+]/\partial[H_L^+])_P$	–
$D_{LH}$	diffusion coefficient of H <sub>3</sub> O <sup>+</sup> in free solution	$\text{dm}^2 \text{s}^{-1}$
$F_H$	rate of H <sup>+</sup> release	$\text{mol dm}^{-2} \text{s}^{-1}$
[H]	concentration of soil acidity titratable to the original soil pH	$\text{mol dm}^{-3}$ soil
[H <sub>L</sub> ]	concentration of H <sub>3</sub> O <sup>+</sup> in the soil solution	$\text{mol dm}^{-3}$ solution
$k_H$	H <sup>+</sup> decomposition rate constant	$\text{s}^{-1}$
$p_{\text{CO}_2}$	partial pressure of CO <sub>2</sub>	atm
pH <sub>i</sub>	initial pH	–
$\lambda_H$	P-H <sup>+</sup> interaction coefficient, $(\partial[P_L^*]/\partial[H_L])_P$	–

# **1. Introduction. Soil constraints to rainfed rice production and strategies to overcome them, with emphasis on phosphate supply**

## **1.1 The need to increase production of rice**

Rice (*Oryza sativa* L.) is essential to the survival of those who live in the most densely populated regions of the world. About 91% of the world's rice is produced and consumed in Asia. Dependence on rice for food energy is much higher in Asia than in other regions. Rice provides between 8 and 79% (average 32%) of the calories consumed by 3.6 billion people in Asian countries. In Africa and Latin America, rice provides 7 to 9% of food energy for 1.3 billion people. Regardless of the region, most rice-dependent countries have high population growth rates, low rice yields and low GNP (except for Japan, South Korea, and China) (IRRI 2000b).

Currently, Asian rice production increases at an annual rate of 1.4%, which is below the continent's population growth rate (Fischer 1999). To meet the projected growth in demand for rice in the year 2025, the International Rice Research Institute (IRRI) estimates that the world's annual rice production will need to increase by 60% above the 1994 production of 485 million tonnes (Fischer 1999). Fischer (1999) also emphasizes that these increases must be achieved against a backdrop of shrinking land area and decreasing availability and increasing cost of production inputs: water, fertiliser, chemicals, labour, and energy. There are also environmental concerns regarding the decline in soil and water quality resulting from injudicious use of fertilisers and agrochemicals in high input, lowland rice farming. The need to increase the world's rice production is therefore urgent, and agronomic practices, such as fertiliser and water use, must be made more efficient.

### 1.1.1 The four main agro-ecosystems that produce the world's rice

There are four main agro-ecosystems within which rice is grown (Table 1.1). These are characterised by elevation, rainfall pattern, depth of flooding and drainage, and by the adaptation of rice to these agroecological factors. Each system, as defined by IRRI (IRRI 2000a), is described in the following paragraphs.

**Table 1.1** Area occupied by the four main rice-producing agro-ecosystems and their yields in 1991 (Cassman and Pingali 1995).

Agro-ecosystem	Area		Average yield (t ha <sup>-1</sup> )	Production	
	(million ha)	(%)		(million t)	(%)
Irrigated	73.9	56	4.89	362	76
Rainfed lowland	38.7	29	2.30	89	19
Upland	10.5	8	1.07	11	2
Deepwater/tidal lowland	10.0	8	1.53	15	3
Total	133.1	100	3.58	477	100

#### 1.1.1.1 Irrigated

Rice is transplanted or direct seeded in puddled soil on levelled, banded fields with water control, in both dry and wet seasons in the lowlands, in the summer at higher elevations, and during the dry season in flood-prone areas. The crop is often heavily fertilised. Under tropical conditions with modern technology, grain yields can reach 5.5 t ha<sup>-1</sup> in the wet season and more than 11 t ha<sup>-1</sup> in the dry season. Irrigated rice makes up 56% of the world's harvested rice area and 76% of world rice production. It provides the major supply for urban consumers. Growth in irrigated rice production has been largely responsible for the recent stability of urban rice supplies and prices.

Most of the land that is suitable for irrigated rice production is already producing irrigated rice – there is little new land available and water reserves are declining (Singh 1999). The yield potential gaps between what is achieved under best management in research stations and what is achieved by the best farmers is narrowing in irrigated rice systems. Therefore

further gains based on technological improvements will be increasingly difficult and more emphasis on rainfed systems is needed.

#### **1.1.1.2 Rainfed lowland**

Rice is transplanted or direct seeded in puddled soil, on level to slightly sloping, banded or dyked fields with variable depth and duration of flooding, depending on rainfall. Soils alternate from flooded to nonflooded; yields vary depending on rainfall, cultivation practices, and use of fertiliser. Rainfed lowland rice makes up 29% of the world's harvested rice area and 19% of world production.

Areas where rainfed lowland rice is the predominant agro-ecosystem are among the world's most densely populated rural regions and home to some of the world's poorest rural and urban populations. The rainfed lowlands must contribute to the production needed to feed expanding urban populations while preserving natural resources and improving the well being of farming families.

#### **1.1.1.3 Upland**

Rice is direct seeded in non-flooded, well-drained soil on level to steeply sloping fields. Crops suffer from lack of moisture and inadequate nutrition, and current yields are very low. Upland rice makes up 8% of the world's harvested rice area and 2% of rice production.

The uplands support millions of people, most of them at the subsistence level. The slash-and-burn agriculture that often follows logging in upland areas opens the way for serious soil erosion and degradation that impacts the lowland watershed. Improved technology is needed that will help rehabilitate degraded uplands and transform them into sustainable agro-ecosystems.

#### **1.1.1.4 Deepwater/Flood-prone**

Rice is direct seeded or transplanted in the rainy season on fields characterised by medium to very deep flooding (50 to more than 300 cm) from rivers and from tides in river mouth deltas. Soils alternate from flooded to non-flooded and may have severe salinity and toxicity



problems. The rice crop grows as the floodwater rises, with harvest after the water recedes. More than 10 million hectares in South and Southeast Asia are subject to various types of uncontrolled flooding. West Africa and Latin America also have some flood-prone riceland.

Rice is often the only crop that can be grown in the flood-prone areas. Yields are low because of problem soils (e.g. high salinity) and unpredictable combinations of drought and flood, and crop failures are common. Yet these low-lying areas support more than 100 million people, most of them in poor farming families, who need sustainable production systems.

In the upland rice agro-ecosystem, the soil remains aerobic throughout the growing season. In the other three systems, the soil may be aerobic during part of the growing season. On average, the rainfed lowland system is aerobic for a longer period than the other two, especially during seedling establishment. Once the soil becomes anaerobic following flooding, the chemistry of the soil changes dramatically – the soil pH rises eliminating any phytotoxic aluminium (Al), and the availability of phosphorus (P) is generally increased, except in highly-weathered soils (see Section 1.3.2.2). This thesis only considers the chemistry of aerobic soils, and so whilst the research presented in this thesis is relevant to sprinkler-irrigated rice and deepwater rice for short lengths of time before the floods come, it is most relevant to upland rice, and rainfed lowland rice during the critical phase of seedling establishment.

### **1.1.2 The need to increase the production of rainfed lowland and upland rice**

Kirk *et al* (1998) stated that the “rainfed lowland and upland rice ecosystems together account for some 45% of the global rice area, but only 25% of global rice production (IRRI 1993). It is estimated that at least 30% of the projected 70% increase in global rice demand by the year 2030 must come from rainfed rice (Scobie *et al.* 1993).” Kirk *et al.* (1998) further argue that traditional upland rice cultivars are well-suited to the soil conditions found in the uplands, being very tolerant to low P and highly acidic soils, relative to other cereals (Garrity *et al.* 1990), but state that upland rice cropping without fertiliser can only

be sustained by shifting cultivation with unrealistically long fallow periods. Efforts should therefore focus on bringing about a gradual improvement in soil conditions. This soil improvement should ultimately be for high value crops, as returns for rice are unlikely to justify the investment. During the soil improvement process, upland rice will be important. In the rainfed lowlands, rice is likely to continue to be important, as farmers generally have no alternative to rice because their land is flooded during the rainy season.

## **1.2 Main factors limiting the yield of rainfed and upland rice**

The main biophysical factors limiting the production of rice are listed below (from Evenson *et al.* 1996).

- ⇒ Insects
- ⇒ Diseases
- ⇒ Weeds
- ⇒ Climate (drought, submergence, cold)
- ⇒ Soil (physical and chemical constraints)
- ⇒ Bioefficiency (plant design, photosynthetic efficiency, growth duration and grain quality)

Obviously all of these factors need improving in order to increase the production of rainfed and upland rice. However serious consideration of the relative importance of each aspect is an enormous question and is outside the scope of this thesis. This chapter covers one aspect – soil-related constraints to rainfed and upland rice production, giving special emphasis to soil acidity and P deficiency, which is the focus of this thesis.

### **1.3 Soil-related constraints to rainfed and upland rice production**

Half of the land area of the tropics is acid upland soils (FAO-Unesco 1971-81). In South East Asia, nutrient stresses are the dominant limiting factor on 59% of the land (Dent 1980). A list of the main soil factors that limit yield on soils of the humid tropics is given below.

- ⇒ acidity related toxicities e.g. high concentrations of monomeric Al and manganese (Mn) in solution
- ⇒ P deficiency
- ⇒ nitrogen (N) deficiency
- ⇒ deficiencies of other nutrients
- ⇒ poor physical properties
  - compaction
  - low water holding capacity

The number and severity of problems increase rapidly if the soil has been left without cover, been eroded, burned or compacted by machinery. The problems listed above are outlined in this section (1.3); subsequent sections (1.4–1.5) discuss management strategies to overcome them.

#### **1.3.1 Acidity related problems**

##### **1.3.1.1 Soil acidity and processes of soil acidification**

The pH of a soil and water suspension is directly related to the activity of hydrogen ions ( $H^+$ ) in the soil solution that occupies part of the soil pore space through which roots have to grow. The activity of  $H^+$  ions in the soil solution is determined by an equilibrium with a larger quantity of  $H^+$  ions and acidic cations associated with organic matter and soil mineral surfaces. There are a number of factors that can contribute to the accumulation of acidity in soils. These are outlined in Table 1.2.

Conceptually, in a perfectly balanced system, processes that generate  $H^+$  would equal processes that consume  $H^+$ , leading to no change in soil acidity. However, in soils,

processes such as leaching (Table 1.2) uncouple these nutrient cycles causing an irreversible build-up of  $H^+$  ions in soil. Soil acidification occurs particularly rapidly in the humid tropics, where nutrient cycles are fast and drainage volumes are large. Also, there is little external renewal from alluvial, aeolian or glacial deposition to buffer this acidification. Examples of how nutrient cycles are uncoupled are given in the following paragraphs.

**Table 1.2 .** Examples of processes affecting soil pH.

<b>Processes that generate <math>H^+</math></b>	<b>Processes that consume <math>H^+</math></b>
cation uptake (plant or microbial)	anion uptake (plant or microbial)
oxidation reactions	reduction reactions
synthesis and dissociation of organic acids and $H_2CO_3$ (produced by dissolution of $CO_2$ )	decarboxylation of organic acids, formation of $H_2CO_3$
nitrification	denitrification
volatilisation of $NH_3$	ammonification of $RNH_2$

<b>Changes in the distribution of <math>H^+</math> between the soil exchange sites and soil solution</b>	
<b>Increase <math>H^+</math> in solution</b>	<b>Decrease <math>H^+</math> in solution</b>
counter-ion desorption of $H^+$	adsorption of $H^+$
adsorption of $OH^-$	desorption of $OH^-$

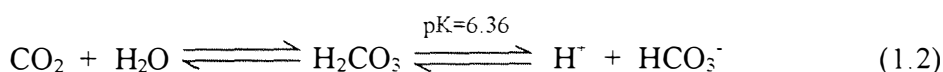
<b>Examples of paired processes* that increase soil acidity<sup>†</sup></b>	
<b>Inputs</b>	<b>Outputs</b>
$NH_4^+$ , $N_2$ , or $R-NH_2$	leaching of $NO_3^-$
elemental sulphur (S) fertiliser	leaching of $SO_4^{2-}$
$NH_4^+$	volatilisation of $NH_3$
synthesis of $R-NH_2$ , $R-SH$	product removal (of $R-NH_2$ or $R-SH$ ) (e.g. harvesting grain)
$NH_4^+$ , $N_2$ , $R-NH_2$ or $CO_2$	accumulation of undecomposed organic matter

\*Inputs and outputs on the same line are paired.

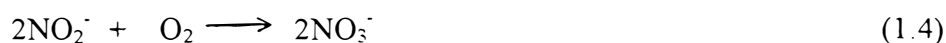
<sup>†</sup> To leave acidity in soil, the form of the element leaving the soil must be more oxidised than the form entering the soil.

*Dissolution of CO<sub>2</sub>*

Carbon dioxide produced by the respiration of plant roots and soil dwelling organisms, and from the atmosphere, reacts with water to produce carbonic acid (H<sub>2</sub>CO<sub>3</sub>). Carbonic acid may then dissociate, depending on the pH, to give a hydrogen ion and bicarbonate ion (HCO<sub>3</sub><sup>-</sup>) as shown in Equation 1.2. If the HCO<sub>3</sub><sup>-</sup> ion is leached this leads to a net build up of H<sup>+</sup> ions in the soil. However, carbonic acid does not dissociate under acid conditions (dissociation constant, pK = 6.36 at 25°C, Lindsay 1979) and most of the CO<sub>2</sub> will be lost as a gas to the atmosphere. Therefore this mechanism is unlikely to cause already acidic soils to become strongly acidic.

*Acidification and the N cycle*

Nitrification of the ammonium ion (NH<sub>4</sub><sup>+</sup>) to nitrate (NO<sub>3</sub><sup>-</sup>) via the action of the soil dwelling bacteria and fungi (Equations 1.3 and 1.4) generates two moles of H<sup>+</sup> per mole of NH<sub>4</sub><sup>+</sup> nitrified.



If a mole of NO<sub>3</sub><sup>-</sup> is removed from the system by being leached below the root zone, this results in a net accumulation of two moles of H<sup>+</sup> in the soil, unless the cation leached with NO<sub>3</sub><sup>-</sup> is H<sup>+</sup> (Bolan and Hedley 2001). Nitrate uptake by roots results in 1:1 molar excretion of OH<sup>-</sup>, thus plant uptake and assimilation of NO<sub>3</sub><sup>-</sup> into protein, followed by removal of the plant material, leaves one mole of H<sup>+</sup> in the soil. The return of plant litter to soil and subsequent ammonification of the plant protein generates OH<sup>-</sup>, which would have completed the cycle and neutralised all the acidity generated through nitrification.

In tropical environments, where there is adequate moisture, nitrification is rapid: the optimum temperature for nitrification is between 25 and 35°C (Tisdale *et al.* 1993). However, as the pH drops below 4–5, nitrification becomes less important as an acidifying process as nitrifying bacteria are less active at low pH (Jackson 1967, Arora *et al.* 1986).

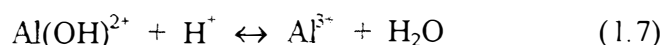
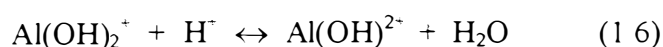
*Rhizosphere pH change – temporary or permanent*

Excess cation uptake causes the plant to release  $H^+$  ions from its roots, in order to maintain electrical neutrality across the root-soil interface. Similarly, anion uptake causes plants to excrete  $OH^-$  or  $HCO_3^-$  (Raven and Smith 1976). The overall balance of cations and anions absorbed by plants can therefore determine whether the plant has a net acidifying or liming effect on rhizosphere soil. The cation-anion balance is largely determined by the species of N taken up by the plant, since N is the only element taken up in large quantities that can be absorbed in either a positive ( $NH_4^+$ ) or negative ( $NO_3^-$ ) form (or neutral in the case of  $N_2$  fixation). Thus, plants that are fed predominantly  $NH_4^+$  will acidify their rhizosphere, whereas  $NO_3^-$ -fed plants will raise their rhizosphere pH (Gahoonia and Nielsen 1992). The  $H^+$  that is excreted to balance cation uptake arises from the dissociation of organic acids (Bolan *et al.* 1991).

The fate of plant material determines whether root excretion of  $H^+$  into the rhizosphere causes a permanent increase in soil acidity. If the plant matter is spatially confined and decomposes where it was synthesized, rhizosphere  $H^+$  will be neutralised by  $H^+$  consuming processes such as ammonification and respiration of negatively charged organic anions ( $RCOO^-$ ). However the removal of plant material, such as grain, will result in a net increase in soil acidity. The more intensive cropping becomes, the greater the removal of plant material, hence the greater the rate of acidification.

**1.3.1.2 pH buffering reactions in acid soils**

In mildly acidic soils, additions of  $H^+$  are buffered by exchange with nutrient cations held on negatively charged surfaces of clay minerals and organic colloid surfaces (Table 1.3). At  $pH < 5$ , the main pH buffering reactions in aerobic highly-weathered soils are associated with the dissolution of aluminosilicate minerals. These reactions are simply represented by the dissolution of gibbsite,  $Al(OH)_3$  (Equations 1.5–1.7).



**Table 1.3.** pH buffering systems in soils (adapted from Johnston *et al.* 1986).

Soil pH	Buffering system
>6.8	Dissolution of free carbonates
4-7	H <sup>+</sup> exchange with nutrient cations held on negatively charged surfaces
3-5.5	Mobilisation of Al <sup>†</sup>

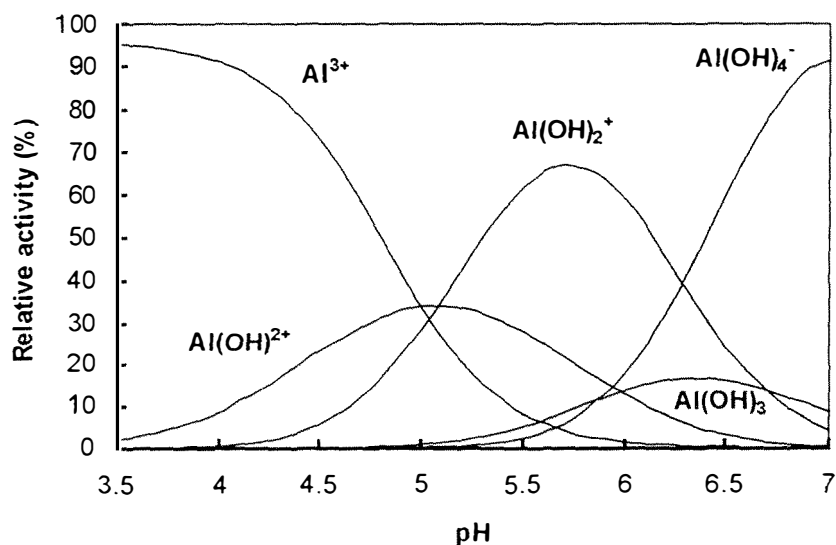
<sup>†</sup> See Equations 1.5 – 1.7

### 1.3.1.3 Acidity related toxicities

#### *Aluminium*

Aluminium comprises 7.1% by weight of the Earth's crust (Lindsay 1979). A considerable amount of Al ions are released into the soil solution during the weathering of aluminosilicate minerals (Section 1.3.1.2), and in acid soils, a substantial proportion of the cation exchange sites may be occupied by Al ions. In solution, Al forms complexes with OH<sup>-</sup>, F<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, and a wide variety of organic ligands. Most researchers agree that the non-complexed Al<sup>3+</sup> ion is toxic to plant growth (Parker *et al.* 1988, Foy 1992, Kinraide 1997). Aluminium complexed with SO<sub>4</sub> or organic ligands is generally considered to be non-toxic to plants (Foy 1992). Research by Kinraide (1997) indicates that AlF<sup>2+</sup> and AlF<sub>2</sub><sup>+</sup> are toxic to wheat roots, although Al-F complexes are less toxic than Al<sup>3+</sup>. The literature has been unclear as to the toxicity of monomeric hydroxy-Al species. Some researchers have used the sum of the activities of Al<sup>3+</sup> and the monomeric hydroxy-Al species as a measure of toxicity (e.g. Bell and Edwards 1986). However, recent research by Kinraide (1997) indicates that monomeric hydroxy-Al is non-toxic to wheat. Solution culture studies indicate that polynuclear hydroxy-Al (Al<sub>13</sub>) is highly toxic to plants (Parker *et al.* 1988), but its role in soil Al toxicity is uncertain given the high affinity of the negatively charged soil surfaces for positively charged Al polymers (Wright 1989). Changes in pH will affect Al speciation (e.g. hydroxy-Al speciation, Figure 1.1), but the most important effect of pH change is that decreasing the pH below ≈5 markedly increases the amount of Al in solution (von Uexküll 1986, see also Section 1.3.1.2).

The symptoms of Al toxicity on plants are swollen, stunted and crooked roots and a lack of feeder roots (von Uexküll 1986) and root hairs (Brady *et al.* 1993). Thus the ability of a plant to take up water and nutrients is impaired. In the plant, free Al binds strongly to the phosphate groups in nucleic acids, so inhibiting cell division (Morimura *et al.* 1978), affects phosphokinase and ATPase activity (Mengel and Kirkby 1982) and inhibits uptake of calcium (Ca) and decreases the permeability of the plasmalemma (Kamprath 1972). Aluminium injury also appears to predispose roots to fungal infection (Ota 1968, as cited in Foy 1971).



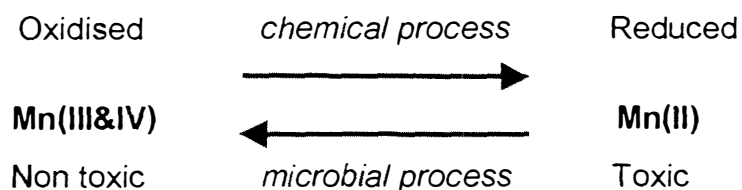
**Figure 1.1.** Relative distribution of the activities of  $\text{Al}^{3+}$  and mononuclear hydroxy-Al species as a function of pH. (Wright 1989).

### Manganese

Manganese is an essential plant nutrient. A solution concentration of 1 – 4  $\mu\text{g mL}^{-1}$  Mn is desirable while concentrations outside this range indicate possible deficiency or toxicity (von Uexküll 1986). At pH less than 5.5 toxicity may develop on soils with a high content of secondary Mn-bearing minerals such as pyrolusite [ $\text{MnO}_2$ ] and magnetite [ $\text{MnO}(\text{OH})$ ].



Below pH 4.8 Mn toxicity may appear together with Al toxicity. Manganese toxicity is characterised by brown spots on older leaves and an uneven distribution of chlorophyll.



**Figure 1.2.** Manganese transformations in *aerobic* soil.

The toxic form of Mn is  $Mn^{2+}$ . The  $Mn^{2+}$  ion is formed from the reduction of Mn(III and IV) as shown in Figure 1.2, which is both a chemical and, particularly under anaerobic conditions, a microbial process. However, the conversion (oxidation) of the phytotoxic species,  $Mn^{2+}$ , to non-toxic Mn(III and IV) forms is solely a microbial process. In most soils these two processes are in a quasi-equilibrium, but when microbial oxidation is stopped by the use of microbial inhibitors, there is an increase in toxic  $Mn^{2+}$  in soil (Sparrow and Uren 1986). Manganese toxicity has been observed in Australian pastoral soils during long hot, dry periods when microbial activity is very low (Siman *et al.* 1974).

### **1.3.2 Low available P and high P-fixation capacity**

#### **1.3.2.1 Phosphorus availability in upland soils**

In large areas of the tropics, P availability is the dominant factor limiting crop production (Nye and Greenland 1960, Sánchez 1976). The vast majority of the world's upland rice is grown on Ultisols, Oxisols and Andepts (Garrity *et al.* 1990) (Acrisols, Ferralsols and Andosols in FAO classification, respectively). Highly weathered tropical soils: Acrisols, Ferralsols, Luvisols, Gleysols and Vertisols, in most cases (about 66%) contain low or very

low amounts of plant available P (Dabin 1980). Andosols also contain low amounts of plant available P, and have a very high P-fixation capacity.

This deficiency of P may not necessarily be the result of low total soil P, but rather that the P present in the soil is so tightly bound (fixed) to the soil particles that the rate at which P is supplied from the soil is too slow to meet plant demand. For P to be taken up by plants, it must be in solution – either as  $\text{H}_2\text{PO}_4^-$  or  $\text{HPO}_4^{2-}$ , depending on the soil pH. Phosphorus in solution is in equilibrium with the mineral phase of the soil; the proportion of P in solution is dependent on the type of minerals present in the soil. The relationship between the type of minerals present in the soil, weathering, and P-fixation is explained as follows (adapted from Von Uexküll 1986):

“As soils weather their Ca contents tend to decrease while reactive Al and iron (Fe) contents increase. With increased weathering there is thus a shift in the control of P solubility from Ca, which gives low P ion concentrations in the soil solution, to Al and Fe which form complex P compounds that are precipitated or strongly sorbed to the clay lattice and amorphous sesquioxides (fixed). Fixation of P is initially rapid and then slows down; fixation capacity is greatest at a pH below 5.5.

The largest amounts of P are fixed by amorphous hydrated oxides of Fe and Al and smaller amounts by crystalline and lattice minerals such as gibbsite, kaolinite and montmorillonite; the more crystalline the material, the lower the reactivity and the smaller the fixation capacity. According to Fox *et al.* (1971), the intensity of fixation by different types of mineral is ranked as follows:

Amorphous oxides > crystalline oxides > 1:1 clays > 2:1 clays”

### 1.3.2.2 Phosphorus availability in flooded soils

In many soils, flooding increases the plant-availability of native and added P both because rates of diffusion increase and because P solubility increases as a result of biochemical reduction processes, particularly reductive dissolution of Fe(III) oxides (Willett 1989, Kirk

*et al.* 1990, Willett 1991). Organic matter also contributes to the P released during flooding, but the reduction of Mn(III and IV) is a minor source (Willett 1989). Therefore, in general, P fertiliser recovery is greater and smaller additions are required in flooded soils. However, much of this easily-extractable P subsequently becomes re-immobilised because it is sorbed on solid phases formed in the course of soil reduction (Ponnamperuma 1972, Willett 1986). Furthermore, the increase in P availability on Ultisols, Oxisols, Vertisols, and certain Inceptisols, where much of the rainfed rice is grown, is generally small (Ponnamperuma 1977). Therefore P deficiency is still an important issue in the rainfed rice ecosystem even once the soils become flooded. Also, rainfed lowland rice is often sown in moist soil before the onset of the main rains, and must therefore become established before the flooding-induced improvement in P-availability.

### **1.3.3 Nitrogen deficiency**

After forest clearing there is generally sufficient N in the soil to meet the needs of the rice crop (von Uexküll 1986). However after a few years the amount of N and organic matter in the soil declines and crops become responsive to N. Traditional rice cultivars are relatively unresponsive to N, but Russel *et al.* (1970) found that the optimum N fertiliser rates for modern high-yielding cultivars was three times higher than traditional cultivars. Prescription-type fertiliser recommendations commonly recommend applying between 60 – 150 kg N ha<sup>-1</sup> to each crop (Raju *et al.* 1993, Murugappan *et al.* 1993).

### **1.3.4 Deficiencies of other nutrients**

Tropical soils may be deficient in any number of the essential elements for plant growth, although the most common elements limiting the growth of rice are N and P. Once these deficiencies have been corrected it is more common for deficiencies of other elements, such as potassium (K) or S, to become apparent (Dobermann *et al.* 1998). Furthermore, as rice production necessarily intensifies in response to population pressure, larger amounts of basic cations and micronutrients are removed. In lowland rice environments deficiencies of zinc (Zn) and copper (Cu) are common (Savithri *et al.* 1999). In sodic and upland and

coarse-textured calcareous soils with a low organic matter content, Fe deficiency may be a problem (Savithri *et al.* 1999).

### **1.3.5 Poor physical properties**

Most problems with soil physical properties relate to soil water holding capacity. In general, the rainfed lowland rice system lacks control over the amount and timing of water, and hence both insufficient and excess water supply are common. Upland rice is susceptible to drought. Flooding and stagnant water are the main problems for rice production on the lowland plains and river floodplains, which account for 50–67% of the total rainfed lowland rice (Tomar 1997). Deep ponding reduces the soil oxygen content, temperature, refracted light intensity, and restricts the development of nodal adventitious roots and leaves (Tomar 1997). Toxins build up in stagnant water, and some essential plant nutrients become unavailable in heavily reduced conditions. A healthy reduced condition can be attained with a water percolation rate of 10–20 mm d<sup>-1</sup> (Mandal 1984, as cited by Tomar 1997).

Drought is the major problem on the terraced slopes, whereas the plateaus may experience either drought or waterlogging. Yield is severely reduced when drought occurs at a critical growth stage. Krishnamoorthy (1979, as cited by Tomar 1997) stated that if the monsoon broke for more than 7–10 d, there would be moisture stress in the crop, which would be more severe in light-textured soils. In soils that are too light or coarse-textured for puddling, upland rice must be grown. Rainfed rice grown in coarse-textured Alfisols and Urtisols loses a considerable amount of water and nutrients by deep percolation. When the rains cease, these soils dry quickly because of low water-holding capacity and high permeability.

Root growth and grain yield also declines with increasing soil bulk density (Tomar 1997). Repeated ploughing of a water-saturated soil also causes the formation of a traffic pan, especially on fine loamy soils (Tomar 1997). Traffic pans have the advantage of increasing the amount of water held in the soil, but they can result in the build-up of phytotoxins and if they are too impermeable.

## 1.4 Strategies to overcome soil constraints to rice production (except P deficiency)

### 1.4.1 Fertiliser use

#### 1.4.1.1 Amelioration of acidity

##### *Liming*

The most common way of ameliorating acid soils, especially in temperate soils, is to raise soil pH by applying lime. Rice is well adapted to acidic soils, and therefore is less responsive to lime than many other crops (Schmidt *et al.* 1990, Fageria *et al.* 1991, 1995). Liming acid soils has the following effects (adapted from von Uexküll 1986):

##### BENEFICIAL EFFECTS

##### i) Reducing Al and Mn toxicity

Raising the soil pH reduces the amount of  $\text{Al}^{3+}$  and  $\text{Mn}^{2+}$  in the soil solution.

##### ii) Addition of Ca to the soil

Calcium is responsible for root elongation in the plant. Incorporation of Ca into the soil promotes root elongation, thus enhancing nutrient and water uptake.

##### iii) Improving P availability

Mineralisation of P is positively correlated with pH (Thompson *et al.* 1954). Liming a soil that is below pH 5.7 can also increase the amount of P in solution (Soltanpour *et al.* 1974) by reducing or eliminating the activity of  $\text{Al}^{3+}$  in the soil solution. Once all the  $\text{Al}^{3+}$  has been removed by precipitation with lime, at a pH of approximately 5.7, further applications of lime will lead to a reduction in P availability (Soltanpour *et al.* 1974). Liming can reduce amount of P fertiliser required by plants (Kamprath and Foy 1985). For example, on a soil with pH 5.1 and Al comprising 49% of the cation exchange capacity (CEC), four times as much superphosphate was required to produce yields of lucerne equal to that of the same soil limed to pH 6.1 with no exchangeable Al (Munns 1965). Increased root extension

allowed by lime will increase uptake of relatively immobile ions like  $\text{H}_2\text{PO}_4^-$ . The reason for increased requirement for P may also have been that a large amount of P was required to remove toxic Al by precipitation as  $\text{Al}_2(\text{PO}_4)_3$ .

iv) Increasing the availability of S and molybdenum

Liming Oxisols or Ultisols increases the availability of extractable  $\text{SO}_4^-$  (Kamprath *et al.* 1956) and enhances the availability of molybdenum (Mo) (Kamprath and Foy 1985).

v) Promoting biological  $\text{N}_2$  fixation

Liming promotes biological  $\text{N}_2$  fixation for several reasons: the availability of P, Ca and Mo (which are necessary for nodulation) is increased, root growth is enhanced, allowing more opportunity for infection, and the detrimental effects of high  $\text{H}^+$  concentration are reduced (Kamprath and Foy 1985). Enhancement of  $\text{N}_2$  fixation is important if legumes are grown to supply N to rice.

vi) Stimulating nitrification

The activity of most micro-organisms involved in nitrification increases with liming (Arora *et al.* 1986, Tisdale *et al.* 1993). Cornfield (1952, as cited by Jackson 1967) found higher concentrations of  $\text{NH}_4^+$  than  $\text{NO}_3^-$  in various incubated soils at a pH less than approximately 5.5.

vii) Enhancing colonization of plants by mycorrhiza

Soedarjo and Habte (1995) found that the colonization of *Leucaena leucocephala* (a tree legume) roots by mycorrhiza was markedly increased as the pH of a Mn-rich Oxisol was increased from 4.3-5.0.

viii) Increasing the CEC

Many tropical soils contain a high amount of clay minerals that have pH-dependent surface charge. These include: crystalline and non-crystalline oxides and hydrous oxides of Al, Fe, Mn, and titanium; allophane, amorphous silica, kaolinite, and halloysite. Whether the surface charge of these minerals and of organic matter is positive or negative depends on pH. Increasing the CEC reduces the susceptibility of bases (such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{K}^+$ ) to leaching (Munson and Nelson 1963).

ix) Changing soil structure

Application of lime to oxidic soils can result in flocculation or dispersion depending on whether liming changes the effective surface charge from positive to zero values (flocculation) or from zero to negative values (dispersion) (Uehara and Keng 1975).

## DETRIMENTAL EFFECTS

### i) Decreasing the availability of K

Liming decreases the availability of K in several ways. Firstly, raising the pH increases the soil CEC causing K to move from the soil solution (where it is available to plants) onto the exchange sites on soil particles. Thus the concentration of K in the soil solution is effectively reduced. Secondly, liming increases the  $\text{Ca}^{2+}$  concentration of the soil solution, which depresses K uptake. Liming also increases the number of K selective sorption sites on clay and organic matter, which at lower pH would have been occupied by positively charged Al hydroxy polymers (Kemmler 1980).

### ii) Decreasing the availability of Mn, Zn, Fe and Cu

Raising the soil pH decreases the availability of these nutrients (Fageria *et al.* 1995), mainly through the precipitation of insoluble hydroxides. Iron deficiency induced by over-liming is particularly a problem in upland rice, which has a large Fe requirement.

### *Gypsum*

Surface application of gypsum (calcium sulphate) has been shown to be an effective ameliorant of subsoil acidity on many different soil types (Shainberg *et al.* 1989). Gypsum, unlike lime, leaches down into the subsoil under excess moisture where it provides Ca and S, and reduces the amount of active aluminium in the soil (Shainberg *et al.* 1989, Sumner 1995). Gypsum was found to be more effective than lime in neutralising subsurface acidity and yields of upland rice in Sitiung, Indonesia (Zaini *et al.* 1994).

#### **1.4.1.2 Correction of N deficiency**

Of the mineral nutrients, the greatest quantity removed with harvested rice grain is N (Dobermann and White 1999). This N demand may be met by the application of chemical fertilisers, or organic manures containing high concentrations of N (Becker 1996). Nitrogen fertiliser has the advantage of requiring much less labour to apply than organic manures, but does require the farmer to spend a relatively large amount of money compared with his/her yearly income, and the farmer must have access to a supply of fertiliser (Palmer 1976). This large investment is usually justified by the large increase in production. However on very P deficient soils, there is no response to N until the P deficiency is corrected.

#### **1.4.1.3 Low basic cations, trace element deficiencies**

These deficiencies are usually corrected for by their application in a chemical fertiliser, although the availability of trace elements may be manipulated by the application of lime (see Section 1.4.1.1).

#### **1.4.1.4 Poor physical properties**

Chemical fertiliser is not normally applied in order to improve the physical properties of acid soils.

### **1.4.2 Organic amendments**

Organic fertilisers require land, labour and other inputs for their production and application. Pandey (1999) emphasized that recent research shows that the effective price of nutrients from organic sources has been found to be higher than that of nutrients from inorganic sources. It is therefore not surprising that the use of green manures to supply N has been decreasing in the last 30 years (Becker *et al.* 1995). However there are other advantages to using green manures and other organic amendments, besides just the amount of nutrients supplied, these are mentioned below.

#### **1.4.2.1 Acidity**

Plants that are high in basic cations will contain equally high molar amounts of organic anions, as the amount of positive charge must be balanced by the amount of negative charge inside the plant. Decarboxylation of these organic anions upon decomposition consumes  $H^+$  (Feng *et al.* 1996). Thus green manures high in basic cations will have a liming effect upon decomposition (Pocknee and Sumner 1997, Tang and Yu 1999). The production of  $NH_4^+$  during the decomposition of N-rich green manures also consumes  $H^+$  (Table 1.2). This may cause a temporary rise in soil pH. Over time, however this is balanced by the uptake of  $NH_4^+$  by plants, and process of nitrification, which releases  $H^+$ . Plant uptake of  $NO_3^-$ , will again release a negative charge, but if the  $NO_3^-$  is lost from the system by leaching there will be a gradual lowering in soil pH.



#### **1.4.2.2 Nitrogen deficiency**

Becker (1996) stated that “green manure legumes for rice based cropping systems show a high average N accumulation of 80–100 kg N ha<sup>-1</sup>, with about 80% being derived from biological N<sub>2</sub> fixation. The average amounts of N accumulated by green manures can entirely substitute for mineral fertiliser N at current average application rates.” Green manure crops grown *in situ* can also act as catch crops for NO<sub>3</sub><sup>-</sup>, reducing leaching losses.

#### **1.4.2.3 Deficiencies of S, basic cations, and trace elements**

Low amounts of S, basic cations or trace elements in the soil can only be supplied by chemical fertilisers, or from organic matter obtained offsite. This is because the growth of any green manure raised *in situ* will also be limited by that particular nutrient, unless the particular species of green manure (perhaps a deep-rooted tree species, Ruhigwa *et al.* 1992) is able to access a particular pool of that nutrient that is unavailable to rice.

#### **1.4.2.4 Poor physical properties**

Organic matter can play a central role in maintaining or increasing soil productivity by improving soil water holding capacity, soil structure, avoiding high soil temperatures and by reducing the danger of erosion (Lal 1979, von Uexküll 1986).

### **1.4.3 Plant breeding strategies to overcome soil constraints**

#### **1.4.3.1 Acidity and Al tolerance**

Most of the problems attributed to soil acidity are caused by high soil solution concentrations of monomeric Al, and to a lesser extent, Mn<sup>2+</sup>. Considerable variation exists between upland rice cultivars in their tolerance to high solution Al concentrations (Howeler and Cadavid 1976, Salinas 1978, Fageria *et al.* 1988c). Salinas (1978) found that the yield of the rice cultivar Pratão Precoce was not affected by Al over the range tested (5 – 63% Al saturation), whereas the cultivars Flotante and Batatais showed decreasing yields with increasing Al saturation. Pratão Precoce also had a low requirement for P, producing 80% of maximum yield at 10 µg available P g<sup>-1</sup> soil under high Al saturation conditions, whereas

other upland rice cultivars required much higher rates of P to produce maximum yields – even under low Al saturation conditions.

Differences in Al tolerance between some plant species and varieties are associated with differing abilities to alter rhizosphere pH. For example, Foy *et al.* (1965, 1967) showed that Al sensitive Monon wheat and Kearney barley induced lower pH values in their growth media than did the Al-tolerant Atlas 66 wheat and Dayton barley cultivars (Table 1.4). Presumably this tolerance mechanism works because the plant-induced pH increase decreases the solubility and potential toxicity of Al. In support of this hypothesis, Clarkson (1970, as cited in Foy, 1971) observed that when the nutrient solution pH was maintained at pH 4.2, Dayton and Kearney barley cultivars appeared equally sensitive to Al. This has also been demonstrated for rice (Subramoney and Saukaranarayanan 1964), where seeds from an acid-soil-tolerant variety raised the soil pH from 3.2 to 5.5 during germination, whereas those from acid-soil-sensitive varieties did not (see also Sivaguru and Paliwal 1993). In the solution culture experiments of Foy *et al.* (1965, 1967) and Sivaguru and Paliwal mentioned above, both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were supplied. Therefore the ability of the plants to alter rhizosphere pH may be due to an ability to select the N species taken up, since root excretion of  $\text{H}^+$  or  $\text{OH}^-/\text{HCO}_3^-$  is largely governed by the form of N absorbed (Section 1.3.1.1). In conditions where the dominant N species was  $\text{NH}_4^+$ , this Al tolerance mechanism might therefore not be possible.

Another mechanism by which plants may tolerate high soil solution Al concentration is root exudation of organic acids. Organic acids form complexes with Al that are non-phytotoxic (Section 1.4.3.1). Miyasaka *et al.* (1991) found that the Al-tolerant snapbean cultivar “Dade” exuded citric acid into the rhizosphere at a concentration that was 70 times as great as that of “Dade” grown without Al, and 10 times as great as the Al-sensitive cultivar “Romano” grown with or without Al. De la Fuente *et al.* (1997) genetically engineered tobacco and papaya plants to exude up to four times the amount of citrate of non-modified clones. These clones had a markedly higher tolerance to Al than the non-modified clones, when grown in solution or media culture containing 300  $\mu\text{M}$  Al; however no soil studies were reported.

**Table 1.4.** Yield data for barley cultivars with differing Al tolerance in relation to plant-induced pH changes in nutrient solution.

Barley variety	Al added ( $\mu\text{g mL}^{-1}$ )	Final solution pH*	Plant yield ( $\text{g pot}^{-1}$ ) <sup>#</sup>	
			Tops	Roots
Dayton	0	6.8	2.41	0.81
Kearney	0	7.2	3.19	1.07
Dayton	3	6.7	2.20	0.84
Kearney	3	4.7	2.40	0.22

Source: Foy *et al.* 1967

\*pH initially adjusted to 4.8 and left unadjusted thereafter

<sup>#</sup>yield of 16 plants grown for 20 d in 9 L of solution.

Thus breeding of cultivars tolerant to acidic, infertile soils shows considerable promise for increasing rice production in less developed countries. Wright (1989) however, argues that breeding plants tolerant to acidic, high Al soils cannot be considered the sole solution to the problem, because if the acidification process continues, a point will be reached where even an Al tolerant plant cannot survive. He therefore recommends some combination of agronomic practices to minimise soil acidification, addition of lime or other soil amendments to reduce Al toxicity and breeding of Al tolerant plants. Moreover, improving the soil fertility will enable farmers to diversify to other, more profitable cash crops.

#### 1.4.3.2 Nitrogen deficiency

There are several breeding strategies that address the problem of N deficiency in rice. Plant breeders have improved the efficiency of N fertiliser use by rice. Cultivar IR8, released in 1965, produced less than 40 kg grain  $\text{kg}^{-1}$  N uptake, whereas cultivars released in 1995 produced almost 55 kg grain  $\text{kg}^{-1}$  N uptake (Fischer 1998). Nitrogen efficiency must continue to be improved in order to reduce production costs and minimise potentially harmful environmental effects. With this in mind, a bold research program is underway at IRRI to develop rice that can fix atmospheric N (Ladha *et al.* 1997). Other breeding strategies to supply N to rice include selection and improvement of leguminous green manures and their rhizobial symbionts, improvement of *Azolla* spp., blue-green algae, and various species of root-associated fungi and bacteria.

#### **1.4.3.3 Improving uptake of nutrients (other than N and P)**

When breeding plants for improved cation or trace element uptake, it is again important to understand the mechanism of plant uptake. Increased root length is a key mechanism that can increase the uptake of immobile nutrients (see Section 1.5.3.2.1 for more detail). Aluminium tolerance in roots will allow more extensive exploration of soils. In addition to Al tolerance (Section 1.4.3.1) and improved P uptake (Section 1.5.3.2.3), organic anions might play an important role in enhancing plant uptake of trace elements. For example, Jones *et al.* (1996a) hypothesised that citrate release may play a significant role in increasing the amount of plant available Fe for grasses grown in acidic soils

#### **1.4.3.4 Breeding plants to tolerate poor soil physical properties**

##### *Drought tolerance breeding*

Many of the soils on which upland rice is grown have a low water-holding capacity and are subject to moisture stress. A dense and deep network of coarse roots is one of the major drought-resistant characteristics for upland rice (O'Toole 1982, cited by Pantuwan *et al.* 1997). Lilley and Fukai (1994, cited by Pantuwan *et al.* 1997) have shown that there are genotypic differences in root depth and resultant water extraction from the soil. Progress in breeding cultivars of both upland and lowland rice with improved drought resistance has been slow (Fukai and Cooper 1995). The difficulties associated with the screening technique are outlined by Suwanavong *et al.* (1997). The presence of a traffic pan that inhibits root growth at approximately 30 cm depth in most lowland rice fields mean that the differences in rooting distribution between cultivars is small. Pantuwan *et al.* (1997) therefore stated that the implications of genetic variation in root system development in rainfed lowland rice are unclear and need to be evaluated.

## **1.5 Strategies to overcome P-related constraints to rice production**

### **1.5.1 Fertiliser use**

Most experiments involving the application of P to highly weathered tropical soils show a response to fertiliser P. Olson and Engelstad (1972) reported that many fertiliser studies in tropical regions showed good responses with appreciable residual effects from minimal rates of P. Other studies, however, indicated a need to satisfy or “quench” the soil’s P-fixing capacity by heavy P dressings before effective crop response occurred (e.g. Singh *et al.* 1973). (See Section 1.3.2 for a discussion on P-fixation, and also Chapter 4). Application of P fertiliser is the most common method of correcting P deficiency. There are 5 forms of P fertiliser in common use: mono- and di-ammonium phosphate, mono- and di-calcium phosphate, and the apatites found in rock phosphate.

The majority of farmers in the humid tropics have little capital (Palmer 1976, Baharsjah and Fagi 1995, cited by Zaini and Fagi, 1999), and would require a loan to purchase P fertilisers, so it is therefore imperative that P fertiliser is used as efficiently as possible. Current recoveries of P fertiliser by upland rice grown in Ultisols or Oxisols is generally low, 5–20% of applied P (Garrity *et al.* 1990), so there is a lot of scope to improve the efficiency of P fertiliser use by rice. Improved efficiency of P fertiliser use would offer significant savings to the farmer, and would also reduce the risk of environmental problems associated with P fertiliser, such as the eutrophication of lakes.

#### **1.5.1.1 Management strategies to improve the efficiency of P fertiliser use by rice**

##### i) Banding/localised placement

The relationship between the concentration of P adsorbed on soil particles and P in solution is often non-linear, particularly in high P-fixing soils. Therefore when the soil P concentration is increased by localised placement there is a greater than linear increase in the amount of P in solution. More P in solution means more P is available to be taken up by plants. Note that banding of slowly soluble rock phosphate fertilisers is not usually recommended because the rate of dissolution of the rock phosphate depends on the supply

of acid and the movement of the dissolution products away from it through the soil, and this is restricted if the fertiliser is banded (Kirk and Nye 1986b).

In this thesis the term “banding” is used in a general sense to include any strategy that concentrates fertiliser in a smaller volume of soil than when the fertiliser is incorporated throughout the plough layer, or broadcast on the soil surface. Common examples of this strategy include the banding of fertiliser with or near the seed using a seed drill, side-dressing fertiliser in a band, or placement of fertiliser granules or briquettes.

ii) Seedling dipping

Roots of seedlings are dipped into a P slurry immediately prior to transplanting. Such a practice has resulted in similar yields with 50% less fertiliser (De Datta *et al.* 1990). However this strategy is only suitable for paddy rice where rice is easily transplanted.

iii) Adding silicate to the soil

Addition of silicate to the tropical soils of Hawaii has been reported to enhance the uptake of fertiliser P by sugarcane. Apparently, silica can substitute for P on the reactive sites in the soil, freeing P for plant uptake (Engelstad 1972).

iv) Placing P in the mulch layer

This avoids contact between fertiliser P and the high P-fixing soil.

v) Changing the soil pH

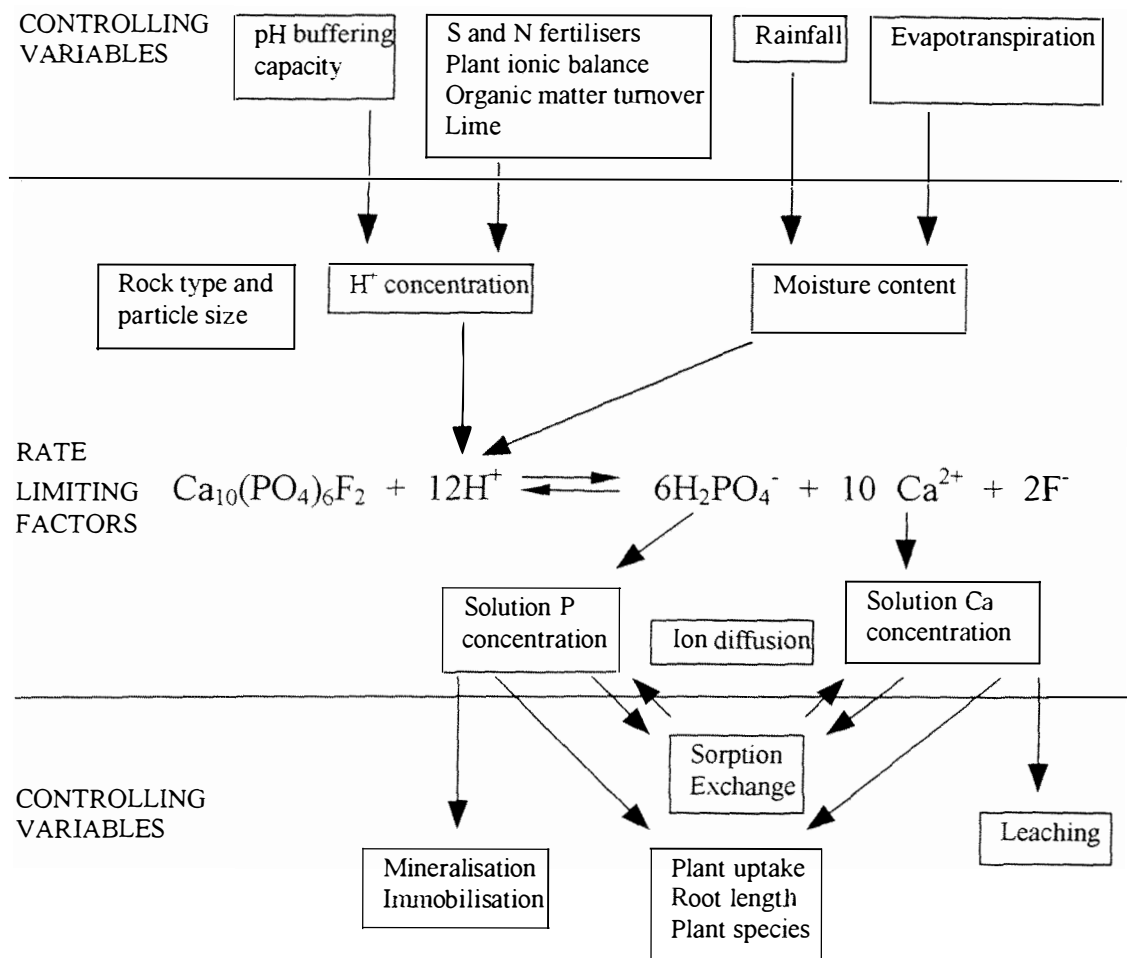
Liming can enhance the availability of organic P, by providing more favourable conditions for mineralisation (Jackson 1967).

vi) Soil or seed inoculation with mycorrhiza or P-solubilising bacteria

Seed coated with a commercial mycorrhizal inoculum – ‘ECOMIC’ (*Glomus fasciculatum*) has recently been developed and has been shown to increase yields of paddy rice (Ortiz and Fernandez 1998)

vii) Use of reactive phosphate rocks (RPRs)

This strategy often improves the economic efficiency of fertiliser use, in terms of kilograms of P taken up by the crop per dollar spent on P fertiliser, as RPRs are generally the cheapest sources of P (Sánchez and Uehara 1980). However this efficiency is highly dependent the rate of dissolution of the RPR, as RPRs are sparingly soluble in water. The rate of dissolution of RPRs is dependent many factors, as shown in Figure 1.3.



**Figure 1.3.** Schematic diagram showing the rate-limiting factors and variables determining phosphate rock ( $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$ ) dissolution (Hedley *et al.* 1995).

Techniques that increase the availability of P from RPRs include:

- ⇒ decreasing the particle size of the RPR
- ⇒ breeding cultivars that excrete large amounts of H<sup>+</sup> or organic acids from their roots (Liu *et al.* 1990)
- ⇒ growing a legume cover crop prior to the food crop (von Uexküll 1986), as legumes have been shown to induce dissolution of RPR (Aguilar and van Diest 1981, Trolove *et al.* 1996)
- ⇒ combination of acid producing fertilisers with RPR e.g. elemental S, the microbial oxidation of which releases acid (Apthorp *et al.* 1987, Ghani *et al.* 1994).
- ⇒ in some cases, incorporating green manure with RPRs (Kanapathy and Thamboo 1960, cited in Davide 1964), [see Chapter 3 for a more detailed discussion on this].

### **1.5.2 Organic amendments**

Large applications of manure from offsite can supply sufficient P to sustain moderate rice yields. For example, Bijay-Singh *et al.* (1996) found that an annual application of 13.8 t poultry manure per hectare produced a yield of 5 t rice grain ha<sup>-1</sup> in a 3 yr trial on a flooded Fatehpur loamy sand. However most farmers in the tropics do not have access to such a resource, or the labour to apply large quantities of manure. The amount of P contained in green manure crops is small, and the organic P is only slowly available to the crop, therefore green manure crops are not grown to supply P. An exception to this is in China, where radish (*Raphanus sativus*) is grown as a green manure crop because it can take up P from not easily soluble sources, such as rock phosphate, more effectively than other plants (Yu 1989). This is due to the high CEC of the root surface and an ability to acidify the rhizosphere. However green-manured crop rotations are only sustainable if *all* the P removed in the crop is returned to the soil (in the form of threshings and excreta), and sufficient time is allowed for the P to be released by mineralisation. Therefore, except in communities where *all* wastes were recycled, P removed by the crop needs to be replaced by the application of P fertiliser.



### **1.5.3 Breeding rice plants with an improved efficiency of P use**

There is no possibility to fix P from the atmosphere as there is with N, therefore the only options that plant breeders have in regards to P, is ensure that existing soil P, and any added fertiliser P, is used as efficiently as possible. Strategies of P efficiency may be divided into two categories, which are defined below:

- ⇒ Internal P efficiency – the ability of the plant to produce more grain while requiring less P
- ⇒ External P efficiency – the ability of the plant to extract more P from the soil.

#### **1.5.3.1 Increasing internal P efficiency**

Differences between rice cultivars in internal P efficiency have been found (Saleque *et al.* 1998), indicating that there is the potential to breed for internal P efficiency. However Kirk *et al.* (1993) are cautious about the strategy of breeding for internal P efficiency alone, because of a likely decrease in the P content of the grain. A high grain P content is important for rapid seedling establishment (Hedley *et al.* 1994), and also for the healthy nutrition of rice consumers, for whom it may be the major source of dietary P (Allaway 1984, as cited by Kirk *et al.* 1993). Grain P content should therefore be kept high, but since the majority of the P absorbed by the plant is ultimately stored in the grain, it seems unlikely that high grain P contents can be achieved by selection for internal P efficiency alone.

#### **1.5.3.2 Increasing external P efficiency**

Mechanisms that enhance the external efficiency of P uptake are mentioned below (adapted from Hedley *et al.* 1994):

- ⇒ P-efficient root structures - ability to develop long, fine, hairy roots
  - ability to multiply roots in P-rich zones
- ⇒ ability to solubilise inorganic P ( $P_i$ ) through changes in pH or through the release of chelating agents
- ⇒ ability to utilise soil organic P ( $P_o$ ) through the release of phosphatase enzymes
- ⇒ ability to associate with mycorrhizal fungi

### 1.5.3.2.1 Phosphorus-efficient root structures

A general response to P deficiency is increased root:shoot ratios. However selection in favour of this would be likely to reduce yields. A better strategy would be to select for a greater absorbing surface per unit mass of roots. Simple mathematics dictate that, for a given root mass, long fine roots provide a greater absorbing surface than short fat ones, as shown in Equation 1.8.

Let  $r$ ,  $l$  and  $\rho$  be root radius, length and density, respectively.

surface area =  $2\pi rl$  and mass ( $m$ ) =  $\rho\pi r^2 l$ .

Therefore surface area =  $2m/(\rho r)$ . (1.8)

Root hairs further increase the amount of absorbing surface. One plant breeding strategy would therefore be to breed plants with long fine hairy roots. According to Kirk *et al.* (1998) this strategy has not been vigorously pursued.

Some plants are able to increase the fineness and surface area per unit dry weight of their roots (Garcia and Ascencio 1992) or the length and density of their root hairs (Foehse and Jungk 1983) when subjected to low P conditions. Wissuwa (2000) identified P-efficient lines of rice that took up two times more P from a P deficient volcanic ash soil than the cultivar Nipponbare. The superior P uptake of the P efficient lines was found to be due to alleles coding for a seven-fold increase in root surface area between days 40 and 150. In contrast the root surface area of Nipponbare less than doubled during this period.

Plants are also able to concentrate their roots in P-fertilised zones (Anghinoni and Barber 1980a). This enables them to take up more P than if their roots were randomly or equally distributed throughout the soil.

### 1.5.3.2.2 Manipulation of rhizosphere pH

The ability of plants to solubilise  $P_i$  by acidifying the rhizosphere has been well documented (Hedley *et al.* 1982, Aguilar and van Diest 1981, Gahoonia and Nielsen 1992, Trollove *et al.* 1996). In these studies, the reason for the acidification was due to an alkaline uptake (excess cation uptake) pattern, which led to the release of  $H^+$  ions to maintain

electroneutrality (the charge balance) inside the plant. In their study on upland rice in an Ultisol, Hedley *et al.* (1994) observed a large decrease in pH in the rhizosphere (0.5 unit) but found that the amount of P uptake that was explained by rhizosphere acidification was small. This emphasizes the point that the amount of P released by acidification of the rhizosphere is dependent on the amount of acid soluble P in the soil. The majority of soils where upland and rainfed rice are grown are highly weathered, and therefore contain minimal amounts of acid soluble P. However rhizosphere acidification is important where acid-soluble forms of P fertiliser are either applied (e.g. RPR, Trolove *et al.* 1996) or form in the soil (e.g. dicalcium phosphate). Acidification of the rhizosphere has been demonstrated to be an important mechanism for P uptake by rice in flooded soils (Saleque and Kirk 1995).

### ***1.5.3.2.3 Release of chelating agents***

#### *a) Mechanism of action*

The ability of low-molecular weight organic acids to increase the amount of P in the soil solution has been well documented (Deb and Datta 1967, Earl *et al.* 1979, Traina *et al.* 1986, Fox *et al.* 1990, Gerke, 1992, 1993b, Bolan *et al.* 1994, Jones and Darrah 1994). Traina *et al.* (1986) identified 3 possible mechanisms why organic acids increase the level of P in solution. These include:

- ⇒ competition for P adsorption sites
- ⇒ dissolution of adsorbents
- ⇒ changes in the surface charge of the adsorbents.

i) Competition for P adsorption sites

Organic anions, particularly citrate, may reduce the amount of P bound to the soil, and consequently increase the amount of P in solution, by competing for adsorption sites (Nagarajah *et al.* 1970, Parfitt 1979, Lopez Hernandez *et al.* 1986, Hue 1991, He *et al.* 1992).

ii) Dissolution of adsorbents or P-containing minerals

Phosphate is released by the dissolution of positively charged surfaces of metal-containing compounds that bind P e.g. Ca phosphates (Dinkelaker *et al.* 1989, Jones and Darrah 1994), Fe or Al oxides (Earl *et al.* 1979, Fox *et al.* 1990, Gerke 1994), or Fe/Al-humic compounds (Gerke 1992). The effectiveness of an organic compound in complexing metal ions is dependent upon the number and position of the carboxylic and phenolic groups in the organic acid.

Associated  $H^+$  ions may also assist in the dissolution process. Jones and Darrah (1994), for example, found that citric acid ( $H^+$  associated citric acid) solubilised more P from a soil rich in Ca-P minerals than that which could be attributed to the sum of the P solubilised by the action of  $H^+$  ions and by Na-citrate.

iii) Changes in the surface charge of the adsorbents

Adsorption of organic anions by variable-charge soils has been shown to increase the amount of negative charge on the surface (Shanmuganathan and Oades 1983), which further decrease the adsorption of nutrient anions, e.g.  $H_2PO_4^-$  (Nagarajah *et al.* 1968).

*b) Influence of soil properties on the solubilising ability of organic acids*

The soil mineralogy determines number and types of phosphate absorbing sites, the amount of variable charge on the soil, and the extent to which the P-sorbing mineral can be solubilised by the organic acid. For example, Earl *et al.* (1979) found a comparatively larger reduction in P sorbed in citrate-treated Fe-rich soils, than in citrate-treated Al-rich soils. Ae *et al.* (1990) grew a range of crops on an Alfisol, which contained mainly Fe-P, and a Vertisol, which contained both Ca-P and Fe-P. Pigeonpea grew well and took up adequate P on the Alfisol, while sorghum, soybean, pearl millet and maize all had very low P contents

and died within a month. On the Vertisol, pigeonpea had a lower P content than all the other crops. Pigeonpea roots were shown to exude piscidic acid, which was not present in soybean and sorghum root exudates. Piscidic acid and some derivatives were shown to release P from  $\text{FePO}_4$ . The mineralogy also affects the diffusion rate of both the organic anion and the metal-organate complex through the soil. These rates determine the volume of soil around a root from which P can be solubilised.

The amount of P solubilised per mole of organic acid added (mobilisation efficiency) is also dependent on the initial P content of the soil. Soils with low P contents are more strongly buffered with respect to P, especially high P-fixing soils, and therefore the mobilisation efficiency of organic acids will be lower on low P soils compared with high P soils (Earl *et al.* 1979, Parfitt 1979).

The microbial population of the soil influences the longevity of organic acids in soil. Root released organic acids may provide energy sources for the rhizosphere microbial population. Conversely, rhizosphere bacteria may stimulate production, or be a source of organic acids. Lin and You (1989) also found that the presence of  $\text{N}_2$  fixing bacteria in the rhizosphere could stimulate the production of organic acids from roots. Rhizosphere bacteria (Kucey *et al.* 1989) and ectomycorrhizal fungi (Graustein *et al.* 1977) can also produce P-solubilising organic acids.

### *c) Relative effectiveness of various organic compounds at releasing P*

In general, organic compounds containing tri-carboxylic acids are more effective at solubilising P than those containing di-carboxylic acids, which are in turn more effective than mono-carboxylic acids. For example, Bolan *et al.* (1994) also found that citric acid, a tricarboxylic organic acid, was more effective at solubilising P from both an allophanic soil and a soil dominated by vermiculite, than dicarboxylic acids. Parfitt (1979) found citrate to be more effective than oxalic acid (a dicarboxylic acid) in desorbing P from goethite. Under some conditions, oxalic acid has been found to be more effective than citric acid. Ström *et al.* (1994) found that oxalic acid extracted markedly more P from an alkaline Rendzic Leptosol ( $\text{pH}_{\text{H}_2\text{O}}$  8.0), than did other organic acids, including citric acid. Nagarajah *et al.* (1970) found that citric acid was more effective than other organic acids in reducing P

sorbed to goethite and gibbsite, but oxalate was markedly more effective in reducing P sorbed to kaolinite.

Fox *et al.* (1990) measured the amount of P solubilised by a range of organic acids from a soil rich in Al-oxides. They found that the amount of P extracted by organic acids was proportional to the stability ( $\log K_{Al}$ ) of the Al-organate reaction product. Below a  $\log K_{Al}$  value of 4, there was no difference in the amount of P extracted by a solution containing organic acids or distilled water.

Soil pH and the charge on the organic acid also plays an important part on the ability of the organic acid/anion to release P from the soil. Nagarajah *et al.* (1970) found that the reduction in the amount of P sorbed on kaolinite, gibbsite or goethite due to the addition of organic acids, is greatest at the pH that corresponds roughly to the second pK value of the organic acid (pH 4 – 6 for many organic acids). Jones and Darrah (1994) showed that citric acid was much more effective at solubilising acid-soluble calcium phosphate than the sum of the effects of Na-citrate and  $H^+$ . The pH also influences the amount of P desorbed because it affects the stability of the reaction products. Iron-citrate complexes are stable below pH 6.8, and Jones *et al.* (1996a) found that the amount of  $Fe(OH_3)$  solubilised by citrate markedly increased as the pH dropped below 6.8. At high pH of approximately 8 and above, citrate is strongly negatively charged ( $3^-$ ): studies at this pH have found that the major mechanism by which citrate release P is by competing with P for adsorption sites (Kafkafi *et al.* 1988, He *et al.* 1992).

#### *d) Amounts of organic acids released*

Amounts of organic acids in soils are often quite low. Citrate concentration in the rhizosphere of upland rice range from 0.6 to 0.07  $\mu\text{mol citrate g}^{-1}$  soil (Kirk *et al.* 1999a). Even in the proteoid root clusters of white lupin, where the total amount of citrate excreted is up to 23% of the total dry mass of the plant, the total citrate concentration only amounts to 1.1  $\mu\text{mol citrate g}^{-1}$  soil (Dinkelaker *et al.* 1989). In these clusters there is a large decrease in available P (due to solubilisation and plant uptake) and an increase in available Fe, Zn and Mn. Experiments by Amann and Amberger (1988) however, suggest that a minimum citrate concentration of 5.3  $\mu\text{mol g}^{-1}$  is necessary to increase the concentration of

P in solution. However in both the experiment of Kirk *et al.* (1999b) and Dinkelaker *et al.* (1989), P uptake was attributed to the release of organic anions. It appears, therefore, that the soil organic acid concentration required for P release is highly variable between soils, depending strongly on factors such as mineralogy and microbial activity, as mentioned above. The lack of consensus on the concentration of citrate required to solubilise P in the rhizosphere may also result from the difficulties in recovering and measuring citrate concentrations in soils.

*e) Factors affecting the amount of organic anions released*

i) Root biomass

First and foremost it should be mentioned that as a general rule the amount of exudates released from roots is proportional to the root biomass (Vancura 1988).

ii) Concentration of root exudates outside the root

Meshkov (1952, 1956, as cited by Vancura 1988) found that the amount of exudates released from maize and pea roots increased by 35–135% and 170–300%, respectively, if the nutrient solutions were renewed (see also Prikryl and Vancura 1980). Similarly, the amount of carbon compounds exuded by roots increases in the presence of micro-organisms (Barber and Lynch 1977, Vancura *et al.* 1977). These observations suggest that the rate of release of carbon compounds from the root may be regulated in an inverse relationship by the concentration of carbon compounds outside the roots.

iii) Rhizosphere micro-organisms

Rhizosphere micro-organisms can have many different effects on the amount and composition of organic anions in the rhizosphere. Meharg and Killham (1995) found that non-root-infecting rhizosphere micro-organisms could enhance root exudation several-fold, depending on population density and composition. O'Keefe and Sylvia (1992) and Azaizeh *et al.* (1995) found that the organic acid composition in the rhizosphere of VAM infected roots was not significantly different from non-VAM-infected roots, whereas Marschner *et al.* (1997) detected fewer compounds in the rhizosphere of mycorrhizal roots, compared to non-mycorrhizal roots. Synergistic interactions also occur: Kim *et al.* (1998) found little difference in oxalate, citrate or 2-keto-D-gluconate concentration in the rhizosphere of tomato plants inoculated with either a P solubilising bacteria (*Enterobacter agglomerans*)

or a VAM (*Glomus etunicatum*) compared to the uninoculated control. However, when the plants were inoculated with both *E. agglomerans* and *G. etunicatum*, the amounts of the aforementioned organic acids released increased several-fold, depending on the age of the plant.

#### iv) Temperature and light

Rovira (1959) and Schroth *et al.* (1966) found that the amount of root exudates released increased as the temperature increased. Sudden temperature changes, e.g. cold shock or heat shock, may also cause increased exudation (Vancura 1967). Light intensity and photoperiod (Vancura 1988) also affect the composition and quantity of root exudates. These factors are important when planning the harvesting of experiments e.g. transferring plants from a glasshouse to a laboratory for harvest.

#### v) Phosphorus stress

The rate of release of organic acids from roots increases when the plant is subjected to P stress. For example, Lipton *et al.* (1987) found that the rate of citrate release from alfalfa roots increased by 182% when grown in nutrient solution containing one tenth of the original phosphate (0.01 mM  $\text{NH}_4\text{H}_2\text{PO}_4$ ) solution. However Kirk *et al.* (1999a) found no consistent effect (across a range of cultivars) of the presence or absence of P on the amount of citric acid released by rice grown in solution culture.

#### vi) Temporary water stress

The amount of root exudates increases when plants are temporarily exposed to water stress (Vancura 1988). Martin (1977a,b) found that zones lacking in water contained larger amounts of carbon exuded in the form of mucigel and in a form originating from the lysis of root tissues, compared with zones that had been well supplied with water. Presumably this is to maintain root-soil contact as the water-stressed root cells lose turgor.

#### vii) High $\text{Al}^{3+}$ concentrations

High solution concentrations of Al have also been shown to increase the release of citric acid from Al-tolerant snapbeans (see Section 1.4.3.1).

#### *f) Mechanism of organic acid/anion release*

The concentration of organic anions in the root is about 1000-fold greater than the concentration in the soil solution, therefore there is a constant low flux of organic anions down the concentration gradient through the cell membrane into solution (Jones 1998).



This rate of efflux can be greatly enhanced by the opening of channels embedded in the lipid bi-layer (Dennis *et al.* 1997). In very P stressed plants, Ratnayake *et al.* (1978) and Graham *et al.* (1981) demonstrated that the root membranes became more leaky (shown by increased  $^{86}\text{Rb}$  efflux (a tracer for K) and a decrease in phospholipid P content), which led to increased exudation of reducing sugars and amino acids. No organic acid analyses were performed to determine whether the membranes had become 'selectively leaky' i.e. there was no comparison between the composition of the reducing sugars and amino acids released, with that of the cytoplasm. Kirk *et al.* (1999a) has shown that the composition of exudates from both P-stressed and non-P-stressed rice roots is different to root cell composition, which suggests active exudation, or 'selective leaking' rather than simple leakage of the cellular contents through the cell membrane. In most 'normal' growing conditions, it is likely that large quantities organic acids are released by active exudation. Gallmetzer *et al.* (1998), studying the efflux of citrate from *Penicillium simplicissimum*, a citrate-producing fungus, found that hyphae grown in solutions treated with the metabolism inhibitors N-ethylmaleimide or sodium azide, had a much higher intracellular citrate concentration than the untreated control, yet markedly lower citrate excretion rates. This led to the hypothesis that citrate exudation is not due to an unspecific change in the permeability of the plasma membrane, nor to simple diffusion of undissociated citric acid, but is mediated by an energy-requiring transport protein.

*g) Form of organic acid released*

Inside the root cell, organic 'acids' would exist as anions, since the pK values of most organic acids are between 3.0 and 4.5, which is well below the cytoplasmic pH of 6.5–7.5 (Smith and Raven 1979). When these organic anions are exuded, these must be balanced by cations in order to maintain electroneutrality inside the cell. In many experiments, the pH of the rhizosphere declines with organic acid release, suggesting that  $\text{H}^+$  is the balancing cation. However under Al toxicity no decrease in  $\text{H}^+$  is observed with organic anion release, and it appears that  $\text{K}^+$  is the balancing cation (Jones 1998).

In the soil, most organic 'acids' will therefore be present as organic anions, since the pH of most soils is above 4.5. However in much of the literature, the term 'organic acid' is used, even though they are actually released as anions, which may or may not be co-transported

with  $H^+$ . In this thesis, the term ‘organic acid’ or ‘organic anion’ has been used in the same way as in the source literature.

#### *h) Root exudates of rice*

Lin and You (1989) studied the composition of exudates from four cultivars of lowland rice and found that the major compounds released were (in order of amount): carbohydrates, organic acids then amino acids, with the level of basic amino acids being higher than acidic amino acids (Table 1.5). Kirk *et al.* (1999b) found that the predominant organic acid released by upland rice grown in soil was citrate. Liu *et al.* (1990) also found that citric acid was the dominant acid released by rice, and that the amount of citric acid released increased in the absence of P. Kirk *et al.* (1999a) also detected citrate release from various rice cultivars grown in solution culture, but found no consistent effect of the presence or absence of P on the amount of citric acid released by different cultivars.

**Table 1.5.** Average composition of root exudates from four lowland rice cultivars. (Lin and You 1989).

Carbohydrate	Avg. amount and (range) (mg g <sup>-1</sup> fresh root)		Organic acid	Avg. amount and (range) (mg g <sup>-1</sup> fresh root)	
fructose	6.7	(3.39 – 9.26)	citric acid	0.88	(0.38 – 1.83)
sucrose	5.5	(9.83 – 1.01)	malic acid	0.58	(0.17 – 1.00)
glucose	0.5	(0.00 – 1.01)	succinic acid	0.48	(0.00 – 1.19)
			lactic acid	0.32	(0.00 – 0.53)

#### *i) Do organic acids enhance plant P uptake in the field?*

Much of the debate over the practical importance of organic acids in enhancing the P uptake of plants grown in soil revolves around their persistence in the soil solution. The more carboxyl groups in the organic acid, the more effective it is in increasing the amount of P in solution (see Section 1.5.3.2.3c); at the same time, the more carboxyl groups the greater the likelihood the organic acid will become bound to the soil surface. Jones and Brassington (1998) found that >80% of the added organic acids (either malate, citrate or oxalate added at 0.25–5  $\mu\text{mol g}^{-1}$  soil) became bound to the soil surface within 10 min. They therefore concluded that this would greatly diminish their effectiveness to mobilise nutrients from the rhizosphere. In addition, organic acids are rapidly consumed in soil.

Studies on the mineralisation of exudates in non-rhizosphere soil have shown that organic anions such as citrate and malate added at realistic rhizosphere concentrations (10 to 100  $\mu\text{M}$ ) have an average half-life of 2–3 h depending on soil type (Jones and Darrah 1994, Jones *et al.* 1996b). In general, rhizosphere decomposition rates are 2–3 fold faster than in non-rhizosphere soil (Jones 1998). Furthermore, organic acids are only effective at mobilising P from some soils. Jones and Darrah (1994) found that citrate mobilised P from only one out of seven soils, this soil having a high Ca-P fraction; even then, only  $\approx 0.03$   $\mu\text{moles}$  of P were mobilised per  $\mu\text{mole}$  of citrate added.

In contrast, Kirk *et al.* (1999b) used a model, which accounted for the binding of citrate to the soil surface and rhizosphere degradation, to show that observed rates of citrate release from upland rice roots were sufficient to explain P uptake that could not be accounted for by diffusion of readily-available P. The reasons for the apparent disagreement in the literature is probably because the rhizosphere conditions are highly variable, and depend on a wide range of factors, including soil type, plant species, the microbial species present and their activity, water content, plant nutrition, soil temperature and weather conditions. More research is required to confirm whether organic acids are important in enhancing the P uptake of plants grown in soil.

#### ***1.5.3.2.4 Release of phosphatase enzymes***

Many plant species release phosphatase enzymes from their roots. Phosphatases release plant-available P ( $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$ ) from organic matter. Phosphatase activity depends on plant age, plant species, and soil type (Tarafdar and Jungk 1987), and shows an increase as rhizosphere  $P_i$  concentrations decrease (Hedley *et al.* 1982, Hedley *et al.* 1994). Tarafdar and Jungk (1987) found a decrease in organic P in the rhizosphere of wheat and clover, which was significantly correlated with phosphatase activity. Other studies, find a small (Gahoonia and Nielsen 1992), or negligible (Trollove *et al.* 1996), depletion of organic P in the rhizosphere, or even an increase (Hedley *et al.* 1982, Zoysa *et al.* 1999). A study by Hedley *et al.* (1994) on the rhizosphere of upland rice grown in an Ultisol found an increase in phosphatase activity next to the root surface, relative to the bulk soil. However, this increase in phosphatase activity did not correspond to a depletion of organic

P in the rhizosphere. They therefore concluded that the P uptake measured in their study was not due to phosphatase release.

#### ***1.5.3.2.5 Association with mycorrhizal fungi***

It has been well documented that mycorrhizal fungi contribute to plant P uptake (see reviews by Powell and Bagyaraj 1984 and Safir 1987). Increased P uptake via mycorrhiza has also been found for upland rice (Sanni 1976, Gangopadhyay and Das 1982, Sharma *et al.* 1988, Pradhan and Mohan 1996). However, a number of studies have suggested that vesicular arbuscular mycorrhizae (VAM) were of minor importance in rainfed rice. Howeler *et al.* (1987) found no response of upland rice to VAM inoculation at a range of soil P concentrations and concluded that VAM were much more important for coarse rooted species such as cassava. Also, early research by Butler (1939) found that VAM were absent from flooded rice. The amount of inoculum is often low if the rice is planted after a flooded crop (Ilag *et al.* 1987), or after a long period of unplanted fallow (Thompson 1991). Also, the rate of root infection by VAM can be slow. Abbott and Robson (1982) found that VAM infection of subterranean clover took at least 2 wk, and more than 6 wk for some VAM species.

Subsequent to Butler's early findings, Iqbal *et al.* (1978) found that transplanted rice seedlings were infected with VAM under flooded field conditions – so rapidly that there was no advantage in transplanting mycorrhizal seedlings. Increases in grain yield of flooded rice as a result of mycorrhizal infection have been reported in both pot (Sivaprasad *et al.* 1990; Secilia and Bagyaraj 1992; Solaiman and Hirata 1996, 1997) and field trials (Iqbal *et al.* 1978; Ortiz and Fernandez 1998). (Note that a yield decrease due to VAM infection was observed in one treatment in the study of Solaiman and Hirata (1995)). Generally, these increases in grain yield have been in the order of 10–30%. Secilia and Bagyaraj (1992) obtained 15–20% increases in rice grain yield in VAM inoculated treatments compared with the non-inoculated control, which had received twice the amount of P fertiliser. The observed increases in grain yield were generally attributed to an increase in the uptake of P, and increases in N and trace element uptake were also observed in some studies. Vesicular-arbuscular mycorrhiza might therefore be beneficial to P uptake in rice. The benefits of

mycorrhiza are likely to increase in situations where initial amount of inoculum is high, e.g. following an upland crop or pasture.

Howeler *et al.* (1987) found that crop responsiveness to mycorrhiza inoculation is highly dependent on a number of factors: the species and strain of mycorrhiza inoculated, the effectiveness of the mycorrhiza already present in the soil, soil P, N, and K status, soil pH, soil temperature and soil moisture. The differences between the results of Howeler *et al.* (1987) and the other researchers listed in the above paragraph highlight the fact that more research needs to be done in order to understand the benefit of mycorrhiza to rice under upland and rainfed conditions. The results of Howeler *et al.* (1987) do highlight, however, that upland rice is more P efficient than various other tropical crops, and that rice is not responsive to mycorrhizal infection in comparison with them. It seems important, therefore, to elucidate the mechanism of P efficiency in rice.

### **1.5.3.3 Examples from rice breeding programs to date**

Fageria *et al.* (1988a,b) tested some 100 upland rice cultivars under low and high P conditions, and found significant differences between cultivars in P uptake, indicating differences in external P efficiency. They also found cultivar differences in a P-use efficiency index based on grain yield at low and high soil P, indicating differences in internal and/or external P efficiency. Sakarung and Zeigler (1991) were fairly successful in breeding upland rice cultivars tolerant to acidic soils. The cultivars performed well in similar environments to that in which they were bred. However, comparing across environments, only a few lines performed well. The majority selected on acid soil in Colombia did not perform well on similar soils in Brazil and Asia. This highlights the need for breeding to be based on an understanding of the tolerance mechanisms (discussed by Kirk *et al.* 1998), so that the results can be extrapolated to other environments, otherwise the breeding process has to be repeated in each environment.

More research is required to fully understand the mechanisms by which plants take up P from the soil, before they can be used to develop an effective screening technique.

One mechanism that looks hopeful, is the release of organic acids from roots. However, due to the difficulties in measuring the quantity of organic acids released into soil, amongst other problems, progress in this area is slow. Genetic engineering may be useful in enabling this trait to be evaluated. De la Fuente *et al.* (1997) genetically modified papaya and tobacco to increase their citrate production four-fold. This was found to significantly increase the Al tolerance of these plants, and research is currently underway in order to evaluate the ability of these genetically modified plants to take up P. If it is successful it may be possible to use this technology to improve the P uptake of rice.

However, breeding alone is not the answer to the problem of P deficiency, because (using the same argument as Wright (1989) for Al-tolerance (Section 1.4.3.1)) it is clear that as more and more P is removed from the soil by the sale of crops, a point will be reached where even the most P efficient plant will not be able to grow. Therefore, a combination of plant breeding and judicious use of fertiliser P is necessary to sustain significant yield increases in the long term.

## **1.6 The purpose of this thesis**

The population of the rice-eating world will continue to increase, and so will their demand for rice. It is clear that some of this increase must come from an increase in rainfed and upland rice production. In order to increase production, a large number of soil constraints must be overcome. This must be done as cost effectively as possible, as most rainfed and upland rice farmers have access to little capital.

Although many nutrient deficiencies can be overcome by the use of leguminous green manures, it is still essential to apply fertiliser P. Phosphorus fertiliser is expensive, and often large applications are required before there is a grain yield response to P, therefore it is important that the efficiency of fertiliser P use by the plant is maximised. The current recovery of fertiliser P by the rice crop is very low (5–20%). There is therefore both a large need and a large potential to increase the recovery of P fertiliser by rice. This may be done either by using improved management techniques, such as banding of fertiliser P, by breeding more P efficient plants, or by a combination of the two strategies.

To make significant progress in developing improved fertiliser management practices, and in breeding a P efficient plant, requires an understanding of the mechanisms by which rice takes up P from the soil. The purpose of this thesis was to investigate the factors that affect the root growth and P uptake of upland rice grown under aerobic conditions in a strongly weathered, high P-fixing soil.

An initial study was conducted in order to assess any effect of Al on root growth. Once it was determined that Al would not significantly affect the study of root growth in the soils used, a number of management strategies and plant strategies for enhancing P uptake were investigated.

Management strategies investigated included the use of leguminous green manure in combination with RPR, and the technique of banding soluble P fertiliser. Plant strategies investigated included the ability of plants to proliferate roots in P-rich zones, mycorrhiza, and particular emphasis was placed on the possible role of organic acids in enhancing external P efficiency.

## 2. Assessing the effect of Al on rice root growth

### 2.1 Introduction

The severity of many of the soil constraints listed in Section 1.3 (except toxicities) can be reduced by an extensive root system. High concentrations of monomeric Al and low Ca, found in many highly weathered tropical soils, restrict the development of such a root system (Ritchey *et al.* 1988, Wright 1989), resulting in reduced yields (Shainberg *et al.* 1989). Rice is particularly tolerant to high concentrations of Al compared with other crops (Wade *et al.* 1988), but yields are still limited by high concentrations of Al in many tropical soils, particularly in South America (Howeler and Cadavid 1976).

Even if Al toxicity is not a problem in the soil in its natural state, Al toxicity may still be induced by the use of fertilisers. Bruce *et al.* (1988) found that increasing the rate of CaSO<sub>4</sub> or CaCl<sub>2</sub> fertiliser increased the ionic strength of the soil solution; this increased the amount of Al displaced into solution, resulting in reduced root growth of soybeans. This may be a particular problem when fertilisers are banded, as increasing the rate of many soluble fertilisers results in more Al being displaced into the soil solution and decreases the pH (Moody *et al.* 1995) – both of these factors exacerbate Al toxicity.

Specifying Al toxic conditions in soils is complicated for two reasons. Firstly, there is a wide variation in the Al tolerance of different crop species, and of varieties within each species (Foy 1988), including rice (Howeler and Cadavid 1976). This is due both to internal plant tolerance mechanisms and to root-induced changes in the soil that ameliorate acidity. Secondly, Al exists in a variety of forms, only some being phytotoxic (as discussed in Section 1.3.1.3). In summary, most researchers agree that Al<sup>3+</sup> is the main mononuclear form causing Al toxicity (Wright 1989, Kinraide 1997). The phytotoxicity of Al is also reduced by the presence of numerous other ions in solution such as Ca<sup>2+</sup> and Mg<sup>2+</sup> (Edmeades *et al.* 1991, Keltjens and Dijkstra 1991), SiO<sub>4</sub><sup>4-</sup> (Hara *et al.* 1999), H<sub>2</sub>PO<sub>4</sub><sup>2-</sup>



(Sarkunan and Biddappa 1984, Gupta and Singh 1989),  $\text{SO}_4^{2-}$  and  $\text{F}^-$  (Cameron *et al.* 1986), and organic anions (Hue *et al.* 1986).

Routine chemical tests do not distinguish between toxic and non-toxic forms, and may falsely indicate Al toxicity (Ahlrichs 1990). The most commonly used indices of Al toxicity are the activity of  $\text{Al}^{3+}$ , or the activity ratio of  $\text{Al}^{3+}:\text{Ca}^{2+}$ , in the soil solution. To accurately obtaining this index would be prohibitively expensive for farmers (especially those in developing countries) because present methods of determining Al speciation involve a large number of analyses to determine the concentrations of all the ions present, both inorganic and organic, then inputting the data into a computer model. More recently, Simpson *et al.* (1997) have made progress in determining  $\text{Al}^{3+}$  and  $\text{Al}_{13}\text{O}_4(\text{OH})_{24}\text{H}_2\text{O})_{12}^{7+}$  species by flow injection using a 1.3 s reaction with 8-quinolinol-derivatised Fractogel. However, such equipment is quite specialised and is not available in many laboratories. Such difficulties led Wright (1989) and Ahlrichs *et al.* (1990) to conclude that an efficient plant bioassay system for identifying Al-toxic soils was important until an effective laboratory procedure could be developed.

Commercial laboratories in New Zealand use 1 M KCl to extract Al, followed by analysis using atomic absorption spectroscopy (AAS). Hume *et al.* (1988) used the 0.02 M  $\text{CaCl}_2$  method of Hoyt and Nyborg (1972) to extract Al, followed by AAS analysis, to establish a critical Al concentration for white clover. These procedures are only partially successful for the reasons discussed above. Hume *et al.* (1988) noted that there were differences between soils in apparent plant tolerance to 0.02 M  $\text{CaCl}_2$  extractable Al, which appeared to be caused by differing C levels in the 0.02 M  $\text{CaCl}_2$  extracts. A laboratory test that shows promise as an indicator of Al toxicity is the amount of Al in soil solution as measured by a short reaction time with pyrocatechol violet ( $\text{Al}_{\text{PCV}}$ ) (Kerven *et al.* 1989, Bartlett *et al.* 1991, Berek *et al.* 1995). The PCV technique developed by Kerven *et al.* (1989) provides an estimate of the amount of complexed (i.e. non-toxic) Al since the reaction of  $\text{Al}^{3+}$  with PCV is virtually instantaneous, whereas not all of the PCV exchanges with the Al-complexed ligand in a time period of 1 min. The  $\text{Al}_{\text{PCV}}$  concentrations measured may be expressed as an  $\text{Al}_{\text{PCV}}:\text{Ca}$  ratio (Manoharan 1997), or as an Al:electrical conductivity ratio (Barton and Carr 1996). Solution Al, as measured by PCV, has also shown a good

relationship with root reduction in the Al-intolerant species lucerne (Bartlett *et al.* 1991, Wang *et al.* 1999). There seems to be no information indicating whether concentrations of  $Al_{PCV}$  are useful for predicting reduced root growth for rice (which is an Al-tolerant crop) in acid soils.

The growth rate of rice roots in acid soils could be a suitable bioassay for evaluating usefulness of the  $Al_{PCV}$  test as an early warning system for poor rice growth. One of the advantages of using root extension rates of germinating seeds as a bioassay method for measuring the effect of Al toxicity on roots is that the complicating effects of nutrient availability on root growth is largely avoided, as all the nutrients the root needs for growth, bar Ca (Presley and Leonard 1948, Rios and Pearson 1964), can be supplied from the seed. Thus, apart from possible Ca deficiency, the effects of the toxicity alone are assessed.

## 2.2 Objectives

The objectives of this first experiment were to evaluate a bioassay and associated simple chemical analyses that could be used to investigate the importance of Al in affecting root growth in rice in highly-weathered tropical soils, particularly the Philippine soil studied in later chapters. Once the bioassay was developed it was also of interest to investigate the possibility that Al toxicity in rice may be caused by localised placement of rapidly soluble fertilisers in acidic soils rich in exchangeable Al.

## 2.3 Materials and methods

### 2.3.1 *Experiment I: The influence of soil characteristics on extractable Al and root elongation*

#### 2.3.1.1 Soils

The soils used for the experiments in this chapter were the topsoil, and different subsoil depths, of the Wharekohe silt loam (New Zealand Soil Classification (NZSC), Perch-gleyed Densipan Ultic Soil; USDA Soil Taxonomy, Typic Albaquult). The Wharekohe soil was chosen because its properties (Table 2.1) are similar to highly weathered Ultisols, which are commonly used for growing upland and rainfed rice in the tropics (Section 1.3.2). The subsoil depths contained low organic carbon and had high Al:Ca ratios – properties in common with many tropical soils where the topsoil has been eroded away following forest clearing (von Uexküll and Mutert 1995). All soils were air-dried and 2 mm sieved.

#### 2.3.1.2 Preparation of root bioassay containers

Air-dried Wharekohe soil from various depths (Table 2.1) was packed into 75 mL tubes (44 mm in diameter by 53 mm deep) with 60 µm pore-diameter mesh at the base. An Egmont brown loam topsoil (NZSC Typic Orthic Allophanic Soil; USDA Soil Taxonomy, Typic Hapludand) was included for a control as it has a very low exchangeable Al concentration, high total carbon, and a high pH, indicating it would have a low soil solution  $\text{Al}^{3+}$  concentration. The soils in the 75 mL tubes were then wet-up from the base to a level where 60% of the porosity was water-filled. This was achieved by standing the tubes overnight in beakers containing a measured volume of distilled water.

**Table 2.1.** Important soil properties of Wharekohe silt loam depths and Egmont brown loam.

Soil	$\rho^{\dagger}$	$\text{pH}^{\dagger}$	Olsen P	P ret. <sup>§</sup>	Total C	Exch. cations <sup>#</sup> (cmol <sub>c</sub> kg <sup>-1</sup> )				
	kg dm <sup>-3</sup>		mg P kg <sup>-1</sup>	%	%	Ca	Mg	K	Al	ECEC
<b>Wharekohe silt loam</b>										
Topsoil (0–14 cm)	0.72	4.6	11.8	7	5.06	0.99	0.91	0.09	1.4	7.6
Subsoil (28–43cm)	0.94	4.7	1.4	29	0.61	0.08	0.47	0.06	8.3	12.2
Subsoil (36–52.5cm)	1.09	5.1	0.5	34	0.30	0.13	0.63	0.06	11.0	15.4
Subsoil (52.5–69cm)	1.05	5.2	0.5	39	0.22	0.22	0.61	0.07	11.3	16.3
Subsoil (96–114cm)	1.04	5.1	0.5	41	0.15	0.29	0.53	0.10	12.4	14.5
<b>Egmont brown loam</b>										
	0.83	5.9	3.0	93	3.07	2.10	0.89	0.09	0.2	5.9

<sup>†</sup> soil bulk density (oven-dry basis) when packed into pots for the bioassay (Experiment I)

\* 1:2.5 soil:water ratio

<sup>§</sup> P retention (Blakemore *et al.* 1987)

<sup>#</sup> Exchangeable cations extracted using 0.01 M silver thiourea (Blakemore *et al.* 1987)

### 2.3.1.3 Plants

Seeds of an Al-tolerant rice cultivar, Azucena (Khatiwada *et al.* 1996), were germinated on wet paper and placed in an incubator under a day/night temperature regime of 28–33°C/18–20°C. When the plumule was just visible, seeds of similar plumule length (approx. 0.5 mm), were transplanted into the tubes containing the different soils. Four seeds were planted just below the surface of each tube and there were two replicates of each treatment. After 44 h growth the seedlings were carefully removed from the soil and their root length measured.

## 2.3.2 Experiment II: Evaluation of extraction methods and measuring techniques for determining the effect of Al on root elongation

### 2.3.2.1 Experiment IIa: The effect of pH on rice root elongation and the evaluation of 0.02 M CaCl<sub>2</sub>-extractable Al to detect Al toxicity

The 96–114 cm depth of the Wharekohe silt loam was selected for this experiment because it contained the highest amount of exchangeable Al. Eleven subsamples (80 g each) of the air-dried subsoil were amended with either NaOH or H<sub>2</sub>SO<sub>4</sub> at rates of 0–6 cmol<sub>c</sub> kg<sup>-1</sup> soil to produce a range of soil pHs from 3.2–6.5, and therefore a range of soil exchangeable Al concentrations. Acid or alkali was applied with a garden sprayer onto a thin layer of soil

that was spread evenly over the bottom of a 2 dm<sup>3</sup> flat-bottomed PVC container. The soil was stirred between each application of approximately 0.75 mL and the sprayer weighed at the start and end of acid/alkali application. The soils were then shaken end-over-end in the 2 dm<sup>3</sup> containers for 3 h before being packed into the 75 cm<sup>3</sup> tubes to a bulk density of 1.0 kg dm<sup>-3</sup>. There was one tube of each treatment. These tubes were then wet-up from the base with distilled water until 60% of the total pore space was water-filled. Pre-germinated rice was planted the following day, as outlined in Section 2.3.1.3, except that there were eight seeds per tube.

After 42 h all tubes were dismantled and the seedlings carefully removed from the soil and the root length measured. The soils were then air-dried and the pH was measured at a soil:solution ratio of 1:2.5 in distilled water. Aluminium and Mn were extracted by end-over-end shaking for 1 h with 0.02 M CaCl<sub>2</sub> (Al<sub>CaCl2</sub> and Mn<sub>CaCl2</sub>) at a 1:2 soil:solution ratio (Hoyt and Nyborg 1972). The solutions were then centrifuged at 9,700 g<sup>†</sup> for 5 min then filtered (Whatman #42).

### 2.3.2.2 Experiment IIb: A comparison of extraction procedures and measurement methods

The acidic treatments (0–3 cmol H<sup>+</sup> kg<sup>-1</sup> soil) were repeated on the same soil (Wharekohe silt loam, 96–114 cm depth) two years later (after my return from conducting studies at IRRI), to determine whether the previously observed decrease in root length could be better explained by the concentration of Al in soil solution extracted by centrifugation and measured by a 1 min reaction with PCV, compared with Al extracted in 0.02 M CaCl<sub>2</sub> and analysed by AAS. The same bioassay procedure was followed as in Section 2.3.2.1, except that pots were used instead of tubes. These were packed in increments of one-eighth, with one-eighth of the soil added then tapped down, then one-eighth of the water applied, then the next one-eighth of soil and so on. This packing technique ensured that there was no movement of acid with the water during the wetting up procedure. Some of the acidity was found to be leached into the bottom of the pot if pots were wet up from above (data not

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<sup>†</sup> Note: the maximum radius was used in calculating the relative centrifugal force.

shown). Duplicate pots were prepared for all treatments, except the 2 cmol H<sup>+</sup> kg<sup>-1</sup> soil treatment where one pot was prepared, as the amount of soil was limited. Pots were kept moist for 12 d before planting. Four pre-germinated seeds of rice (cv. Azucena) and four pre-germinated seeds of lucerne (*Medicago sativa* L. cv. Otaio) of equal radicle length were planted per pot. Lucerne was chosen to provide a contrast between Al-tolerant (rice) and Al-intolerant (lucerne) species.

After root measurement the soil solution was extracted by centrifugation at 10,000 rpm (12,000 g) (Reynolds 1984) for two runs of 10 min. The soil solution was analysed for: pH, conductivity, Ca by AAS, and Al – by AAS and also by spectrophotometry, using a 1 min reaction time with PCV (Kerven *et al.* 1989).

The remaining soil was air-dried then extracted with 0.02 M CaCl<sub>2</sub> and analysed for Al by AAS as in Experiment IIa, and by PCV as above. These additional Al analyses were conducted to compare the effect of the different extraction methods (centrifugation and CaCl<sub>2</sub> extraction), and the different measuring techniques (PCV and AAS).

### **2.3.3 Experiment III: The effect of fertiliser amendment on soil Al concentration and root elongation**

The 36–53 cm depth of the Wharekohe silt loam was chosen for this experiment as it had the highest exchangeable Al:Ca ratio (Table 2.1). The air-dried soil was sieved (< 2 mm) and the fertilisers for the various treatments (listed in Table 2.2) were thoroughly hand-mixed with the soil. Duplicate pots were then packed, planted and the soil and roots analysed as in Experiment IIb.

The rates for the low fertiliser treatments were the same (in μg g<sup>-1</sup> soil) as those used by Fageria *et al.* (1988b), who grew rice in a dark red Latosol of low nutrient status, and high exchangeable Al. The rates in the high fertiliser treatments correspond to 140 kg N, 100 kg P and 110 kg K per hectare (to 5 cm depth), which are near the maximum rates commonly recommended for rice (Grist 1975). The N fertiliser would normally be split into two or three applications, although farmers monitored by the IRRI Mega Project in West

Java were observed to mostly apply N as a single application (IRRI 1997), which would reduce the labour requirements. The high urea and high  $\text{Ca}(\text{NO}_3)_2$  treatments therefore represent a 'worst case scenario' in terms of the likelihood of broadcasting fertiliser inducing Al toxicity in the soil. Banding of fertilisers, however, may produce much higher nutrient concentrations in the soil.

**Table 2.2.** Fertiliser treatments for Experiment III.

Label	Nutrient	Form of nutrient	Rate of element <sup>†</sup> ( $\mu\text{g g}^{-1}$ soil)
Control	None	–	–
Lime	Ca	$\text{CaCO}_3$	2500
MCP	P	Monocalcium phosphate (MCP)	200
	Ca	MCP	129
MCP+lime	Ca	$\text{CaCO}_3$ and MCP	2629
	P	MCP	200
Low urea	N	Urea	66
	K	$\text{K}_2\text{SO}_4$	160
	S	“	76
	P	MCP	87
	Ca	$\text{CaCO}_3$ and MCP	2629
Low $\text{Ca}(\text{NO}_3)_2$	N	$\text{Ca}(\text{NO}_3)_2$	66
	K	$\text{K}_2\text{SO}_4$	160
	S	“	76
	P	MCP	87
	Ca	$\text{CaCO}_3$ , $\text{Ca}(\text{NO}_3)_2$ and MCP	2723
High urea	N	Urea	280
	K	$\text{K}_2\text{SO}_4$	218
	S	“	89
	P	MCP	200
	Ca	$\text{CaCl}_2$ and MCP	529
High $\text{Ca}(\text{NO}_3)_2$	N	$\text{Ca}(\text{NO}_3)_2$	280
	K	$\text{K}_2\text{SO}_4$	218
	S	“	89
	P	MCP	200
	Ca	$\text{Ca}(\text{NO}_3)_2$ and MCP	529

<sup>†</sup> All fertiliser rates calculated per gram of oven-dried soil.

### **2.3.4 Experiment IV: Use of the bioassay to evaluate root growth in an Ultisol from Cavinti, Philippines**

The bioassay technique was used to evaluate root growth in the soil that was used for P uptake studies in the Philippines. The soil used was an isohypothermic palehumult from Cavinti, Laguna, Philippines (chemical properties are listed in Table 4.1). The methodology followed was the same as for Experiment IIb, and the 36–53 and 96–114 cm Wharekohe subsoil depths were used as controls. It should be noted that although the soils were both packed to the same air-dried bulk density, the moisture content of Cavinti soil was higher than the Wharekohe subsoil, therefore the oven-dry bulk density of the Cavinti soil was 9.7% lower than the Wharekohe subsoils.

### **2.3.5 Statistics**

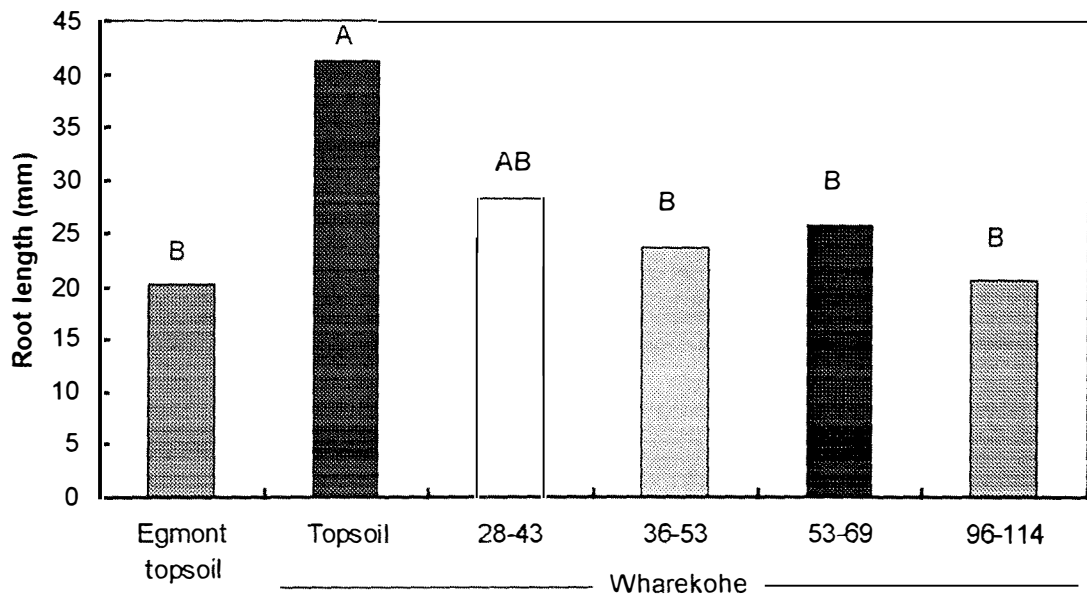
All root length data were square root transformed because the variance was proportional to the square root of the mean (except for Experiment 2.3.1 where it was not necessary). Treatment differences were tested for significance using the SAS<sup>®</sup> System (1989) to conduct analysis of variance (ANOVA) and multiple comparisons. Each root was treated as a replicate as there was no significant pot effect. In this thesis, results reported as being significant are significant at  $P < 0.05$ , unless otherwise stated.

## **2.4 Results and Discussion**

### **2.4.1 Experiment I: The influence of soil characteristics on root elongation**

The root length of rice seedlings grown in Wharekohe topsoil was significantly ( $P < 0.01$ ) longer than in the other soil materials (Figure 2.1). This difference is probably due to the very low bulk density of the Wharekohe topsoil (Table 2.1). Root lengths were not significantly different between the other soil materials, which had similar bulk densities to each other. To compare the effects of soil chemistry on root elongation between different soils it is therefore important to pack each pot to the same bulk density. The reason for the low root elongation in the Egmont soil, in spite of its low bulk density, is unclear. The soil pH, Ca, and exchangeable Al (Table 2.1) do not appear limiting.





**Figure 2.1.** Root elongation in samples of Egmont brown loam and various depths of Wharekohe silt loam. Treatments denoted by different letters are significantly different using Duncan's Multiple Range Test (DMRT) at  $P < 0.05$ .

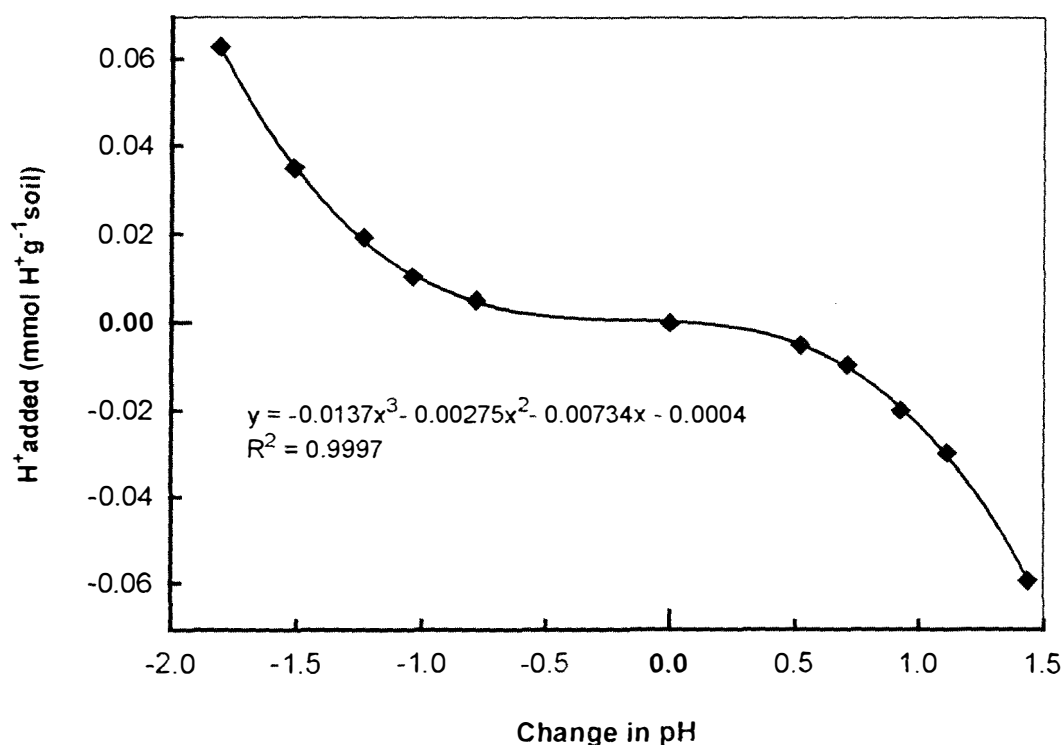
A comparison of rice root length in the Wharekohe subsoil depths with the Egmont topsoil (which has a low concentration of exchangeable Al) suggests that the subsoil depths do not contain high concentrations of phytotoxic Al, although the proportion of Al on the cation exchange complex is  $>65\%$  (Table 2.1). Therefore, phytotoxic Al concentrations would need to be increased by acidification in order to evaluate the bioassay as a means of detecting toxic concentrations of Al. It was also of interest to raise the pH of the subsoil, to determine whether root lengths were being slightly reduced by phytotoxic Al.

## 2.4.2 Experiment II: Evaluation of extraction methods and measuring techniques for determining the effect of Al on root elongation

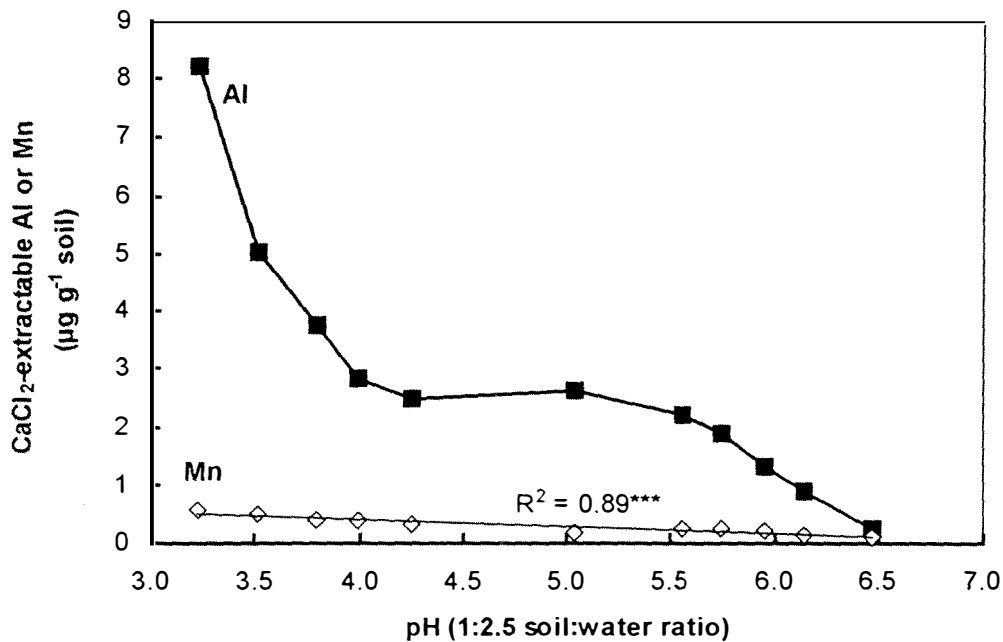
### 2.4.2.1 Experiment IIa: The effect of pH on rice root elongation and the evaluation of 0.02 M CaCl<sub>2</sub>-extractable Al to detect Al toxicity

#### a) Soil pH buffering and changes in CaCl<sub>2</sub>-extractable Al and Mn with pH

Figure 2.2 shows the pH buffer curve for the Wharekohe silt loam subsoil (96–114 cm depth). The pH buffer power, obtained from the slope of the curve at a particular pH, is 0.008 mmol H<sup>+</sup> pH<sup>-1</sup> kg<sup>-1</sup> soil between pH 4.3 and 5.6, but 0.08 mmol H<sup>+</sup> pH<sup>-1</sup> kg<sup>-1</sup> soil outside this range. The low buffering power of the Wharekohe subsoil at pH 4.3–5.6 is due to its low organic matter content and low content of basic cations (Table 2.1). The rise in buffer power at the acidic end (pH <4.0) is due to the dissolution of Al (Figure 2.3, also see Section 1.3.1.2). Fox (1982) also found that highly-weathered Puerto Rican Ultisols and Oxisols had low buffering around pH 5, and that the pH buffer power rose at low pH, except in the Oxisols, where much of the Al had been weathered from the soil.



**Figure 2.2.** pH buffer curve of Wharekohe silt loam subsoil (96–114 cm depth).

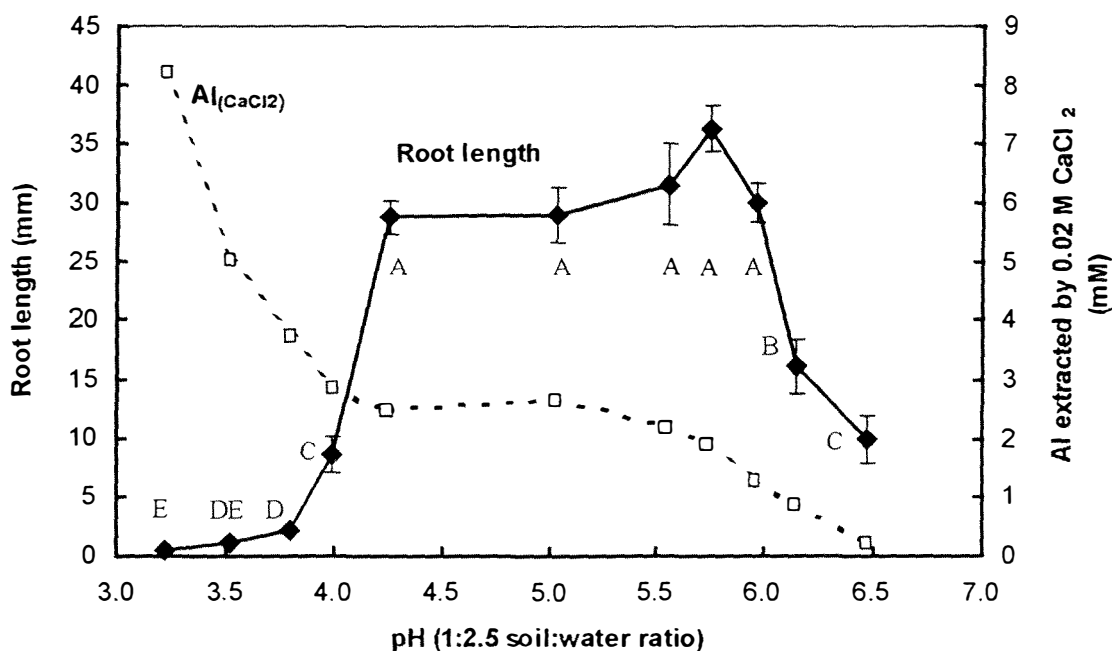


**Figure 2.3.** The effect of soil pH on 0.02 M CaCl<sub>2</sub>-extractable Al and Mn in Wharekohe subsoil (96–114 cm depth). A linear regression line has been plotted for the Mn data. Data are means of two replicates. \*\*\* The R<sup>2</sup> is significant at P<0.001.

The CaCl<sub>2</sub>-extractable Al concentration of Wharekohe subsoil increased markedly upon acidification to pH <4.0, and decreased upon alkalization above pH 5.5 (Figure 2.3). Calcium chloride extractable Mn also increased as soil pH decreased (Figure 2.3). The Mn<sup>2+</sup> concentrations in the pH-adjusted Wharekohe subsoil were very low (Bansal and Nayyar 1999) and would have had no deleterious effect on root elongation.

*b) The effect of soil pH on root elongation*

Soil pH had a highly significant (P<0.0001) effect on root elongation of rice seedlings. The optimum pH range for root elongation in rice seedlings was between 4.3 and 6.0 (Figure 2.4). Outside this range root elongation rates declined rapidly.



**Figure 2.4.** The relationship between pH, 0.02 M CaCl<sub>2</sub>-extractable Al, and root length of rice seedlings after 42 h growth in Wharekohe silt loam subsoil (96–114 cm depth). Data are means  $\pm$  SE of eight roots. Points denoted by a different letter are significantly different by DMRT at  $P < 0.05$ .

The rapid decrease in root elongation at a soil  $pH_{(H_2O)}$  above 5.7 (Figure 2.4) indicates that unfavourable chemical conditions for root growth developed as the pH (and presumably the ionic strength) of the soil solution rose following the addition of NaOH. The root reduction may be due to the activity of Ca<sup>2+</sup> in the soil solution decreasing as the activity of Na<sup>+</sup> increased (Bruce *et al.* 1988). The reason for the decrease in root extension was not pursued as the main focus of this chapter was on root extension in acid soils.

The rapid decline in root elongation rate below pH 4.2 is unlikely to be due to the effects of high H<sup>+</sup> concentration *per se*, as solution culture experiments in the absence of toxic concentrations of Al, with adequate Ca supplied, have shown no difference in the dry weight of rice roots or shoots when grown at pH 3.5, 4.3 or 5.0 (Thawornwong and van Diest 1974). An unfavourable Ca<sup>2+</sup> activity ratio was also not the cause of the rapid decline in root length below pH 4.2, as the Ca<sup>2+</sup> activity ratio increased with increasing H<sup>+</sup> addition (Table 2.3), presumably due to displacement of Ca<sup>2+</sup> by H<sup>+</sup>. The increase in soil solution

electrical conductivity (EC) with increasing acid addition (up to  $1.8 \text{ mS cm}^{-1}$  in the  $3 \text{ cmol H}^+ \text{ kg}^{-1}$  soil treatment of Experiment IIb (Table 2.3)), is also probably not the main reason for the observed decrease in root elongation. Moody *et al.* (1995) investigated the effect of soil solution EC, resulting from high  $\text{NH}_4$  and  $\text{NO}_3$  fertiliser concentrations, on soybeans, and found root length was little affected below  $6 \text{ mS cm}^{-1}$ . The high EC of the solution may have contributed to the decrease in root length in the high HCl treatments, but, unlike the decline at the alkaline end, which occurred at  $3 \text{ cmol OH}^- \text{ kg}^{-1}$  soil, the marked decrease in root length occurred at an addition of only  $1 \text{ cmol H}^+ \text{ kg}^{-1}$  soil. The rapid decline in root elongation rates with decreasing pH was most likely caused by the exponential increase in Al (Figure 2.4), for reasons discussed below, and in Section 2.4.2.2.

The inhibition of root extension was not linearly related to  $\text{Al}_{\text{CaCl}_2}$  (Figure 2.5). A linear regression of root length against  $\text{Al}_{\text{CaCl}_2}$  for the data of Experiment IIa over the range of acid additions ( $0\text{--}6 \text{ cmol H}^+ \text{ kg}^{-1}$  soil) gave a coefficient of determination ( $R^2$ ) of only 0.46, which was not significant at  $P < 0.05$ . An exponential relationship fitted the data better ( $R^2$  of 0.92) (Figure 2.5). However an exponential relationship is not a very sensible indicator: an increase in Al concentration of only 11% (between pH 5.0 and 4.0) corresponded to a dramatic 70% decrease in root length (Figure 2.4). Moreover, the critical  $\text{Al}_{\text{CaCl}_2}$  concentration at which root length rapidly decreased ( $2.7 \text{ mM}$ ) was higher than all previously published critical concentrations, except those of Tanaka and Navasero (1966) (Table 2.4), and roots grown in soil containing  $2.6 \text{ mM}$   $\text{CaCl}_2$ -extractable Al showed no decrease in root elongation. Presumably this is because  $0.02 \text{ M}$   $\text{CaCl}_2$  extracted a high amount of non-toxic Al in addition to  $\text{Al}^{3+}$ , particularly from the less acidic treatments. The poor relationship between  $\text{Al}_{\text{CaCl}_2}$  and Al-toxicity is also highlighted by von Uexküll (1986) in his review on fertiliser use in tropical soils (see also research by Manoharan *et al.* 1996). It was therefore important to find an analytical procedure that more accurately measured the concentration of phytotoxic Al species in soil (see Section 2.4.2.2).

**Table 2.3.** Soil solution chemical parameters that are important in explaining the root length observed in Experiments IIb, III and IV. Also given is the percentage difference in Al concentration\* measured using PCV compared with AAS†. The data are means of two replicates.

Soil, and soil treatment	pH	Conductivity ( $\mu\text{S cm}^{-1}$ )	Al <sub>PCV</sub> conc. (mM)	Ca conc. (mM)	Ca activity* (mM)	Al:Ca conc. ratio	% difference between Al <sub>PCV</sub> and Al <sub>AAS</sub> †
<b>Wharekohe subsoil (96–114 cm depth)</b>							
0 cmol H <sup>+</sup>	4.93	110	0.002	0.05	0.04	0.04	-22
0.5 cmol H <sup>+</sup>	4.06	390	0.144	0.19	0.15	0.75	-23
1 cmol H <sup>+</sup>	3.81	670	0.639	0.39	0.27	1.68	-15
2 cmol H <sup>+</sup>	3.60	1210	2.850	0.65	0.40	4.39	-1
3 cmol H <sup>+</sup>	3.46	1770	5.280	0.80	0.46	6.58	3
<b>Wharekohe subsoil (36–53 cm depth)</b>							
Control	4.85	180	0.004	0.06	0.05	0.06	-10
Lime	5.19	160	0.006	0.22	0.19	0.03	20
MCP+lime	5.11	180	0.003	0.25	0.21	0.01	-3
MCP	4.43	190	0.003	0.15	0.13	0.02	-27
Low urea	4.45	1030	0.040	2.56	1.64	0.02	-11
Low Ca(NO <sub>3</sub> ) <sub>2</sub>	4.18	2000	0.215	7.96	4.41	0.03	3
High urea	3.39	4330	3.730	9.61	4.34	0.39	-20
High Ca(NO <sub>3</sub> ) <sub>2</sub>	3.25	6000	7.320	16.99	6.99	0.43	-9
<b>Cavinti soil</b>	5.65	33	0.0005	0.02	0.00	0.02	1

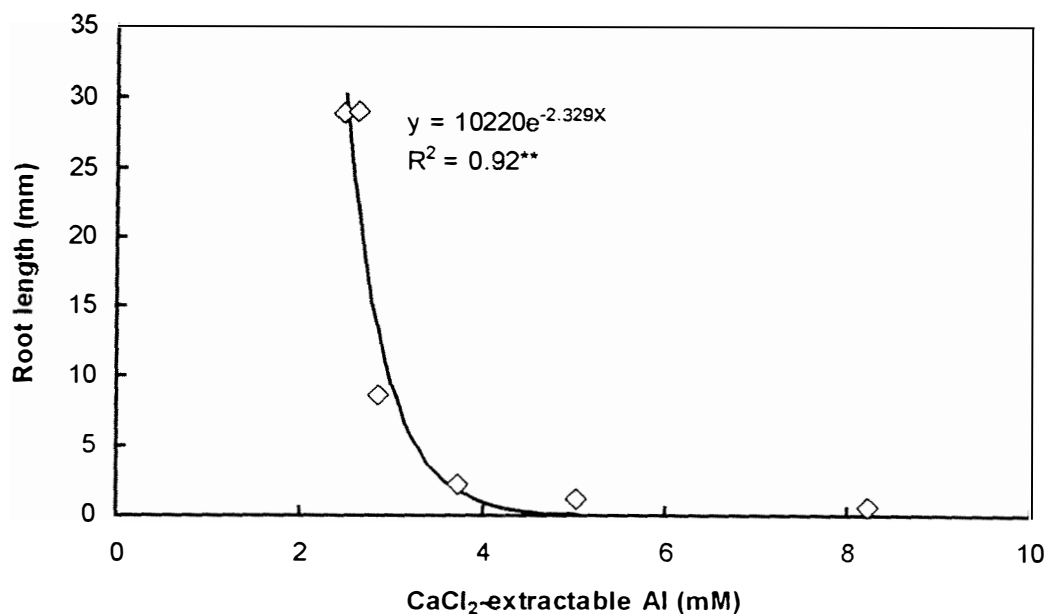
\*Al in 0.02 M CaCl<sub>2</sub> extracts.

†([Al] measured by PCV – [Al] measured by AAS)/[Al] measured by AAS × 100

\* Ca activity ( $\gamma$ ) was calculated by first estimating the solution ionic strength ( $I$ ) from the conductivity ( $EC$ )  $I=0.0120EC-0.0004$  (Gillman and Bell 1978, as cited by Wolt 1994), then using the Davies equation

$$\text{(Lindsay 1979) to estimate } \gamma: \log \gamma_i = -0.509Z_i^2 \left( \frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3I \right)$$

where  $Z$  is the valency of the ion ( $Z=2$  for Ca).

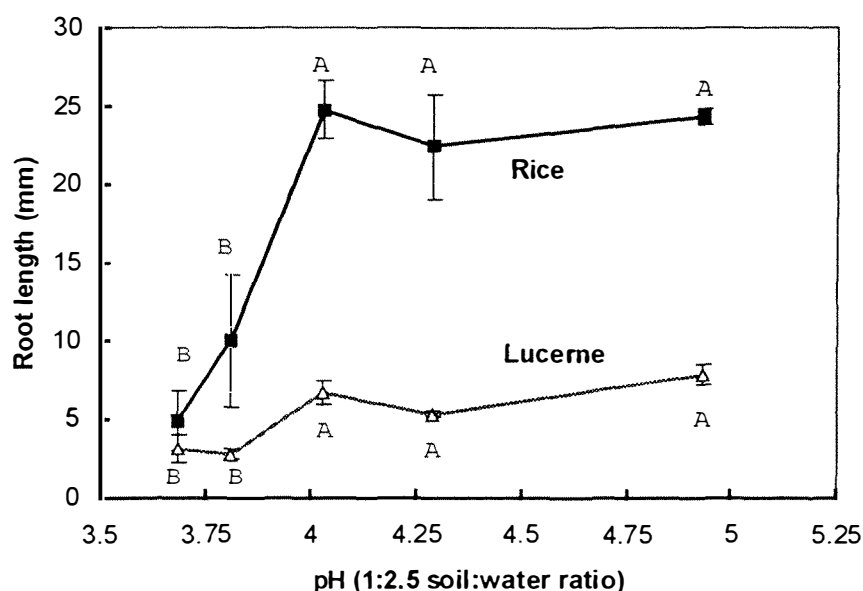


**Figure 2.5.** The relationship between rice root length and Al extracted by 0.02 M CaCl<sub>2</sub> for Experiment IIa. Data are means of eight roots. \*\* The R<sup>2</sup> is significant at P<0.01.

#### 2.4.2.2 Experiment IIb: A comparison of extraction procedures and measurement methods

##### a) Rice and lucerne root elongation.

As in the earlier experiment (Section 2.4.2.1), there was a rapid and highly significant ( $P < 0.0001$ ) reduction in rice root elongation with increasing acidity (Figure 2.6). The pH at which root elongation sharply declined (4.0) was slightly lower in the second experiment (Figure 2.6), compared with the first experiment (Figure 2.4). This suggests that there was a small change in the soil phytotoxic Al concentration during the two years of storage. Alternatively the concentration of phytotoxic Al may have been reduced by the longer incubation (12 d versus 1 d), and consequent higher microbial activity, in the second experiment. Microbial activity may have reduced the amount of phytotoxic Al by releasing organic acids, or by direct uptake of Al.



**Figure 2.6.** The relationship between soil solution pH and root length for rice and lucerne seedlings grown in Wharekohe silt loam subsoil (96–114 cm depth). Data are means  $\pm$  SE of eight roots. Points in each curve denoted by different letters are significantly different by DMRT at  $P < 0.05$ .

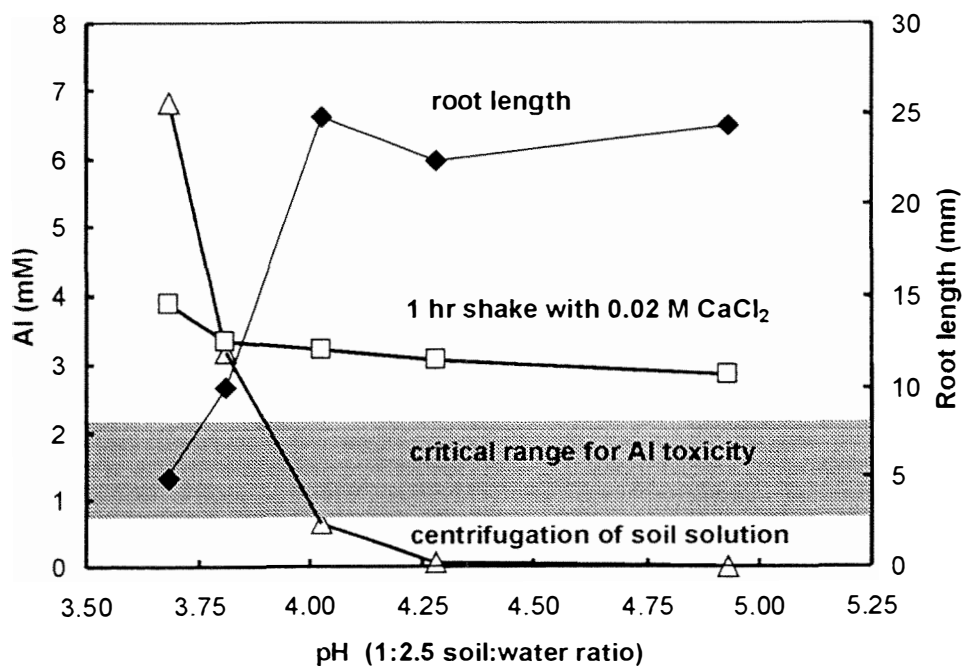
Lucerne root growth was significantly ( $P < 0.0001$ ) depressed below pH 4.0 (Figure 2.6). Lucerne roots were very short – all  $< 17$  mm. The root was often much thinner than the hypocotyl and in some cases roots were so short that the seedlings fell over when the pots were moved. This indicates that none of the soil pHs were suitable for lucerne growth. Bringans (1971) stated that lucerne grows best at a soil pH between 6.5–7.0. The fact that lucerne is a much smaller seed (with lower nutrient stores) than rice would also explain its slower root elongation rate, due to a greater dependence on nutrient uptake from the soil.

*b) Comparing extraction procedures:  $\text{CaCl}_2$ -extractable Al and soil solution Al.*

The decrease in root length was much better explained by the concentration of Al in the soil solution, as extracted by centrifugation, than by that in the  $\text{CaCl}_2$  extracts (Figures 2.7 to 2.9). Therefore the Al concentration of the soil solution must more accurately reflect the concentration of phytotoxic Al. Calcium chloride extraction gives a poor indication of phytotoxic Al because it extracts a significant amount of Al that is not in the soil solution, as shown by the large amounts extracted at  $\text{pH} > 4.3$  in Figures 2.3 and 2.7.



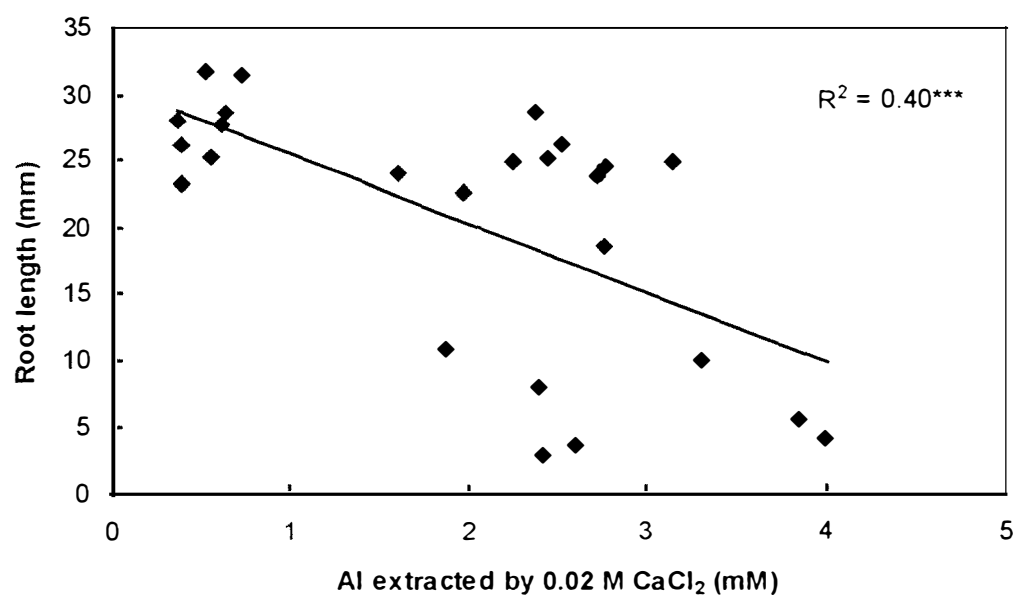
A sharp increase in soil solution Al concentration corresponds to a sharp decrease in root elongation rates (Figure 2.7), meaning that soil solution Al is likely to be a good diagnostic test for phytotoxic Al. A strong correlation between root length and the concentration of Al in the soil solution agrees with the findings of Bartlett *et al.* (1991) who obtained a strong linear relationship between depression of lucerne root length and soil solution Al measured by PCV. Ritchey *et al.* (1988) also obtained a highly significant linear relationship between root length and soil solution Al concentration measured by inductively coupled plasma – atomic emission spectroscopy (ICP–AES). Furthermore, the critical Al concentration at which root elongation rates are affected (0.7 to 2.9 mM) measured in the soil solution extracts (Figure 2.7) agrees with the majority of research on Al for rice grown in solution culture (Table 2.4). The extraction of soil solution by centrifugation is also simple to do. The centrifugation method is therefore the preferred method for extracting rice-toxic Al.



**Figure 2.7.** The effect of soil pH on rice root elongation and soil Al concentration (as recovered by centrifugation or extracted by 0.02 M  $\text{CaCl}_2$ ) in Wharekohe silt loam subsoil (96-114 cm depth). The shaded rectangle indicates the range of critical concentrations for Al toxicity in rice, as given by the majority of studies in Table 2.4. Data are averaged for two measuring techniques (AAS and PCV).

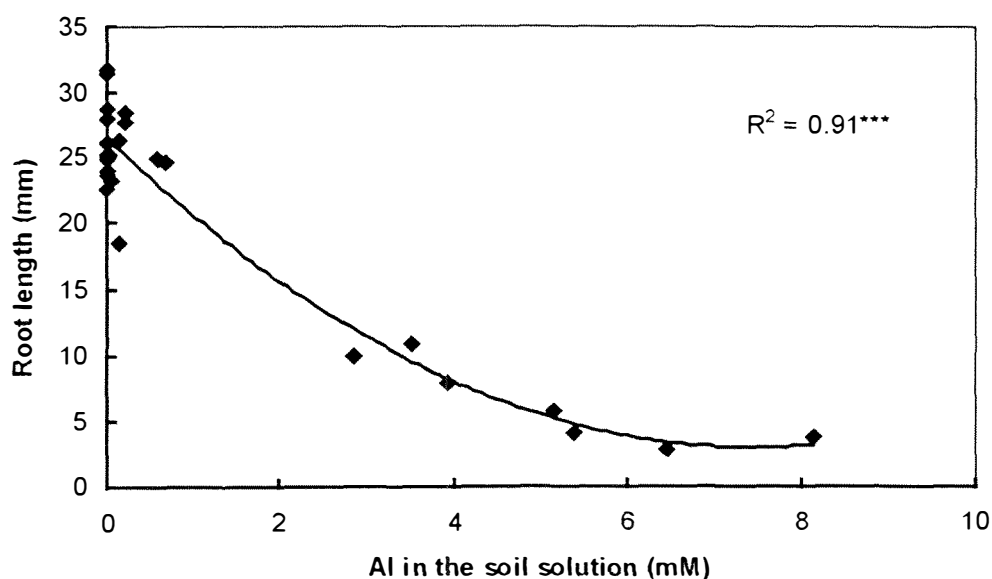
**Table 2.4.** Reported critical Al concentrations above which rice growth was affected in solution culture experiments. Updated from Coronel *et al.* (1990).

Sol'n Al conc. (mM)	Sol'n salt conc. (mM)	Variety	Authors
0.037	0	British Guiana var. 6047	Cate and Sukhai (1964)
3.71	9.2	Peta	Tanaka and Navasero (1966)
0.002	1.4	Puang ngeon	Thawornwong and van Diest (1974)
1.11	9	screening level for 830 varieties	Howeler and Cadavid (1976)
2.22	12.6	110 varieties	Konzak <i>et al.</i> (1976)
0.74	1.1	7 varieties	Martinez (1976)
0.74	7	Al-sensitive IPEACO 162	Fageria <i>et al.</i> (1988c)
2.22		Al-tolerant Fernandes	
0.07		Al-sensitive Cica4 and OS4	Coronel <i>et al.</i> (1990)
0.37		Al-tolerant IAC3	
0.74			de Paula Ferreira <i>et al.</i> (1998)

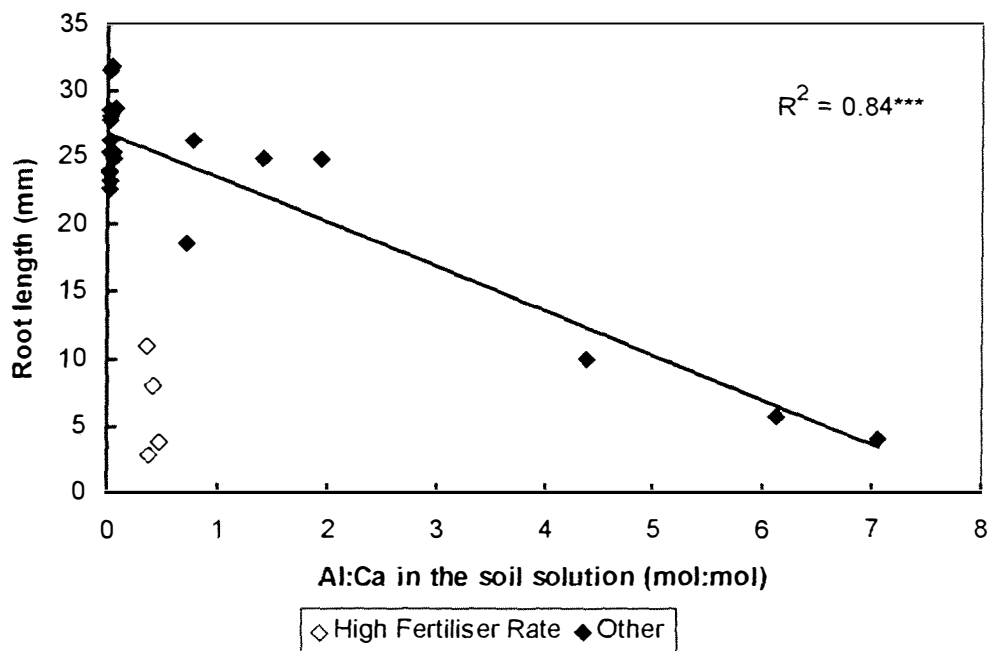
**Figure 2.8.** The relationship between the concentration of Al extracted in 0.02 M CaCl<sub>2</sub> (as measured by PCV) and root length of rice grown in Wharekohe subsoil. Data are from Experiments IIb and III and are means of four root lengths. \*\*\* The R<sup>2</sup> is significant at P<0.001.

c) *The use of the Al:Ca ratio as an indicator of Al toxicity*

A high  $R^2$  value (0.84) was also obtained for the regression of reduction in root length against the molar Al:Ca ratio in the soil solution (Figure 2.10). The Al:Ca ratio described well the barley root elongation data of Manoharan *et al.* (1996) (Al:Ca activity ratio), and the root elongation of lucerne (Wang *et al.* 1999) (molar Al:Ca ratio). However, the molar Al:Ca ratio was not a good indicator of Al toxicity in soil solutions from treatments that had a very high Ca concentration (high urea and high  $\text{Ca}(\text{NO}_3)_2$  treatments, Figure 2.10), due to the application of high rates of soluble Ca-containing fertiliser. The data in Figure 2.10 show a critical Al:Ca ratio of approximately 3 for the Al-tolerant cultivar Azucena. This is much higher than the critical ratio of  $\ll 0.1$  obtained for lucerne (Wang *et al.* 1999) and indicates that rice is much more tolerant to Al than lucerne; this is also suggested by the poor root growth of lucerne in this study.



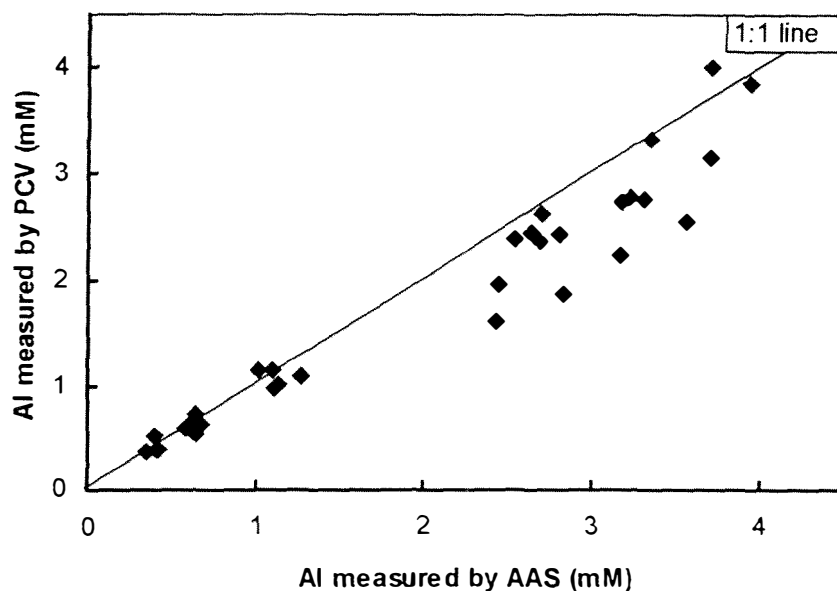
**Figure 2.9.** The relationship between the concentration of Al in soil solution (as measured by PCV) and root length of rice grown in Wharekohe subsoil. Data are from Experiments IIb and III and are means of four root lengths. \*\*\* The  $R^2$  is significant at  $P < 0.001$ .



**Figure 2.10** The relationship between the  $Al_{PCV}:Ca$  ratio in the soil solution and root length of rice grown in Wharekohe subsoil. Data are from Experiments IIb and III and are means of four root lengths. The treatments with high fertiliser rates (high urea and high  $Ca(NO_3)_2$ ) are shown separately from the other treatments and have been omitted from the regression analysis. \*\*\* The  $R^2$  is significant at  $P < 0.001$ .

*d) Comparing Al measurement methods: PCV and AAS*

There was a small but highly significant ( $P < 0.0001$ ) difference in measured Al concentration between the PCV and AAS methods (Figure 2.11). The correlation between the two methods was 0.97. The PCV method measured, on average, 12% less Al than AAS.



**Figure 2.11.** The relationship between Al measured by AAS by spectrophotometric analysis of Al reacted with PCV. Data are from Experiments IIb, III and IV.

There was a highly significant ( $P < 0.01$ ) measurement technique by treatment interaction. Most treatments showed a good agreement between the Al concentration measured by AAS and that measured by PCV, except for higher pH ( $\text{pH}_{\text{H}_2\text{O}} > 4.25$ ) unfertilised Wharekohe subsoils, and MCP or urea fertilised treatments. For these treatments PCV gave a lower measured Al concentration than AAS (Table 2.3). The lower Al concentration measured by PCV is probably because there was more organic matter present in solution in these treatments. Kerven *et al.* (1989) found that a smaller proportion of the total Al in solution reacts with PCV when the concentration of organically complexed Al is high. In contrast, AAS does not distinguish between inorganic Al in solution and organically complexed Al. Fan and MacKenzie (1993) found that the concentration of soil organic matter in the soil solution was increased by the addition of urea and/or triple superphosphate.

The close agreement between the measurements made by PCV and AAS indicates that there was little organically complexed Al in Wharekohe subsoil. Therefore no calibration or adjustment was necessary to account for organically complexed Al, as written in the

method of Kerven *et al.* (1989). The very low total C readings of the subsoils (Table 2.1) also suggest that the amount of organically complexed Al in solution is likely to be very small.

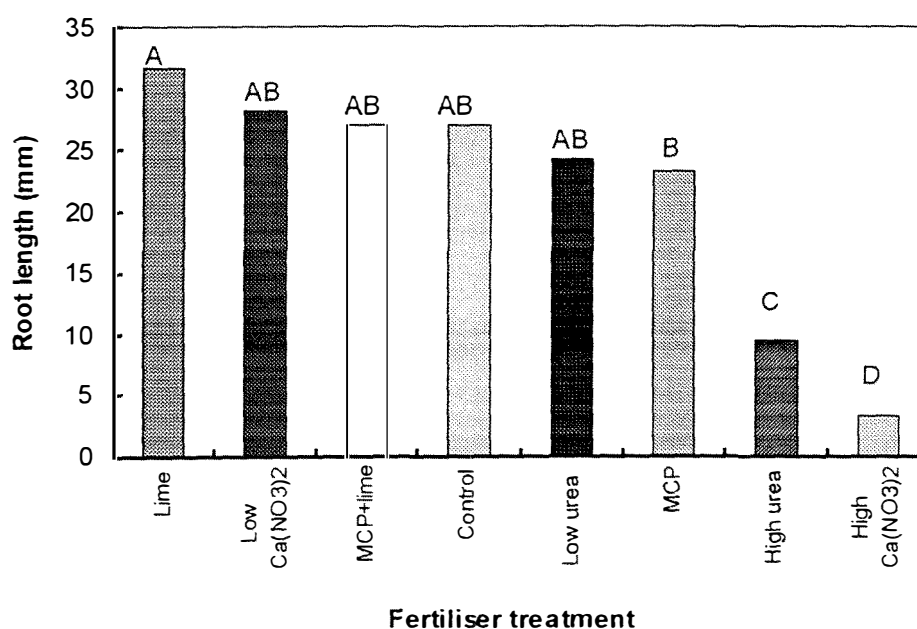
Considering the advantages of each measurement technique, AAS is quick (each analysis takes only a few seconds compared with the 1 min reading time required for the PCV method of Kerven *et al.* (1989)), it requires less sample (approximately 2 mL whereas the PCV method requires 3 mL), and the standard curve covers a much wider range (20–4070  $\mu\text{M}$  for the 396.2 nm wavelength, compared with 0.3 to 20  $\mu\text{M}$  for PCV). However these ranges highlight the fact that the PCV method is able to measure lower concentrations than the AAS. The greater sensitivity would be important for measuring solution Al values for Al-sensitive species and varieties; e.g. Thawornwong and van Diest (1974) reported that a solution concentration of only 1.9  $\mu\text{M}$  Al markedly affected the growth of rice cultivar Puang ngeon. Therefore, for Al-tolerant plants, the faster AAS measuring technique would be more suitable, but for Al sensitive plants, the more sensitive PCV method is recommended.

### **2.4.3 Experiment III: The effect of fertiliser amendment on soil Al concentration and root elongation**

#### *a) Rice root elongation*

Except at very high rates of N, fertiliser addition had no significant effect on rice root elongation, although there was some indication (significant with DMRT but not Scheffe's Test) that roots were longer in the liming treatment than with MCP alone (Figure 2.12). The longer root length in the liming treatment might have been due to the combined effect of increased pH and Ca supply. The liming treatment had a pH of 5.19, compared with 4.43 in the MCP treatment (Table 2.3). Increasing the pH would have reduced the  $\text{Al}^{3+}$  activity (Figure 1.1). The low concentrations of Al measured in the MCP and lime treatments (3–6  $\mu\text{M}$ ) might slightly depress root extension, as they are still greater than 2  $\mu\text{M}$ , which was found to limit root growth in the cultivar Puang ngeon (Thawornwong and van

Diest 1974). The supply of Ca might also have been important because the control soil had a low  $\text{Ca}^{2+}$  concentration (Table 2.3). Bruce *et al.* (1988) found a large decrease in relative root length of soybeans in soils where the concentration of  $\text{Ca}^{2+}$  in the soil solution was below approximately  $50 \mu\text{M}$ . However Tanaka and Tadano (1973) found no Ca deficiency symptoms in rice until the solution Ca concentration dropped below  $25 \mu\text{M}$ , however high solution concentrations of Al interfere with Ca uptake (Section 1.3.1.3).



**Figure 2.12.** Root length of 42-h-old rice seedlings grown in Wharekohe silt loam subsoil (36–53 cm depth) amended with different fertilisers. Treatments denoted by a different letter are significantly different as tested by DMRT at  $P < 0.05$ .

The high rates of soluble fertiliser used in the high urea and high  $\text{Ca}(\text{NO}_3)_2$  treatments resulted in much higher soil solution conductivities than the other treatments ( $4\text{--}6 \text{ mS cm}^{-1}$ ) (Table 2.3). The high concentration of ions in solution appears to have displaced monomeric Al and  $\text{H}^+$  ions into solution (Table 2.3) causing a distinct, highly significant depression of root elongation in the high N treatments (Figure 2.12). The concentrations of soil solution Al in these high N treatments (Table 2.3) were ten times the critical limit

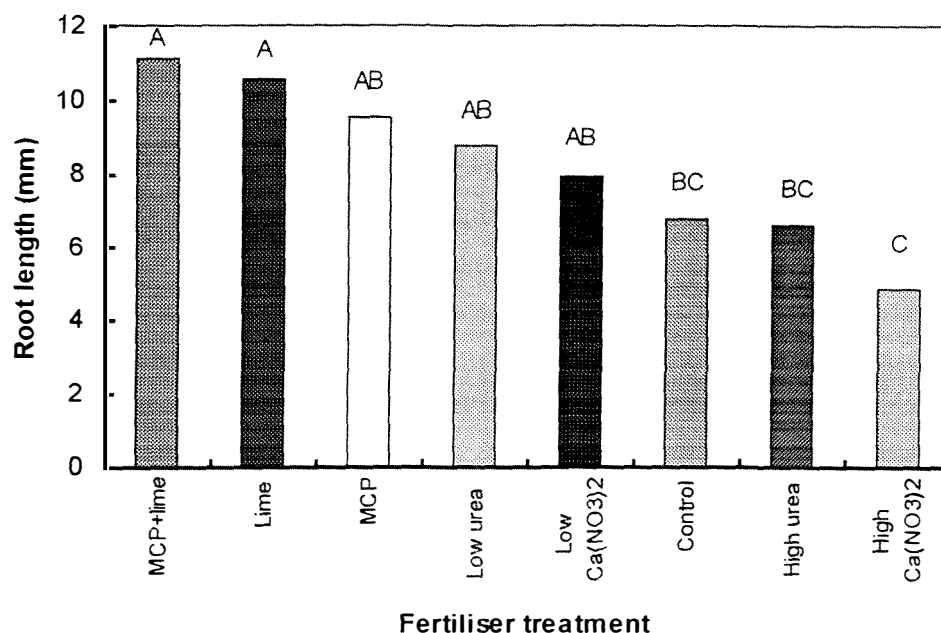
of 0.37 mM Al cited by Coronel *et al.* (1990) for Al-tolerant rice grown in nutrient solution. This scenario has also been observed in the field. Singh *et al.* (1985) stated that the application of N fertiliser alone appeared to aggravate Al toxicity causing reduced yields of floating rice grown in an acid sulphate soil. These results highlight the fact that high-analysis N fertiliser should not be placed in high concentration near the seed in acidic soils, but should be mixed through an adequate volume of soil or else incorporated after liming the soil to avoid exacerbating Al toxicity.

As mentioned in Section 2.4.2.2c, the  $Al_{PCV}:Ca$  ratio was a poor predictor of Al toxicity when high amounts of readily-soluble Ca fertiliser were applied (Figure 2.10). This is because the high application of soluble Ca (as  $CaCl_2$  or  $Ca(NO_3)_2$ ) markedly increased the soil solution Ca concentration while the concentration of Al in solution remained high (Table 2.3), hence the  $Al_{PCV}:Ca$  ratio was greatly decreased. This suggests that the solution Al:Ca ratio should be treated with caution as an indicator of Al toxicity when concentrations of Ca in solution are unusually high (> 9 mM).

#### *b) Lucerne root elongation*

The results for lucerne were not as clear as for rice, probably because lucerne root elongation was inhibited in all treatments in these acidic soils (see Section 2.4.2.2a), but they did show the same trends (Figure 2.13). Analysis of variance showed that there were significant differences between the treatments at  $P \leq 0.01$ . Duncan's Multiple Range Test showed that root lengths in the control, and both high N treatments were significantly shorter than the MCP+lime treatment, and that the high  $Ca(NO_3)_2$  treatment was significantly lower than all of the fertilised treatments, except the high urea treatment.





**Figure 2.13.** Root lengths of 42-h-old lucerne grown in Wharekohe silt loam subsoil (36–53 cm depth) amended with different fertilisers. Treatments denoted by a different letter are significantly different as tested by DMRT at  $P < 0.05$ .

#### **2.4.4 Experiment IV: Use of the bioassay to evaluate root growth in an Ultisol from Cavinti, Philippines**

Rice root length (elongation) in Cavinti soil (18.4 mm) was significantly lower than in the 36–53 cm depth of the Wharekohe subsoil (26.7 mm), but not significantly different to the 96–114 cm Wharekohe depth (24.3 mm). The decrease in root elongation was not due to a high concentration of Al in solution but was probably due to the very low solution Ca concentration (Table 2.3). The measured Ca concentration of 21  $\mu\text{M}$  in the soil solution of Cavinti soil is below the critical concentration of 25  $\mu\text{M}$  for Ca deficiency in rice as determined by Tanaka and Tadano (1973).

## **2.4.5 General discussion**

### **2.4.5.1 Evaluation of the bioassay technique**

The root elongation bioassay technique used was similar to that of Karr *et al.* (1983) and Ahlrichs *et al.* (1990). Ahlrichs *et al.* (1990) made no mention of the effects of bulk density on the assay, although they used a wide range of soils. Experiment 1 indicates that, in order for the observed differences to be due to the effects of soil chemistry alone, it is important to pack the soil in each pot to the same bulk density. Another preliminary study (data not shown) indicated the importance of maintaining an identical moisture content, as well as bulk density, in each pot.

The root elongation bioassay was quick to perform, the slowest part being the placing of the seeds in holes in the soil, as it was easy to damage the emerging root or plumule. A subsequent modification involved using a larger pot (100 cm<sup>3</sup>). The seeds were then simply laid on the top of the soil then covered with a small amount of air-dried soil, which was then wet up to the same moisture content as the rest of the pot. This modified technique was much faster and was less likely to damage the germinating seeds.

Using the root elongation bioassay to evaluate different soil chemical constraints is simple and inexpensive. It is useful for comparing different treatments on the same soil type. However comparisons across different soil types are valid only when packed to the same bulk density and moisture tension. The root bioassay technique may be a useful field technique to quickly and cheaply assess the lime requirement for a soil. The soil could be incubated with different amounts of lime in order to ascertain how much is needed for adequate root elongation. A bioassay does not, however, answer the question as to why the soil limits root elongation.

Further testing would need to be carried out in order to ascertain whether the observed trends in root elongation were also reflected in the root growth and subsequent yields of older plants. The literature suggests that such a bioassay should be successful. Seedlings

appear to be the most sensitive indicator of Al toxicity, as Coronel *et al.* (1990) found that 2-wk-old rice seedlings were more sensitive to Al than 4-wk-old seedlings. Furthermore, research by Howeler and Cadavid (1976) has shown a good correlation between the amount of reduction in root length in Al-rich solution culture and rice grain yields of the same cultivars grown in Al-rich soil in the field.

#### **2.4.5.2 The use of high rates of N fertiliser at sowing**

The reduction in root elongation in the high N fertiliser treatments, highlights the importance of using smaller, split applications, as opposed to a single, heavy application of N fertiliser. Splitting the N application also reduces leaching losses and ensures adequate N is present in the soil when plant demands increase at booting. Deep placement of urea supergranules is recommended for rainfed rice in some situations, as is banding of N fertilisers near seed rows for direct-seeded rice (Mohanty *et al.* 1999). The results above emphasize the importance of allowing some distance between the band and the seed. These problems are avoided with the recent development of controlled-release urea (urea coated with a polymer which allows for the slow release of N), which means that the N fertiliser can be placed with the seeds without salt injury to seeds and roots (Mohanty *et al.* 1999). High rates of soluble N fertiliser should not be banded near roots or together with P in potentially Al-toxic soils, since exploration of the P-rich band by roots is important for P uptake.

## **2.5 Conclusions**

The pH and concentrations of exchangeable basic cations and Al of the Wharekohe silt loam subsoil depths were similar to those of the highly weathered Philippine Ultisol used in later chapters. The Wharekohe subsoil was therefore a suitable surrogate for evaluating rice root elongation as a bioassay for Al toxicity. Aluminium did not limit rice root elongation in the Wharekohe silt loam, unless the soil was acidified or high rates of N fertiliser were applied. Acidification of Wharekohe soil caused an exponential increase in both soil solution and CaCl<sub>2</sub>-extractable Al at a pH below 4.0–4.25. Aluminium did not limit root

growth in the highly weathered soil from Cavinti, Philippines. However the Cavinti soil contained a very low concentration of Ca, which appears to be the reason for the significantly shorter rice root length compared with the Wharekohe subsoil.

The concentration of Al in the soil solution, as extracted by centrifugation, showed a strong linear relationship with root length, in contrast to Al extracted by 0.02 M CaCl<sub>2</sub>. The critical concentration of Al measured in the soil solution, above which root elongation was depressed (0.7–2.9 mM), agreed well with critical concentrations found by the majority of solution culture studies. Centrifugation is therefore preferred to 0.02 M CaCl<sub>2</sub> as a means of extracting phytotoxic Al. The molar ratio of Al:Ca in the soil solution was also a good predictor of Al-toxicity in the Wharekohe subsoil, except where high rates of soluble Ca fertiliser had been added to the soil (soil solution Ca concentrations > 9 mM). Further experiments should be conducted to evaluate Al<sub>PCV</sub> and the Al<sub>PCV</sub>:Ca ratio as predictors of Al toxicity using a range of soil types and rice cultivars.

The difference between AAS and PCV in their measured Al concentration was small (12%) in these low organic matter soil depths, though statistically significant. Thus both methods worked equally well to identify Al toxic soils.

The bioassay provides a quick and simple method for determining the toxicity of a soil to root growth, and a basis to compare the two methods of measuring phytotoxic Al.

High rates of N fertiliser (equivalent to 140 kg N ha<sup>-1</sup> in the top 5 cm of soil) were detrimental to root growth – increasing the solution ionic strength and causing strong acidification, which led to Al toxicity in the Wharekohe subsoil. Therefore high rates of soluble fertiliser N should be split into several dressings in potentially Al-toxic soils. Furthermore, high rates of soluble N fertiliser should not be banded near roots or together with P fertiliser in potentially Al-toxic soils, since exploration of the P-rich band by roots is important for P uptake.

### 3. Phosphate fertiliser management for rice in aerobic soil I. Interactions between phosphate rock and organic amendments

#### 3.1 Introduction

Soil acidity and N and P deficiency are major factors constraining aerobic rice production in the tropics and subtropics (Chapter 1). Preliminary studies at Massey University were designed to investigate methods of studying these factors, prior to more detailed study at **IRRI** in the Philippines. In Chapter 2 the main problem associated with soil acidity – Al toxicity – was investigated. Chapter 3 now focuses on the provision of nutrients, particularly N and P.

The strategy recommended by von Uexküll and Mutert (1995) to supply these key growth-limiting nutrients to highly-weathered acidic tropical soils is to use RPR (or its equivalent as triple superphosphate and lime), and a leguminous green manure. Reactive phosphate rocks have a number of advantages over other P fertilisers: they are generally cheaper per unit P (Sánchez and Uehara 1980), they have a higher P content than superphosphate (which reduces transport costs), and they have a liming effect upon dissolution (Sinclair *et al.* 1993, Loganathan *et al.* 1995). Possible disadvantages to using RPRs is that they are only suitable for use on soils with a pH of < 5.5 (Apthorp *et al.* 1987) (although most highly weathered tropical soils have a pH well below this). Phosphate rocks also need to be reactive (solubility in 2% citric acid of > 30%) and very finely ground (< 150 µm diameter) to ensure that they release P quickly to meet the needs of the establishing crop (Bolan *et al.* 1990).

There are also a number of advantages in using green manures as a source of N: they require a minimal amount of capital, seed supplies can be maintained by the farmer without needing to rely on a distribution network, they may increase soil moisture retention, and reduce soil bulk density and compaction (Wade 1978, as cited by Sánchez and Salinas

1981). The decomposition of green manures may lime acidic upland soils (Section 1.4.2.1), and can detoxify phytotoxic  $Al^{3+}$ .

The decomposition of organic matter to produce organic acids has been shown to reduce Al toxicity, which is a common problem in acidic soils. Wong and Swift (1995) found that the addition of humic and fulvic acids to soil decreased the activity of  $Al^{3+}$  in an Oxisol and an Ultisol. Berek *et al.* (1995) found that the incubation of leaves from three leguminous species with a red-yellow podzolic soil from Indonesia markedly decreased the concentration of monomeric Al, resulting in significant increases in soybean growth. In contrast, rice straw, sugarcane leaves, and *Imperata* tops had a much smaller effect on soil monomeric Al concentration.

Disadvantages of the use of green manures include the opportunity cost of growing a crop with no observable economic return, and the labour cost of incorporating the green manure crop. If the crop is grown offsite, disadvantages include transportation and application costs, and the depletion of nutrients at the site of green manure production. Furthermore, they may harbour pests or disease, especially if used as mulch. These disadvantages may discourage farmers from using green manures (Phiri *et al.* 1994), unless the benefits considerably outweigh the costs.

There are additional benefits in using green manures in combination with RPR. The application of a large amount of organic matter and/or P fertiliser may increase the size of the microbial (McLaughlin and Alston 1986, Parfitt *et al.* 1994) or organic P (Zhang *et al.* 1999) pools, where P is temporarily protected from soil fixation and may become available to the plant through mineralisation. Research by Perrott *et al.* (1990) and Kouno *et al.* (1994) suggests that microbial biomass can be a significant source of P to plants. Similarly Harrison (1987), in his review on soil organic P, states that “the vast majority of the evidence indicates that it [organically-bound P] is important in plant nutrition”. However three studies cited by Harrison (1987) as well as Hedley *et al.* (1994) and Crews (1995) suggest that organic P is of little or no significance. For example, Hedley *et al.* (1994) observed no depletion of organic P in the rhizosphere of upland rice grown in a high P-fixing Ultisol, although no organic amendments were used in this study. To the author’s

knowledge there have been no rhizosphere studies to determine what happens to the organic P fraction when a large amount of organic material has been added to the soil. Therefore, it is also of interest to measure the distribution of P amongst the organic and microbial P pools following the addition of a large amount of organic matter to soil, and to determine whether significant plant P supply is derived from organic P in a soil amended with organic materials.

Some authors have suggested that an additional benefit of using green manures in combination with RPRs is that they might help to solubilise P rocks (Tian and Kolawole 1999). When green manures and RPRs are incorporated together, there are several mechanisms by which organic manures can influence the dissolution of RPRs:

- ⇒ The production of  $\text{NH}_4^+$  during organic matter decomposition consumes protons (van Breeman *et al.* 1983) which would raise the pH and slow dissolution of RPRs. However in the rhizosphere, plant uptake of  $\text{NH}_4^+$  would cause plant roots to release acid (Gahoonia and Nielsen 1992) and this would promote the dissolution of RPR. Similarly, nitrification produces acid, which would also enhance RPR dissolution (Apthorp *et al.* 1987, Barbarick *et al.* 1990). (Proton production/consumption processes in the soil system are listed in Table 1.2).
- ⇒ The addition of organic matter stimulates microbial activity, some species of which can solubilise RPRs (Kucey *et al.* 1989, Narsian *et al.* 1994, Whitelaw 2000).
- ⇒ Organic manures high in Ca may reduce the dissolution of RPR (Smithson 1999).
- ⇒ Organic acids produced during decomposition may accelerate the dissolution of RPR by reducing the soil Ca concentration, through chelation of Ca (Drake 1964, Chein 1979). Organic acids may also reduce the soil  $\text{H}_2\text{PO}_4^-$  concentration, through the formation of organic acid-Al/Fe-P complexes, although the soil solution  $\text{H}_2\text{PO}_4^-$  concentration is generally much lower than the solution Ca concentration, and therefore less likely to be limiting RPR dissolution.

The overall effect of organic manure (OM) on RPR dissolution is therefore not clearcut: this list indicates that some factors promote dissolution, and others reduce it. Zaharah and Bah (1997) stated that “It has been established that organic animal manures generally enhance the utilization of P fertilizers (Singh *et al.* 1993). However, there is little

information on the use of green manures in improving the solubility of phosphate rocks.” In the study of Zaharah and Bah (1997) however, RPR dissolution was not measured, but rather plant P uptake was used as an indicator of P dissolution. A P fractionation of green manure/RPR/soil mixtures would enable direct measurement of the amount of P released and the amount of undissolved RPR remaining in the soil. Also, to better understand the effects of different organic materials on RPR dissolution, it is important to measure soil pH, as the decomposition of organic materials can have a large effect on soil pH (Section 1.3.1.1). Zaharah and Bah (1997) did not measure soil pH, despite the fact that there was almost a two-fold difference in the N concentration in the green manures. Moreover, it is important to measure the pH of rhizosphere soil, as rhizosphere pH may differ markedly from bulk soil pH and accelerate RPR dissolution (Trollove *et al.* 1996, Zoysa *et al.* 1999). Phosphorus fractionation and rhizosphere pH measurements are therefore necessary to provide direct evidence about whether green manures can enhance RPR dissolution.

## 3.2 Objectives

The objectives of this experiment are twofold:

- i) to determine the overall effect of the addition of green manure in combination with RPR on the dissolution of RPR, and the resulting growth of upland rice;
- ii) to determine by chemical fractionation and chloroform fumigation, the fate of P released from RPR and green manure in the soil.

## 3.3 Materials and methods

### 3.3.1 Soil

The soil used was a 40:60 mix (by weight) of 2 mm sieved Wharekohe silt loam topsoil and subsoil (28–43 cm depth) (for important soil chemical properties see Table 2.1). This soil mix approximates the relatively common scenario where a tropical soil has lost most of its topsoil due to erosion after forest clearing, or through poor husbandry (von Uexküll and Mutert 1995).



### 3.3.2 Amendments

#### 3.3.2.1 Phosphate rock

The phosphate rock used for the experiment was ‘as received’ Sechura reactive phosphate rock from Peru, which is one of the most reactive commercially available phosphate rocks used in New Zealand (13.7% P, 5.3% soluble in 2% citric acid, < 0.01% soluble in water, Mackay *et al.* 1984). The Sechura RPR was sieved to < 250  $\mu\text{m}$  particle size before use (yield 97%).

#### 3.3.2.2 Organic manures

Tree lucerne (*Chamaecytisus palmensis*) leaves and wheat (*Triticum aestivum*) straw were dried to a constant weight at 45 and 60°C, respectively. The organic amendments were then ground to less than 1 mm. The concentrations of N and P in the organic matter (Table 3.1) were determined by Kjeldahl digestion (Twine and Williams 1971), and K, Ca and Mg by atomic absorption (Ca and Mg) or emission (K) spectroscopy following digestion in nitric acid. The ash alkalinity of each amendment was determined by ashing 500 mg samples at 550°C for 3 h, then adding excess (50 mL) of standardised 0.1 M HCl for 1 h. The supernatant was filtered (Whatman #42), and a 10 mL aliquot was back-titrated against standardised 0.1 M NaOH.

**Table 3.1.** Content of major nutrients in the organic amendments.

	N	P	K	Ca	Mg	C	C:N	Ash alkalinity
	mg g <sup>-1</sup> DM					%		cmol OH <sup>-</sup> /kg <sup>-1</sup> DM
Wheat straw	5.3	0.9	9.1	2.4	0.6	46	87	3.5
Tree lucerne	35.2	2.1	14.4	10.8	1.4	48	13	16.4

#### 3.3.3 Treatments

The soil was amended with organic materials and RPR in a two-factor design (Table 3.2). The organic amendments were added at a rate of 32 mg DM g<sup>-1</sup> soil ( $\equiv$  9 t DM ha<sup>-1</sup>, assuming that the plants could only access nutrients from the upper cell, see Figure 3.2). In

the tree lucerne treatment this equated to  $1.1 \text{ mg N g}^{-1} \text{ soil}$  ( $320 \text{ kg N ha}^{-1}$ ), which is about the maximum amount of N that could be expected to accumulate in a single leguminous crop (Meelu *et al.* 1994). The RPR was added at a rate of  $200 \text{ } \mu\text{g P g}^{-1} \text{ soil}$  ( $57 \text{ kg P ha}^{-1}$ ). There were four replicates of each treatment.

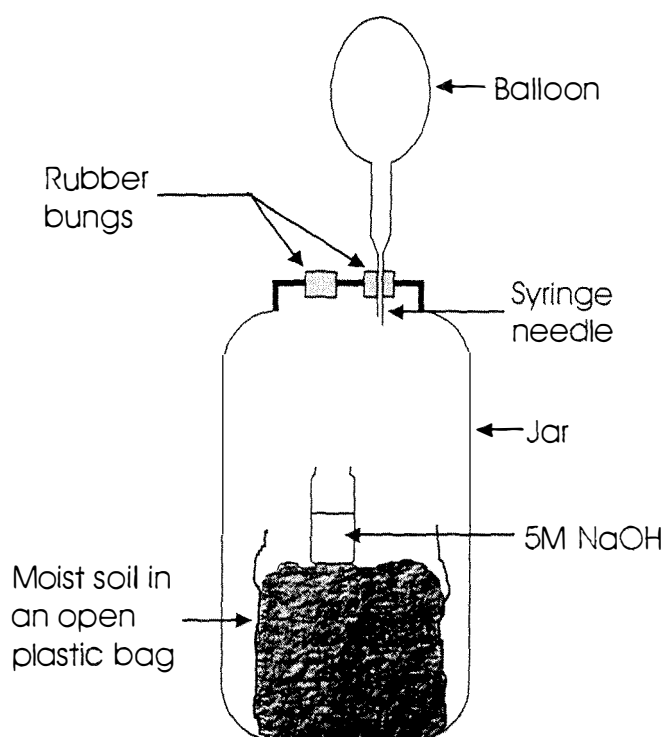
**Table 3.2.** The soil treatments.

Symbol	Treatment
Con.-P	Soil, no organic amendment, no RPR
Con.+P	Soil, no organic amendment, + RPR
TL-P	Soil + tree lucerne leaves, no RPR
TL+P	Soil + tree lucerne leaves + RPR
WS-P	Soil + wheat straw, no RPR
WS+P	Soil + wheat straw + RPR

### 3.3.4 Incubation study

The soil treatments described above were wet up to 43%<sup>†</sup> moisture and incubated in an aerobic atmosphere in sealed jars at 27°C for 4 wk (Figure 3.1), using the method of Jerez *et al.* (1988). A small balloon was filled with a small amount of air until just turgid; to monitor the amount of oxygen required. When the balloon became flaccid additional O<sub>2</sub> gas was injected into the jar through a rubber bung. Carbon dioxide was collected in a NaOH trap and the amount of CO<sub>2</sub> respired determined by titration every 1 to 8 d. Control jars, containing no soil, were used to correct for the background CO<sub>2</sub> content of the air in the jars.

<sup>†</sup> all % moisture calculations in this thesis are in g water g<sup>-1</sup> oven-dry soil

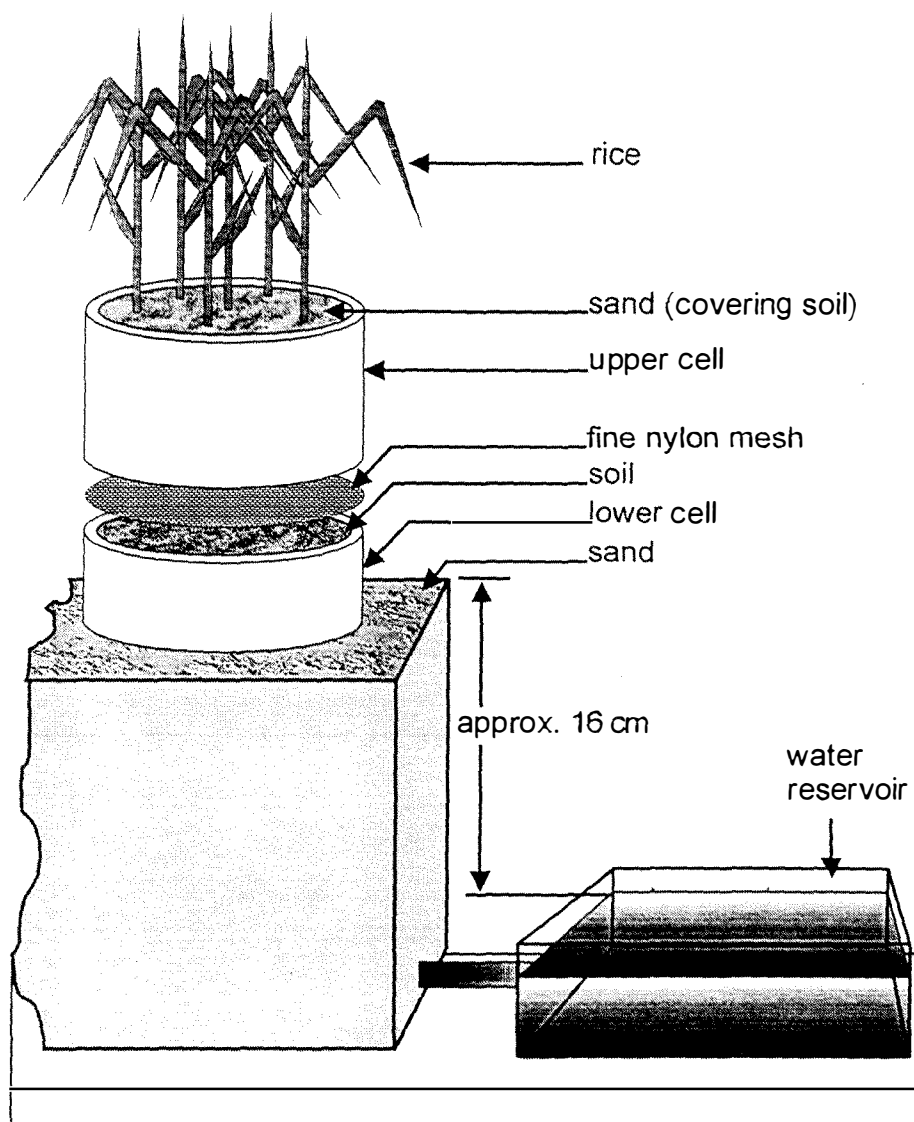


**Figure 3.1.** The incubation system for measuring microbial respiration (after Jerez 1988).

### 3.3.5 Rice growth experiment

After 6 wk the soil was air-dried, then wet up for approximately 4 d, then air-dried again, which simulates wetting and drying cycles in the field. It was then packed into both upper and lower cells of Root Study Containers (RSCs) (Figure 3.2) to a bulk density of  $0.95 \text{ kg air-dried soil dm}^{-3}$ . Root study containers were used in order to measure soil pH and P depletion in the rhizosphere (see Hedley *et al.* 1994). Briefly, a RSC consists of a vertical, soil-filled PVC tube that is separated into two sections (top section 82.5 mm diameter by 30 mm deep, basal section 75 mm diameter by 25 mm deep) by a fine nylon mesh. Plants are grown in the top section. The roots of these plants are unable to penetrate the fine nylon mesh so form a dense root mat over the mesh. The soil immediately below the mesh (at the top of the lower cell) is therefore rhizosphere soil.

The RSCs were placed on moist sand for 5 d to wet up before planting. However, because the soil had become very dry (only 2.5% moisture) it was hydrophobic, so additional water was added from above during planting. Pre-germinated seeds of a traditional rice cultivar (Azucena) were sown on the 14<sup>th</sup> of November 1996 at a rate of seven seedlings per pot. Four of the six replicates were planted; the remaining two were unplanted. Plants were grown under plastic (in order to maintain a humid environment) in a glasshouse with an average max:min temperature regime of approximately 35°C:15°C. The RSCs were placed on moist sand and additional water supplied twice daily (pots were watered every morning



**Figure 3.2.** The experimental system showing a root study container (exploded view) on a moist sand bath.

to 43% by weight) to prevent the pots from drying out. Plants were then removed, or more seedlings of the same age were planted, to maintain 6 plants per RSC.

Twenty-five days after sowing it was observed that some roots were growing out of the bottom of the RSCs into the sand. The reason why the roots had grown through the mesh was because the incorrect nylon mesh had been supplied (pore size 60  $\mu\text{m}$  instead of 24  $\mu\text{m}$ ). It was decided to harvest the pots in the hope that it might still be possible to observe rhizosphere effects before root proliferation became too great in the lower cell. No root mat had formed in the control or wheat straw treatments, but a loose root mat had developed over the mesh in the tree lucerne treatments. The RSCs were taken apart and the lower section was placed in a piston microtome and 6 thin slices were cut from the soil adjacent to the root mesh. The first four slices were 0.5 mm thick and the remaining two were 3 mm thick. Shoots were oven-dried at 68°C for 2 d.

### **3.3.6 Soil analysis**

#### **3.3.6.1 Ammonium and nitrate**

Soil samples were taken from the entire depth of the upper cell using a cork borer, 18 d after planting. These samples were immediately extracted at a 1:2 soil:solution ratio with 0.02 M  $\text{CaCl}_2$  by shaking end-over-end for 1 h. The samples were then centrifuged at 9,770 g for 5 min, filtered (Whatman #42) and analysed by autoanalyser for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (adapted from Blakemore *et al.* 1987).

#### **3.3.6.2 Soil pH and P fractionation**

The pH of the thin slices of soil from two planted replicates of each treatment was measured at a 1:5 soil:water ratio and a phosphate fractionation (Table 3.3), designed to measure RPR dissolution (Tambunan *et al.* 1993), was also conducted on these soils. The procedure for the P fractionation was as follows. The soil was ground with a mortar and pestle, then a 0.75 g soil sample was shaken end-over-end for 30 min with 30 mL of a 0.5 M NaCl + 1 M tri-ethanol amine (TEA) solution adjusted to pH 7.0 with 2 M HCl. This step was included to remove soluble and exchangeable Ca and thus avoid available P

forming calcium phosphates forming during the Olsen extraction (Tambunan *et al* 1993). The extracts were centrifuged (7,700 *g* was used throughout the fractionation procedure), filtered and analysed for P. The method of Murphy and Riley (1962) was used to determine reactive orthophosphate in all extracts.

The soil pellet was resuspended and shaken end-over-end for 30 min with 15 mL of 0.5 M HCO<sub>3</sub> adjusted to pH 8.5 (Olsen *et al.* 1954), centrifuged, filtered, and the filtrate analysed for P. The soil was again shaken end-over-end with 30 mL of 1 M NaOH for 16 h. The solution was then centrifuged, filtered and inorganic P (*P<sub>i</sub>*) was determined using a 2 mL aliquot of the filtrate. A correction was made for colour in the filtrate by subtracting the reading of a solution prepared using Murphy and Riley reagent prepared without the ascorbic acid. Total NaOH-P in the filtrate was determined by digesting a 5 mL aliquot at 280°C with 8 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and repeatedly adding 0.5 mL of 30% H<sub>2</sub>O<sub>2</sub> until the solution was clear. The solutions were made to 50 mL, neutralised, and the P determined as above. Organic P (*P<sub>o</sub>*) was calculated as the difference between total NaOH-P and NaOH-*P<sub>i</sub>*.

**Table 3.3.** A modified P fractionation procedure to measure soil and fertiliser P fractions.

Name	Extractant	Form of P extracted
NaCl/TEA-P	0.5 M NaCl/TEA pH 7.0*	Soil solution and some readily exchangeable P
Olsen P	0.5 M NaHCO <sub>3</sub> pH 8.5#	HCO <sub>3</sub> <sup>-</sup> -exchangeable P
NaOH- <i>P<sub>i</sub></i>	1 M NaOH*	P associated with negatively charged oxide surfaces via exchangeable cations
NaOH- <i>P<sub>o</sub></i>	Digest the 1M NaOH w/ H <sub>2</sub> SO <sub>4</sub> /H <sub>2</sub> O <sub>2</sub> then subtract the 1M NaOH- <i>P<sub>i</sub></i> †	The more labile organic P
0.5 M H <sub>2</sub> SO <sub>4</sub> -P	0.5 M H <sub>2</sub> SO <sub>4</sub> †	Ca bound P + some P held within oxide crystals
Residual P	Concentrated H <sub>2</sub> SO <sub>4</sub> /H <sub>2</sub> O <sub>2</sub> †	Residual P

\* Tambunan *et al.* (1993). #Olsen *et al.* (1954). †Hedley *et al.* (1994).

The soil was again shaken end-over-end for 16 h with 30 mL of 0.5 M H<sub>2</sub>SO<sub>4</sub>, centrifuged, and the supernatant analysed for P to determine the Ca-P fraction (comprised almost entirely of undissolved RPR). The residual P was determined by digesting the soil with H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> as described above.

### 3.3.6.3 Microbial P

Paired 1 g (moist weight) soil samples were taken from the top section of each pot. One of each pair was treated with 2 mL of chloroform while the other remained untreated. Both samples were then left in a fume cupboard overnight to evaporate the chloroform, then extracted the following day by shaking with 20 mL of 0.5 M NaHCO<sub>3</sub> for 30 min, centrifuged (7,700 g for 10 min), filtered (Whatman #5), and analysed for P (Murphy and Riley 1962).

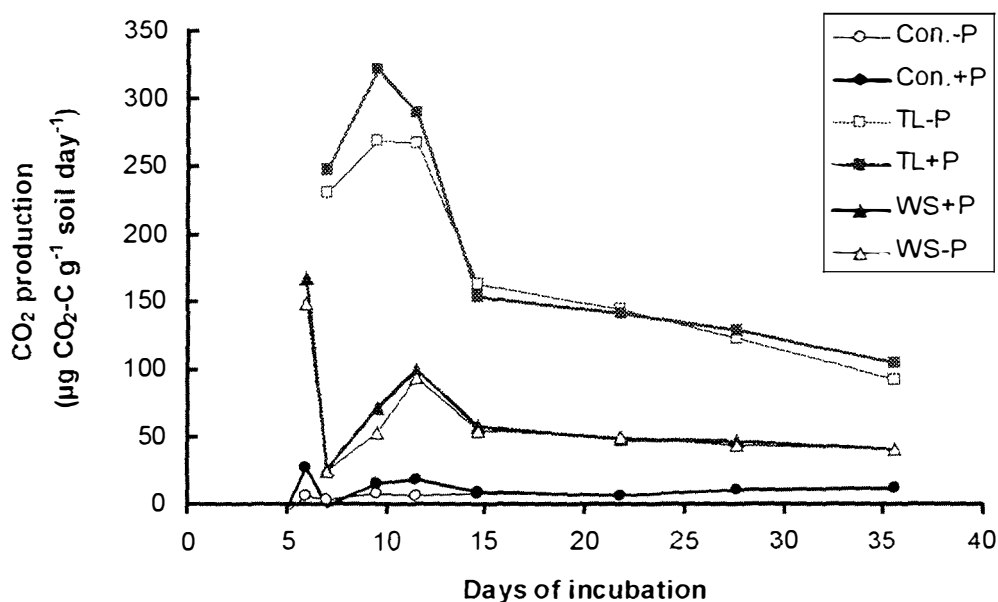
### 3.3.7 Statistics

Unless otherwise stated, the experiments were analysed by ANOVA, with two-factors: organic manure type and  $\pm$ P. For the incubation study, date was treated as a repeated measure, and for the rhizosphere soil pH data distance from the rhizosphere was treated as a repeated measure. The effect of RPR addition on soil pH was analysed separately for each organic amendment by conducting a t-test, with the data paired for each distance from the rhizosphere. For the P fractionation experiment the data were averaged across the six slices, as there was no detectable depletion of any soil P fraction by the roots. The microbial P data were analysed using a t-test (data were paired for each pot) to determine if there was a significant increase in soil P concentration after treatment with chloroform.

## 3.4 Results and discussion

### 3.4.1 Incubation study

The soil respiration rates were highly significantly different between the three organic matter treatments (Figure 3.3). The difference between the wheat straw and the control treatments indicates that C was limiting microbial activity. Higher rates, measured in soil amended with tree lucerne, were associated with a higher nutrient content per unit C (Table 3.1). The addition of P had no significant effect on respiration rates, except on day 12 and day 36, when the addition of P increased respiration. It therefore appears that P was not limiting microbial activity, except at peak respiration rates (near day 12). Note that no CO<sub>2</sub> production data are shown in Figure 3.3 for the first 6 d of the experiment due to a low concentration of NaOH used in the traps and a long time period (5 d) before CO<sub>2</sub> production was measured. Subsequently, the NaOH concentration in the trap was increased to 5 M and CO<sub>2</sub> production measured more frequently (Figure 3.3).



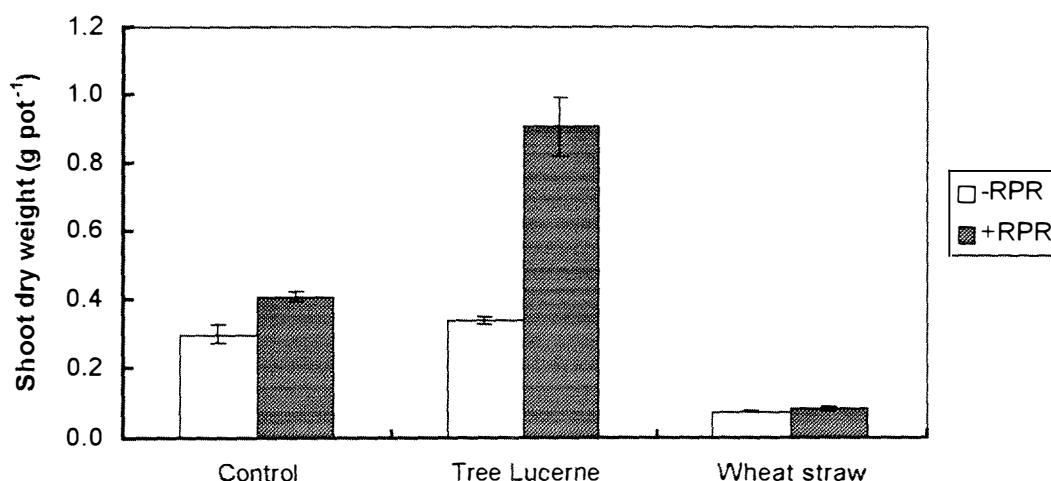
**Figure 3.3.** CO<sub>2</sub> production from soil and organic amendment mixtures in the presence or absence of P (Sechura RPR) incubated at 43% moisture. (See Table 3.2 for treatment key)



### 3.4.2 Rice growth experiment

#### 3.4.2.1 Dry matter production and plant N status

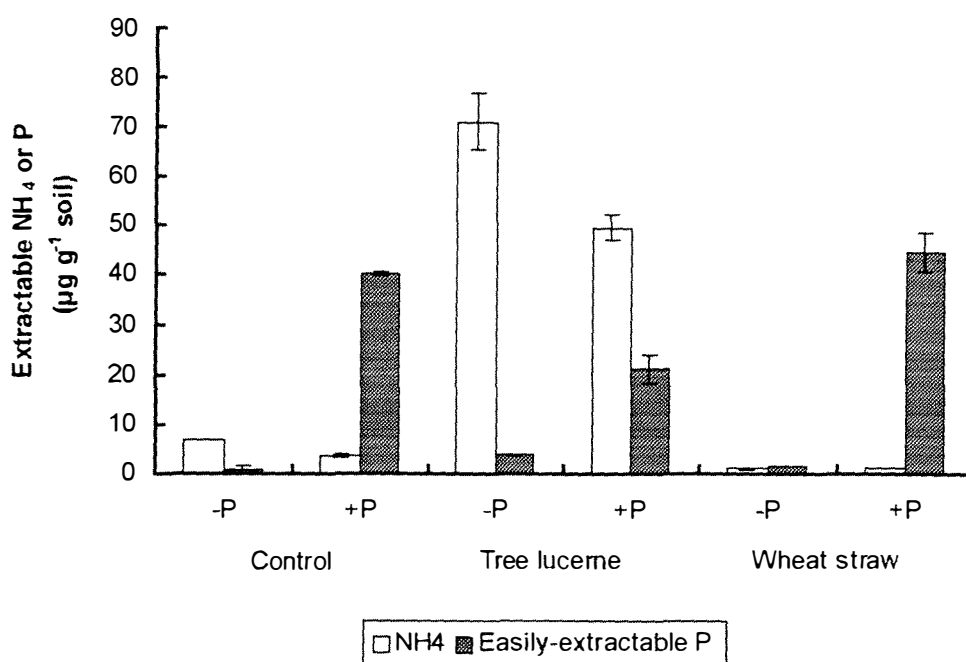
The effects of OM addition, P addition, and the interaction between OM type and P addition, were all highly significant ( $P < 0.0001$ ). Tree lucerne increased rice yields and wheat straw depressed yields relative to the control (Figure 3.4). The addition of P increased yield. The magnitude of the response to P strongly depended upon the different N source or sink effects of the amendments (OM  $\times$  P interaction). Tree lucerne acted as a source of  $\text{NH}_4\text{-N}$ , whereas wheat straw was a sink (Figure 3.5). Rice growth was strongly limited by both N and P – only the TL+P treatment had sufficient N and available P (Figure 3.5) for reasonable growth.



**Figure 3.4.** The effect of different organic matter amendments and RPR on shoot dry matter yields of upland rice after 25 d of growth. Data are means  $\pm$  SE,  $n=4$ .

Rice plants growing in the tree lucerne treatments were dark green in colour, the control treatments were light green, with the Con.+P treatment slightly paler, and the two wheat straw treatments were very pale. Decreasing colour probably reflected decreasing plant N status. Ammonium concentrations in  $\text{CaCl}_2$  extracts of soil decreased in a similar manner to the decrease in leaf colour:  $\text{NH}_4^+$  concentration of tree lucerne  $>$  control  $>$  wheat straw

treatments (Figure 3.5). The lower  $\text{NH}_4$  concentration in the wheat straw treatments is consistent with mineral N immobilisation during the initial stages of wheat straw decomposition. The lower  $\text{NH}_4$  concentration of the RPR-fertilised tree lucerne and control soils compared with their unfertilised counterparts is probably due to the increased growth, and hence  $\text{NH}_4$  uptake, of these treatments.



**Figure 3.5.** 0.02 M  $\text{CaCl}_2$ -extractable soil  $\text{NH}_4$  concentrations 18 d after sowing, and easily-extractable P (NaCl/TEA-P plus Olsen P) 25 d after sowing. NB: soil  $\text{NO}_3^-$  concentrations were negligible ( $< 0.1 \mu\text{g g}^{-1}$  soil). Data are means  $\pm$  SE of four replicates ( $\text{NH}_4$ ) or two replicates (P).

### 3.4.2.2 The ability of organic amendments to supply adequate N and P for the growth of aerobic rice

The large difference in rice growth between the TL-P and TL+P treatment (Figure 3.4) indicates that tree lucerne was able to supply adequate N for the establishment of aerobic rice in Wharekohe silt loam, but that rice was still highly deficient in P. Wheat straw did not supply adequate amounts of N or P to rice, and created a sink for N, shown by the lower yields and lower available nutrient concentrations in the wheat straw treatment compared with the control.

Studies of  $^{15}\text{N}$ -labelled green manures incorporated into aerobic soil have shown that 12–38% of the N in green manures is taken up by the subsequent crop (Gong and Li 1986, Seiter and Horwath 1999, Wivstad 1999). As a general rule, the succeeding crop took up a greater proportion of N from young, annual, leguminous green manures, compared with older stalky tissues. For example, 38% of the N in sweet clover was taken up by wheat/perennial ryegrass in the pot experiment of Wivstad (1999), but only 12% of the N in alder prunings was taken up by the subsequent sweetcorn crop in the field experiment of Seiter and Horwath (1999). A  $^{32}\text{P}$  study by Vig *et al.* (1989) showed that the succeeding maize crop took up only 5–9% of the P from incorporated *Sesbania aculeata*.

The value of tree lucerne green manure to supply N and P to aerobic rice grown in the field was estimated as follows. Firstly, it was assumed that 25% of the N in the added tree lucerne leaves would be taken up by rice, and 7% of the P. Then, based on the nutrient concentrations of tree lucerne in Table 3.1, a rice crop could be expected to take up around 63 kg N ha<sup>-1</sup> and 1.3 kg P ha<sup>-1</sup> from the 9 t DM ha<sup>-1</sup> added as a green manure. A relatively good grain yield for rainfed or upland conditions of 4.5 t rice ha<sup>-1</sup> would require 74 t N ha<sup>-1</sup> and 17 t P ha<sup>-1</sup>, (assuming 16.5 kg N and 3.75 kg P are required for a tonne of rice grain yield (Grist 1975)). Therefore, whilst almost all of the rice crop N requirements could be supplied by incorporation of 9 t tree lucerne DM ha<sup>-1</sup>, only 8% of the crop requirements for P would be met. An application of 110 t tree lucerne DM ha<sup>-1</sup> would be necessary to meet the crop P demand. This quantity would be totally impractical in any farming system, and would result in a large oversupply of N. In a 35 year experiment on a thin black chernozemic soil in Saskatchewan, Canada, Campbell *et al.* (1996) observed that fertiliser improved soil quality, while absence of fertiliser, combined with frequent fallowing, led to soil degradation. The inclusion of a legume green manure crop in the rotation failed to maintain soil fertility because legumes do not supply P (they only fix N). The present study and the literature therefore indicate that green manures may be able to provide a substantial portion of the crop N requirements, but that they are unable to supply sufficient P. These results highlight the need to use P fertiliser in combination with green manures for adequate growth of food or cash crops.

### 3.4.3 Liming or acidifying effects of the soil treatments

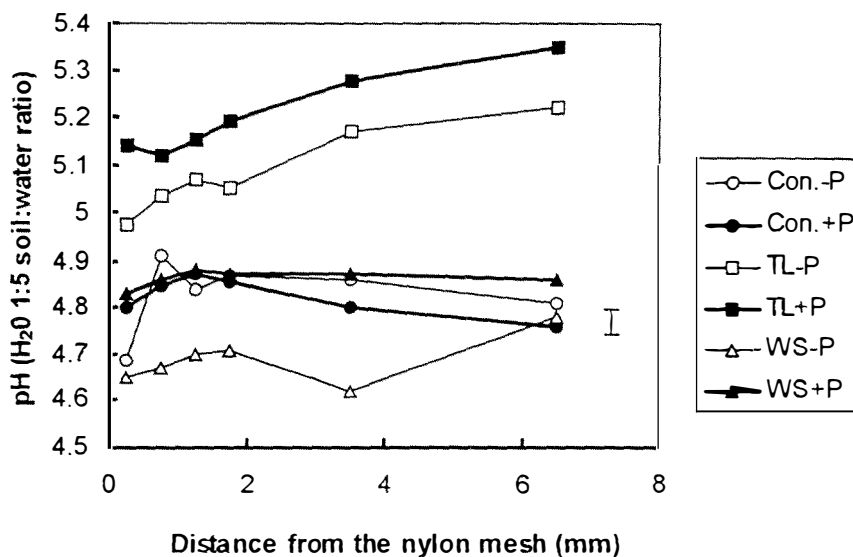
#### 3.4.3.1 Bulk soil pH

The effect of the various amendments on the bulk soil pH is shown by the pH of the soil slices taken 6–8 mm below the nylon mesh (Figure 3.6). These measurements show that the addition of tree lucerne has significantly increased the bulk soil pH by 0.4 of a unit relative to the control treatment. This pH increase is due to the decarboxylation of organic anions (the high alkalinity of the ash for tree lucerne (Table 3.1) indicates a high concentration of cation balancing organic anions), and the production of  $\text{OH}^-$  during ammonification (as discussed in Section 1.3.1.1). There was probably no acidification due to nitrification, since no  $\text{NO}_3^-$  was detected in either the planted or unplanted pots. There was no significant effect of wheat straw on soil pH, partly due to the large variance in Con.-P soil pH. The apparent difference between the WS-P and Con.-P treatments shown in Figure 3.6 is due to one replicate of the Con.-P treatment having a high soil pH.

The addition of RPR (+P) significantly increased the soil pH of the tree lucerne and wheat straw treatments (by approximately 0.13 pH units), although there was no significant difference in the control treatment, presumably due to the large variance in Con.-P soil pH. The observed increase in pH is due to the liming effect of phosphate rocks (Sinclair *et al.* 1993, Loganathan *et al.* 1995).

#### 3.4.3.2 Rhizosphere pH

Plants were harvested early because the roots had penetrated the nylon mesh (see Section 3.3.5), so no root mat had had formed above the nylon mesh in the wheat straw or control treatments. There were therefore no observed changes in rhizosphere soil pH in these treatments (Figure 3.6). However a loose mat of roots had formed in the faster growing tree lucerne amended treatments, and a significant decrease in soil pH next to the root plane was evident for both tree lucerne treatments (Figure 3.6). This pH decrease was most probably the result of high  $\text{NH}_4^+$  uptake by the tree lucerne amended treatments (Section 1.3.1.1) –  $\text{NH}_4^+$  concentrations were high for both TL treatments (Figure 3.5) whereas  $\text{NO}_3^-$  concentrations were negligible.



**Figure 3.6.** Changes in soil pH with distance from the nylon mesh. (See Table 3.2 for treatment key). The bar shows the average standard deviation for the data points,  $n=2$ .

#### 3.4.4 Phosphorus fractionation

None of the fractions analysed showed any detectable depletion of P in soil slices taken within 2 mm of the nylon mesh. This is because roots penetrated the nylon mesh. Hence no root mat was formed on the mesh in the control and wheat straw treatments. In the tree lucerne treatments a loose root mat formed (see Section 3.3.4) but the proportion of roots comprising the root mat was too small<sup>†</sup> to result in a significant depletion of P in the soil. The data presented below are therefore an average of all six soil slices for two replicates.

<sup>†</sup> Experience with previous rhizosphere experiments suggests that 10 % of the total root length might accumulate above the mesh after 25 d of growth. But since the roots penetrated the mesh, occupying both cells, this proportion would reduce to approximately 5%.

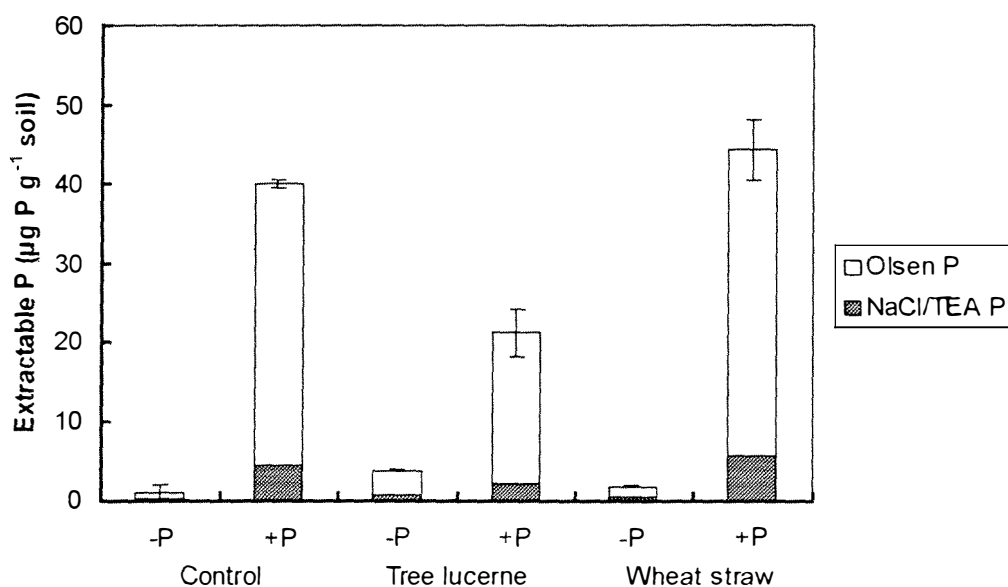
The total P uptake would be approximately  $(0.9 \text{ g shoot DM} + 0.6 \times 0.9 \text{ g root DM}) \times 0.2\% \text{ P} = 2.9 \text{ mgP pot}^{-1}$ . 5% of the 2.9 mg P would be taken up by the root mat P, half of this from below the mesh i.e.  $72 \mu\text{gP pot}^{-1}$ . In the 0.5 mm slice below the mesh there is 2.1 g of air dried soil, so the amount of depletion would be in the order of  $35 \mu\text{gP g}^{-1}$  soil, which, when distributed amongst the different P fractions, was found to be undetectable with the given amount of error, and only two replicates analysed.

### 3.4.4.1 NaCl/TEA-P plus Olsen P

The sum of the NaCl/TEA-extractable P plus Olsen P fractions can be expected to provide a relative indication of the plant-available P status of the soil. The addition of Sechura RPR resulted in a large increase in these two fractions, which shows that Sechura RPR rapidly dissolves in the acidic Wharekohe soil, and suggests that RPR is a suitable P source for upland rice in acidic soils. This was confirmed by the large growth response to P in the presence of high N (Figure 3.5)

The variation in easily-extractable P concentrations (Figure 3.7) for RPR fertilised treatments reflect the differences in soil pH. The wheat straw and control treatments had a lower soil pH than the tree lucerne treatment, and therefore more P was released. Easily-extractable P concentrations for the non-RPR-fertilised treatments reflect the P content of the amendments (Table 3.1).

There was an extremely high correlation (0.99) between the NaCl/TEA-P and the Olsen P fractions. This is good evidence of exchange of P between these P fractions during the 3 wk of the experiment, i.e. that an equilibrium exists between these fractions.

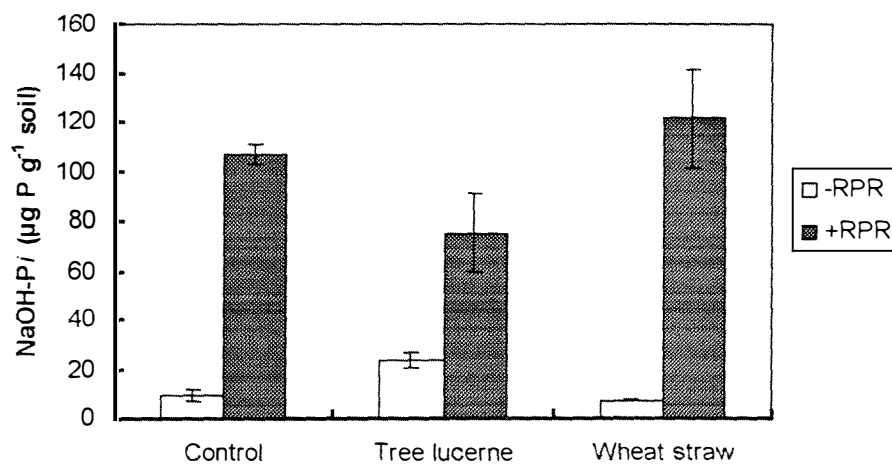


**Figure 3.7.** NaCl/TEA-extractable P and Olsen P values for Wharekohe silt loam amended with different organic materials and/or RPR. Data are means  $\pm$  SE,  $n=2$ .

#### 3.4.4.2 1 M NaOH-P<sub>i</sub>

The addition, and subsequent dissolution, of Sechura RPR caused a large increase in the amount of NaOH-P<sub>i</sub> in the soil (Figure 3.8). Approximately 75% of the dissolved RPR was adsorbed onto hydrous oxide surfaces (measured as NaOH-P<sub>i</sub>) with 25% remaining in the easily-extractable (NaCl/Olsen-extractable) P<sub>i</sub> pool (Figure 3.7). The very strong correlation (correlation coefficient 0.99) between the NaOH-extractable P<sub>i</sub> and easily-extractable P<sub>i</sub> again suggests that the Olsen and NaOH-P<sub>i</sub> extractable fractions were in equilibrium over the 3 wk experimental period.

The concentrations of NaOH-P<sub>i</sub> measured in the non-RPR-fertilised treatments, like the easily extractable P<sub>i</sub>, reflect the concentration of P contained in the organic amendments (Table 3.1), with tree lucerne containing twice as much P as wheat straw.

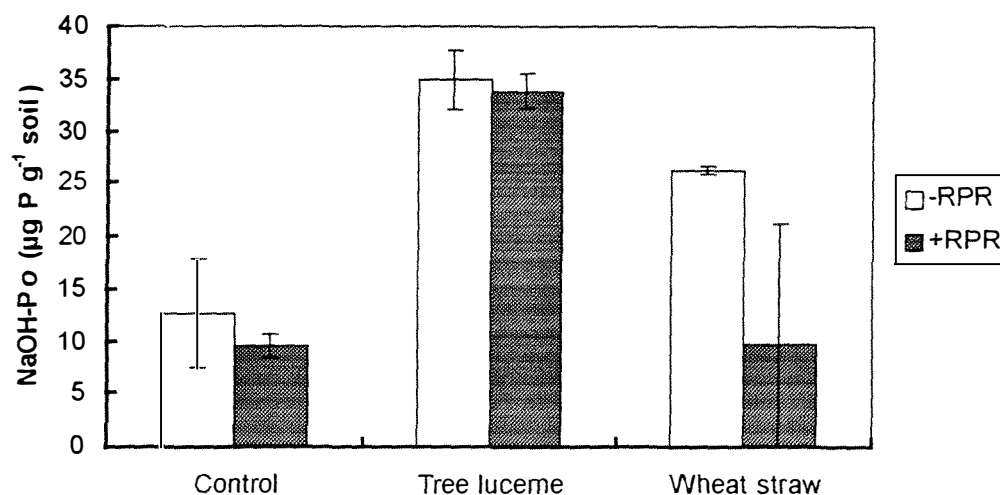


**Figure 3.8.** Inorganic P concentrations in the 1 M NaOH fraction of Wharekohe silt loam amended with different organic materials and/or RPR. Data are means  $\pm$  SE,  $n=2$ .

#### 3.4.4.3 1 M NaOH-P<sub>o</sub>

The concentrations of NaOH-P<sub>o</sub> measured in each treatment (Figure 3.9) reflect the P concentration of the added organic matter (Table 3.1). The WS+P treatment is the only exception to this trend, being much lower than the WS-P treatment. However this is partly

due to one of the replicates giving a particularly low value, which is reflected in the large standard deviation. The large addition of organic material more than doubled the soil  $P_o$  content in the short term (Figure 3.9).



**Figure 3.9.** Organic P concentrations in the 1 M NaOH fraction of Wharekohe silt loam amended with different organic materials and/or RPR. Data are means  $\pm$  SE,  $n=2$ .

Table 3.4 gives approximate calculations to explain the observed increases in  $P_o$  in the soil. These were made as follows. The amount of P added in the organic amendment was calculated from the dry weight of material added ( $32 \text{ mg g}^{-1} \text{ soil}$ ) and its P content (Table 3.1). Half of the P in the added tree lucerne is assumed to be  $P_o$ , and three quarters of the P in the wheat straw (Harrison 1987, Appendix P). Some  $P_o$  will be converted to  $P_i$  by microbial decomposition, calculated as follows. The amount of organic material decomposed was estimated from the total  $\text{CO}_2$  production. This was calculated from the area under the curves in Figure 3.3, extrapolated to time = 0 and to harvest (assuming linear changes), subtracting the  $\text{CO}_2$  produced in the control treatments, and assuming that total C metabolised was 1.4 times the  $\text{CO}_2$  produced (Stotzky 1965). The amount of  $P_o$  metabolised was taken to be directly proportional to the amount of C metabolised (Dalal 1979), and of the  $P_o$  metabolised, 60% was assumed to become  $P_o$  in microbial biomass



and 40%  $P_i$  (Harrison 1987, Appendix M). The increase in  $P_o$  in the soil was then estimated from the  $P_o$  added less that converted to  $P_i$ . The calculated increases in  $P_o$  agree with the measured increases shown in Figure 3.9 to within the standard error of the mean (except for the WS+P treatment).

These calculations show that the increases in soil % C were smaller than the increases in NaOH- $P_o$ , particularly for tree lucerne, due to large losses of C as  $CO_2$  by microbial respiration. An increase in soil C content of 5 to 6 mg C  $g^{-1}$  soil, which has a native C content of 24 mg C  $g^{-1}$  soil, is however, still significant. Thus there appears to be potential to increase the soil organic P concentration markedly, at least in the short term, by applying organic amendments. The increases in soil organic C, however, will be smaller, particularly if the organic amendment is readily decomposed by micro-organisms e.g. tree lucerne.

**Table 3.4.** Approximate calculations showing the fate of added C and  $P_o$  from tree lucerne (TL) and wheat straw (WS) 65 d after addition to a Wharekohe silt loam topsoil:subsoil mix. The % of the total C or  $P_o$  added is shown in brackets.

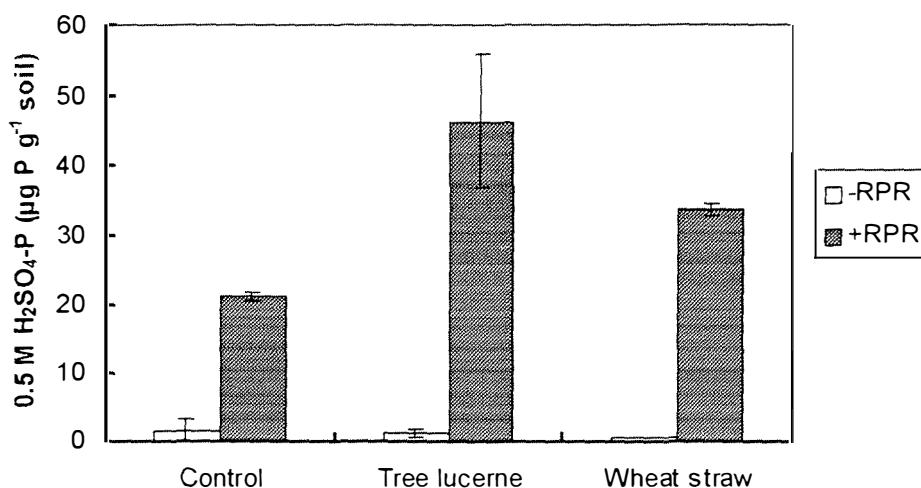
Treatm't	C added	CO <sub>2</sub> -C respired	C metabolised by microbes	P added	$P_o$ added	$P_o$ untouched by microbes	$P_o$ in microbes	$P_o$ remaining after 65 d
	(mg P $g^{-1}$ soil)			$\mu g g^{-1}$ soil				
TL+P	15	7.0	10 (65%)	67	34	11.8	14.2	26 (77%)
TL-P	15	6.5	9 (61%)	67	34	13.2	13.3	26(79%)
WS+P	15	2.8	4 (27%)	29	21	15.6	3.7	19 (91%)
WS-P	15	2.6	4 (25%)	29	21	15.9	3.5	19 (91%)

The addition and dissolution of RPR did not change the concentration of P extracted in the NaOH- $P_o$  fraction. In order for fertiliser P to become incorporated into the NaOH- $P_o$  fraction it must pass through the microbial P pool. In a review on organic P, Harrison (1987) states that "It is generally considered that immobilisation of P is likely to surpass mineralisation when the C:P ratio in soil is 200:1", although there was considerable variation in the literature. The tree lucerne has a C:P ratio of 230:1, and the wheat straw, 510:1. Therefore there should not be much immobilisation of fertiliser P in the tree lucerne

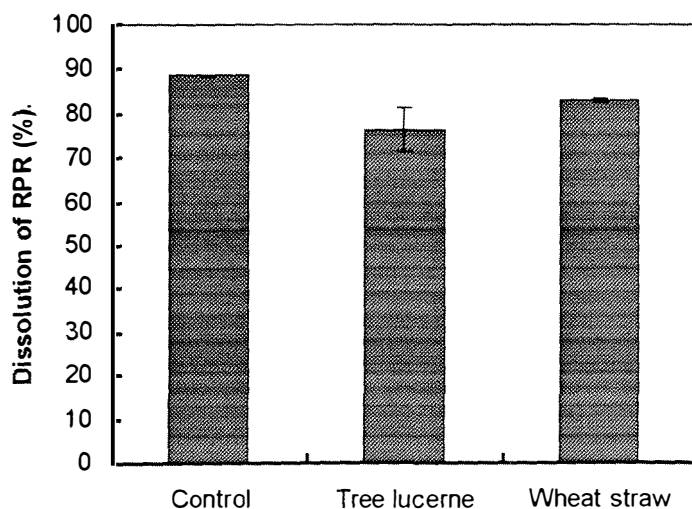
treatment, but immobilisation of fertiliser P could be expected in the wheat straw amended treatment. However there was not much microbial activity in the wheat straw amended treatment (Figure 3.3 and Table 3.4). In addition, organic P may have been underestimated since the acidification of alkaline extracts to  $\text{pH} < 4.0$  (following the addition of the Murphy and Riley reagent) can cause substantial release of inorganic P from the organic matrix (Gerke and Jungk 1991). Gerke and Jungk (1991) therefore recommend the addition of polyethylenimine to the soil extract as a flocculant, followed by ultrafiltration through 20,000 D cellulose acetate membranes prior to inorganic P determination of soil extracts.

#### 3.4.4.4 0.5 M $\text{H}_2\text{SO}_4$ -P

The concentration of 0.5 M  $\text{H}_2\text{SO}_4$ -P (mainly Ca-bound P) in the unfertilised soil was very low ( $< 2 \mu\text{g P g}^{-1} \text{ soil}^{-1}$ , Figure 3.10). The concentration of 0.5 M  $\text{H}_2\text{SO}_4$ -P in the RPR fertilised treatments therefore represents undissolved RPR. The addition of tree lucerne has therefore resulted in significantly less dissolution of RPR than in the other treatments (Figure 3.11). Reasons for the lower dissolution of RPR in the tree lucerne treatment are discussed in Section 3.4.6.



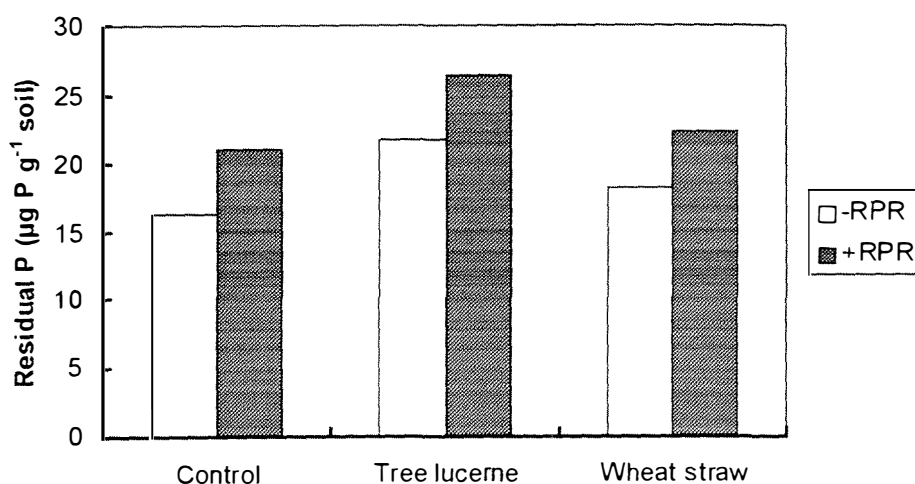
**Figure 3.10.** P concentrations in the 0.5 M  $\text{H}_2\text{SO}_4$  fraction of Wharekohe silt loam amended with different organic materials and/or RPR. Data are means  $\pm$  SE,  $n=2$ .



**Figure 3.11.** Estimated<sup>†</sup> dissolution of Sechura RPR after 13 wk incubation in Wharekohe silt loam amended with different organic materials. Data are means  $\pm$  SE,  $n=2$ .

#### 3.4.4.5 Residual P

The concentration of residual P, measured by digestion with  $H_2SO_4$  and  $H_2O_2$ , in each treatment was low, around  $20 \mu\text{g P g}^{-1}$  soil (Figure 3.11). The TL+P treatment was slightly higher at  $26 \mu\text{g P g}^{-1}$  soil and the Con.-P treatment slightly lower at  $16 \mu\text{g P g}^{-1}$  soil.

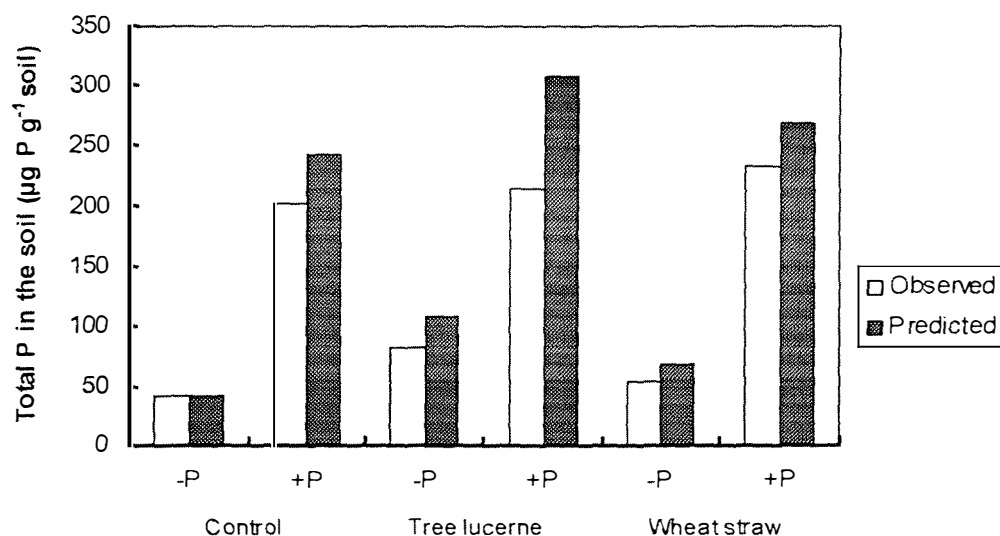


**Figure 3.12.** P concentrations in the residual fraction of Wharekohe silt loam amended with different organic materials and/or RPR.

<sup>†</sup> Estimated as:  $(\text{P added as RPR} + \text{original soil } H_2SO_4\text{-P content} - H_2SO_4\text{-P}) / \text{P added as RPR} \times 100$

#### 3.4.4.6 Recovery of P

The recovery of P from all six soil P fractions was 14 – 31% less than that predicted by adding the amount of P in the RPR and in the OM to that present in the control soil (Figure 3.12). The agreement is particularly poor with the TL+P treatment. One reason for this difference is that a small amount (< 5%) of P in undecomposed organic matter was removed when the extracts were filtered. An improvement would be to use ashless filter papers and digest the filter papers with the residual fraction. The difference between the predicted and measured P concentrations might also reflect poor mixing of the organic matter or fertiliser with the soil, or P losses during the digestion process.



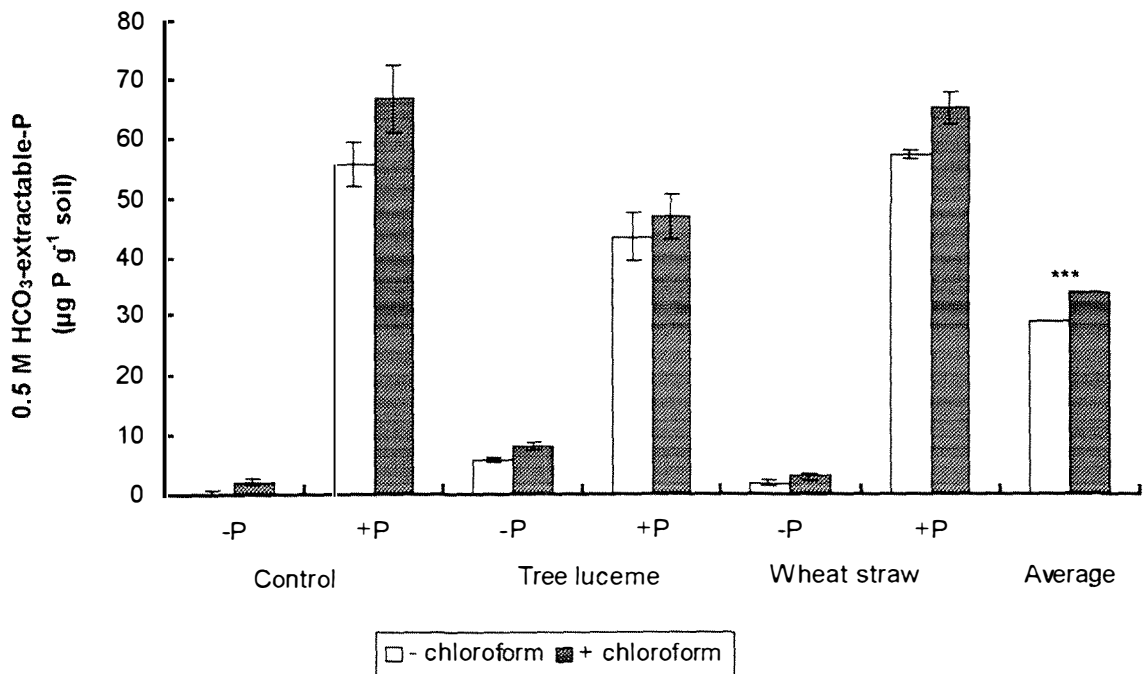
**Figure 3.13.** The total amount of P recovered from the soil (the summation of all six fractions) and that predicted by adding the concentration in the control soil, plus what was added as RPR and/or organic matter, for each treatment.

#### 3.4.5 Soil microbial P

Treating the soils with chloroform significantly ( $P < 0.001$ ) increased the concentration of  $\text{HCO}_3^-$ -extractable P (Figure 3.13). Chloroform treatment released an average of 17% more  $\text{HCO}_3^-$ -extractable P, or  $5 \mu\text{g P g}^{-1}$  soil. However the actual concentration of P contained in the microbial biomass would have been *at least*  $13 \mu\text{g P g}^{-1}$  soil, because Hedley and Stewart (1982), using a soil of comparatively low P retention (Chernozem), found that a

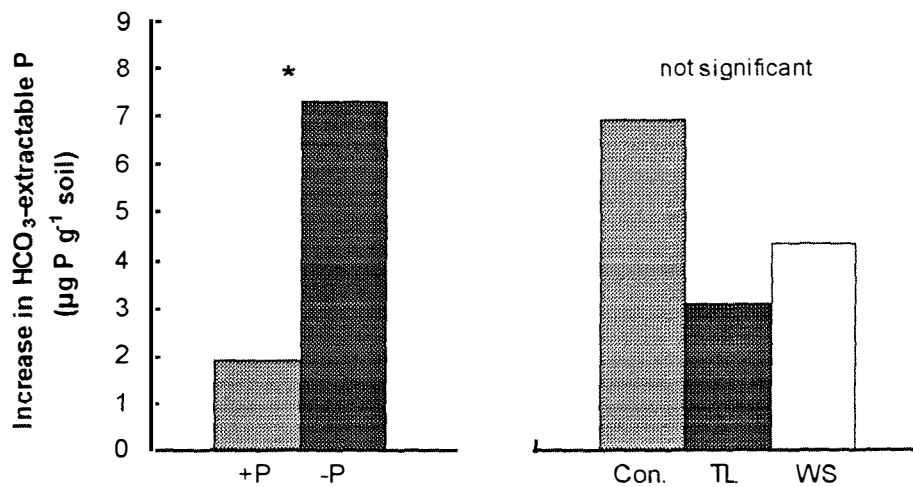
similar chloroform technique measured only approximately 38% of the total P contained in the micro-organisms. (A large proportion of the P released is strongly bound to the soil and not recovered in the 0.5 M HCO<sub>3</sub> extractant.)

The application of P to the soil increased the soil microbial P content (significant at  $P < 0.1$ ) i.e. the RPR-fertilised treatments released 5  $\mu\text{g P g}^{-1}$  soil more microbial P than non RPR-fertilised treatments (Figure 3.14). The extra 5  $\mu\text{g P g}^{-1}$  soil is most likely due to luxury uptake of P by the existing microbial population, rather than by an increase in microbial biomass, as the CO<sub>2</sub> data (Figure 3.3) showed only a very small increase in the respiratory activity of the microbial population in response to P. Perrott *et al.* (1990) observed that micro-organisms have a large capacity for luxury uptake of P – in their study in a high-producing pasture the P concentration of the microbial biomass varied from 1.5 to 3% depending on the season.



**Figure 3.14.** A comparison of the amount of 0.5 M HCO<sub>3</sub>-extractable P in soils treated (+) and not-treated (-) with chloroform. Data are means  $\pm$  SE,  $n=4$ . \*\*\* The difference between + and - chloroform is highly significant by t-test at  $P < 0.001$ .

There was no significant difference between the organic amendments in the amount of microbial P recovered (Figure 3.14). From the CO<sub>2</sub> production data in Figure 3.3, however, it may have been expected that the greatest increase in microbial P should be in the organic matter amended treatments (as found in the study of Kouno *et al.* 1994). However Figure 3.3 also shows that the microbial activity receded over time. After 13 wk of incubation and two cycles of wetting and drying, any difference in the size of the soil microbial populations (reflected in the concentration of P released) between the treatments appears to have disappeared. So whilst it is likely that net immobilisation or mineralisation may have occurred when the organic matter was first added (depending on the C:P ratio of the added organic matter (Harrison 1987)) there was no significant effect of organic matter addition on soil microbial P content in the long term in this study.



**Figure 3.15.** The increase in HCO<sub>3</sub><sup>-</sup>-extractable P following chloroform fumigation in treatments with (+P) and without (-P) the addition of rock phosphate (averaged across amendment), and with different organic amendments (averaged across P treatment) (Con.=control, TL=tree lucerne, WS=wheat straw). \* Significant at P<0.1.

### **3.4.6 General discussion – the effect of green manure addition on RPR dissolution**

#### **3.4.6.1 pH and Ca effect**

Since the soil moisture content and RPR particle size were constant between the treatments, pH and Ca and P concentrations would have been the most important soil variables governing RPR dissolution (Khasawneh and Doll 1978, Kirk and Nye 1986a). The 12% slower RPR dissolution in the tree lucerne treatment compared with the control soil could be explained by the 0.4 of a unit increase in pH, which was probably due to ammonification and decarboxylation of organic anions (Yan *et al.* 1996). The soil Ca concentration may have also played a significant role in the amount of RPR dissolved, as the tree lucerne contained four times more Ca than the wheat straw (Table 3.1). Research by Smithson (1999) indicated that the significant decrease in Minjingu RPR dissolution, following the addition of *Tithonia diversifolia* as a green manure, was due to the high Ca content of the plant material. The more Ca supplied by the green manure the less the equilibrium favours the formation of the dissolution products –  $\text{Ca}^{2+}$  and  $\text{H}_2\text{PO}_4^-$  (Figure 1.3), i.e. the less RPR dissolves. The solution P concentration is unlikely to have had a significant effect on RPR dissolution since the NaCl/TEA-extractable P was very low in all treatments (Figure 3.7).

#### **3.4.6.2 Other factors that may affect RPR dissolution**

Chelation of Ca by hydrolysed organic matter does not appear to have been an important factor because RPR dissolution was fastest in the treatment with no organic matter, and slowest in the treatment with tree lucerne. Nor were differences in microbial activity important: the  $\text{CO}_2$  production data indicate that microbial activity was greatest in the treatment with tree lucerne where dissolution of RPR was slowest. Carbon dioxide did not cause acidification (and hence more RPR dissolution) in these treatments since the soil pHs (4.6–5.4) were well below the pK value for the dissociation of  $\text{H}_2\text{CO}_3$  (Equation 1.2) and most of the  $\text{CO}_2$  would have left the soil as gas. The production of organic acids during the decomposition of OM was also not a plausible explanation for the observed RPR dissolution, as more RPR dissolved in the control soil, than the soil to which tree lucerne was added.

### 3.4.6.3 Strategies for green manure and RPR use

A high N content in green manure is important for rice growth but it is not beneficial to the dissolution of RPR. In very acidic soils such as the Wharekohe silt loam this would not be a problem, as the addition of high-N organic matter did not cause the soil pH to rise above 5.5: above pH 5.5 the rate of RPR dissolution is significantly reduced (Apthorp *et al.* 1987). In this experiment the Olsen P value of  $21 \mu\text{g P g}^{-1}$  soil for the tree lucerne amended treatment would still be adequate for moderate rice yields (Liming Xiong, pers. comm.). For soils with a pH of 5.0 or above, where large amounts of high-N organic manure are to be applied in combination with RPR, there are a number of strategies to overcome possible detrimental effects of an increase in pH on RPR dissolution. Firstly, the RPR can be applied to grow a leguminous green manure crop (Beri and Meelu 1980, von Uexküll 1986). This is a particularly good strategy when the rice is to be grown on the same soil following the legume crop. Many legume species that are actively fixing  $\text{N}_2$  acidify their rhizosphere (Bolan *et al.* 1991) and thus enhance the dissolution of RPR (Aguiler and van Diest 1981, Trolove *et al.* 1996). This dissolved P is then either taken up by the green manure, where it can become available to the plant upon decomposition, or will move into the NaOH-P<sub>i</sub> fraction, where it is in equilibrium with the easily-extractable P.

If the organic manure is not grown *in situ*, then it may be a useful strategy to apply the RPR a month or two before the application of the manure to allow time for dissolution to occur. The RPR could also be ploughed into the soil, whereas the OM simply applied to the surface as mulch. Surface mulching is generally beneficial although during wet periods mulching is better done in rows with the mulch kept off the stem to reduce the risk of disease (von Uexküll 1986). In high P-fixing soils, Hands *et al.* (1995) suggest that it is better to incorporate the RPR into the mulch layer to avoid P fixation. The RPR is then taken up very efficiently by the mycorrhizal and fine root network, which forms in the mulch.



### 3.5 Conclusions

This study has shown that the application of the high N-containing green manures with RPR is likely to slow the dissolution of RPR. This is caused by a pH increase due to decarboxylation of organic anions, ammonification of the green manure, and the high Ca content in some green manures. In acidic, highly-weathered soils (with a low Ca concentration), CO<sub>2</sub> produced by OM decomposition and the chelation of Ca by organic acids appears to be less important than soil pH in determining the amount of RPR dissolved. The addition of an amendment containing low ash alkalinity, and low N and Ca concentrations such as wheat straw is likely to have little effect on soil pH or RPR dissolution.

On highly weathered soils, green manure alone is not sufficient to meet the P requirements of the crop, so the addition of P fertiliser is also necessary. The addition of a crop residue with a high C:N ratio is likely to reduce the soil N supply and decrease crop growth.

Sechura RPR dissolved rapidly in the acidic Wharekohe soil, indicating it would be suitable as a fertiliser for aerobically-grown rice on acidic soils. In Fe- and Al-rich soils, most of the dissolved RPR-P will become sorbed to hydrous Fe and Al surfaces (NaOH-*P<sub>i</sub>*). The addition of RPR does not necessarily translate into an increase in NaOH-*P<sub>o</sub>* in the short term (<2 months). Reactive phosphate rock addition can however, increase microbial P, which should enhance the availability of P to plants.

Organic amendments, at least in the short term, increase the amount of organic P in the soil. An increase in organic P does not necessarily correspond to an increase in the amount of microbial P in the soil. The large initial differences in microbial activity are likely to disappear rapidly as the readily decomposable organic material is quickly broken down in the warm temperatures of tropical fields.

## 4. Phosphate fertiliser management for rice in aerobic soil II. Soil P sorption relations and the effect of fertiliser banding

### 4.1 Introduction

The results of Chapter 3 show that it is possible to supply N to rice via organic manure. However even at the high rate applied ( $9 \text{ t DM ha}^{-1}$ ), the amount of P supplied in the organic manure ( $57 \text{ kg P ha}^{-1}$ ) was grossly inadequate for growth on these highly weathered soils. Other researchers also recommend supplying P fertiliser along with green manures (e.g. von Uexküll 1986, Hands *et al.* 1995, Lathvilayvong *et al.* 1995). Nitrogen therefore, can be supplied to the rice crop with minimal capital outlay, however some capital outlay will be required for P.

Since capital is one of the most limiting resources to farmers of the humid tropics (Baharsjah and Fagi 1995, cited by Zaini and Fagi 1999), it is important to pursue strategies which could increase the efficiency of fertiliser P use by the rainfed, and upland, rice crop. Plant mechanisms and management strategies that may enhance P efficiency have been discussed in the Introduction (Section 1.5). The Introduction also highlighted the importance of understanding these mechanisms, both in order to significantly improve the rate of progress in developing P efficient cultivars, and to be able to extrapolate the results of one experiment into different environments.

Banding or localised placement of fertiliser is one useful management strategy for improving plant P uptake in high P fixing soils (Section 1.5.1.1). Similarly, the release of organic acids by roots was one plant mechanism that showed promise for improving plant P uptake, and has the potential to be improved through plant breeding or genetic engineering (Section 1.5). It is possible that by combining both P efficient management strategies with P efficient cultivars, significant benefits could be achieved in terms of increased plant yields,

farm incomes, and reduced risk of adverse environmental effects. The following chapters (research conducted at IRRI) investigate the banding of P fertiliser, plant P uptake mechanisms, and the interactions between them.

*Investigating the banding of fertiliser P: adsorption/desorption isotherms*

The extent to which banding of P fertiliser increases the concentration of P in the soil solution, and hence its availability to plants, depends on the shape of the P adsorption isotherm. In low P-fixing soils the P adsorption isotherm shows a steady increase in soil solution P concentration as more P is added. In such soils the banding of P has no advantage over incorporating P throughout the soil, and may sometimes be detrimental, as the root system is more concentrated in the P-rich zone, which reduces the total volume of soil explored by the roots and may reduce the uptake of water and other nutrients besides P (Garrity *et al.* 1990). However in high P-fixing soils, the shape of the P adsorption isotherm is highly non-linear – initially there is negligible increase in the amount of P in solution as more P is added, and only at high rates of P addition does the concentration of P in solution increase. Hence there is often no response to P fertiliser addition in high P-fixing soils until unaffordably high rates of P are added. In such situations the banding of P fertiliser is by far the preferred option. Therefore in order to study banding in highly weathered, high P-fixing tropical soils it is important that the nature of the relationship between solution P concentration and P added (the P adsorption isotherm) is known.

In addition to knowing the amount of P in the soil solution, it is important to know the capacity of the soil to sustain the concentration of P in solution as P is removed from the soil. This parameter is measured by the soil buffer power ( $b_p$ ).

$$\text{In equation form: } b_p = \frac{d[P]}{d[P_L]} \quad (4.1)$$

where [P] is the concentration of P in the whole soil;  
and [P<sub>L</sub>] is the concentration of P in the soil solution.

The P buffer power was also a necessary parameter to obtain for the P uptake models that are used in Chapters 5 and 9.

## 4.2 Objectives

The purpose of this chapter was to experimentally determine the P adsorption isotherm and the P buffer power of the high P-fixing Cavinti soil, and to examine how they are influenced by the addition of citrate. The amounts of Fe, Mn, or Al released into solution by citrate were studied in order to understand the mechanisms by which citrate released P from Cavinti soil.

## 4.3 Method

### Soil

The soil used for all of the studies conducted in the Philippines is an isohypothermic palehumult collected from a permanent pasture site in Cavinti, Laguna, Philippines. Important properties of this soil are shown in Table 4.1.

**Table 4.1.** Important characteristics of Cavinti soil.

Characteristic	Value	Units
pH (1:2.5 soil:water ratio)	4.5	—
Clay <sup>†</sup>	70	%
Silt <sup>†</sup>	26	%
Sand <sup>†</sup>	4	%
Organic C	2.4	%
Total N	0.21	%
Olsen P	1.4	µg g <sup>-1</sup>
P retention	83	%
Exchangeable SO <sub>4</sub>	0.19	cmol <sub>c</sub> kg <sup>-1</sup>
Exchangeable K <sup>#</sup>	0.11	cmol <sub>c</sub> kg <sup>-1</sup>
Exchangeable Ca <sup>#</sup>	<0.1	cmol <sub>c</sub> kg <sup>-1</sup>
Exchangeable Mg <sup>#</sup>	0.47	cmol <sub>c</sub> kg <sup>-1</sup>
Exchangeable Na <sup>#</sup>	0.30	cmol <sub>c</sub> kg <sup>-1</sup>
Effective CEC <sup>‡§</sup>	10.9	cmol <sub>c</sub> kg <sup>-1</sup>
Exchangeable Al <sup>†*</sup>	8.3	cmol <sub>c</sub> kg <sup>-1</sup>
Active Fe <sup>†*</sup>	2.8	%
Active Mn <sup>†*</sup>	0.52	%

<sup>†</sup> from Hedley *et al.* (1994).

<sup>#</sup> Extracted by 1 N ammonium acetate

<sup>§</sup> (K + Na + Ca + Mg) + Al extracted by 1 M KCl.

\* Extracted by 1 M KCl.

\* Extracted by 0.02 M EDTA.

### **4.3.1 Phosphate adsorption isotherm**

Portions (2 g) of air-dried Cavinti soil (2 mm sieved) were weighed into 30 mL Oak Ridge centrifuge tubes. Added to these tubes was added 6 mL of 0.01 M CaCl<sub>2</sub> containing a range of P (as KH<sub>2</sub>PO<sub>4</sub>) concentrations from 0 to 10,000 µg P g<sup>-1</sup> soil. The pH of each solution had been adjusted to the native soil pH of 4.3 (in 0.01 M CaCl<sub>2</sub>) using 13 mM H<sub>2</sub>SO<sub>4</sub>. There were three replicates of each treatment. The solutions were shaken end-over-end overnight for 16 h, centrifuged at 7,640 g (8,000 rpm) for 5 min and the solutions analysed for P by spectrophotometry at 882 nm wavelength (Murphy and Riley 1962).

### **4.3.2 The effect of increasing P addition on the P buffer power**

#### *Soil preparation*

Monocalcium phosphate was added to 14 g portions of sterilised<sup>†</sup> (γ irradiation 25 kGy), 2 mm sieved Cavinti soil at rates of 0, 250, 500, 1000, 2000, 3000, 4000, 5000 and 6000 µg P g<sup>-1</sup> soil (referred to as P<sub>0</sub>, P<sub>250</sub> etc). The fertiliser was thoroughly hand-mixed with the soil then shaken end-over-end. The soils were incubated at approximately field capacity (deionised water was added at 30 mL per 100 g soil) in an aerated humid chamber at 25°C for 6 wk. The soils were mixed every 7–10 d and a very small amount of water added to maintain the exact water content.

#### *Desorption isotherms*

After incubation, 1.3 g of moist soil from each P level was weighed into an appropriate-sized container and  $x$  mL of 0.01 M CaCl<sub>2</sub> (containing 50 mg L<sup>-1</sup> HgCl<sub>2</sub>) was added ( $x = 25, 50, 100, 250, 500$  or 1000 mL). Each solution added had been adjusted to the native soil pH, which had been previously measured (Figure 4.4). The solutions were shaken end-over-end for 16 h, centrifuged at 17,200 g (12,000 rpm), filtered (Whatman #5), the pH measured and P determined using the method of Murphy and Riley (1962). In order to plot P desorption isotherms; the change in the P concentration in the whole soil was calculated as the amount of P desorbed into solution.

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<sup>†</sup> The soil was sterilised because the isotherm data was required for use in Chapter 9.

### 4.3.3 Phosphate desorption by citrate

Monocalcium phosphate was added to 2 mm sieved, air-dried, sterilised ( $\gamma$  irradiation 25 kGy) Cavinti soil at rates of 500  $\mu\text{g P g}^{-1}$  soil (P500) and 5000  $\mu\text{g P g}^{-1}$  soil (P5000). The soils were prepared as described in Section 4.3.2, except that the incubation time was 4 wk, after which the soil was air-dried (25°C) then 2 mm sieved.

The P desorption isotherms were conducted in a 3 factor design: 2 soil P rates  $\times$  6 citrate concentrations  $\times$  6 solution volumes, and each treatment was duplicated.

Citrate<sup>†</sup>-CaCl<sub>2</sub> solutions of six different citrate concentrations (0, 0.1, 0.25, 0.5, 1, and 2 mM) were prepared by adding reagent grade citric acid to 0.01 M CaCl<sub>2</sub>. The pH was adjusted with NaOH to the equilibrium soil pH of each soil, 4.3 for P500 soil and 4.7 for P5000 soil. Mercuric chloride was also added to the citrate-CaCl<sub>2</sub> solutions at 2 mg L<sup>-1</sup> to retard microbial activity, as there would have been some reinfection of micro-organisms during incubation. Portions (2 g) of P-fertilised soil (either P500 or P5000) were weighed into appropriate-sized containers and then  $x$  mL of the citrate-CaCl<sub>2</sub> solution was added to each container ( $x$  = either 50, 100, 250, 500, 1000, or 2000 mL). These soil suspensions were then gently shaken (approx. 10 rpm) end-over-end for 16 h. After shaking, an aliquot (approximately 50 mL) of the soil-solution suspension was centrifuged at 17,200  $g$  for 5 min and the supernatant filtered (Whatman #5). The equilibrium pH was measured, then the filtrates were acidified with 0.055 mL of 5 M H<sub>2</sub>SO<sub>4</sub> per 5.5 mL of solution, covered with parafilm and frozen. The concentration of P in the filtrate was determined by spectroscopy using the method of Murphy and Riley (1962). A 4 cm cuvette was used for the P500 soil solutions. Additional samples were taken and analysed by the Analytical Services Laboratory at IRRI for Fe and Mn by AAS and Al by (ICP–AES).

Further samples of the supernatant were 0.45  $\mu\text{m}$  filtered (Acrodisk<sup>®</sup> LC PVDF) then analysed for citrate by high performance liquid chromatography (HPLC). Citrate was measured using a Shimadzu HPLC, by isocratic elution through a nonpolar reverse-phase column (RP8 Lichrocart<sup>®</sup> 250-4 Lichrospher<sup>®</sup> 100, 5  $\mu\text{m}$ ). The stationary phase of the

<sup>†</sup> The charge on the citrate depends on the pH. At pH 4.3 the word citrate refers to a solution that contains 80% citrate<sup>1-</sup> and 20% citrate<sup>2-</sup>. The dissociation constants for citric acid are shown in Table 6.3.

column is an octyl carbon chain. The operating conditions were: a mobile phase of 18 mM  $\text{KH}_2\text{PO}_4$  adjusted to pH 2.1 with  $\text{H}_3\text{PO}_4$ , the flow rate was  $0.8 \text{ mL min}^{-1}$  at a temperature of  $28^\circ\text{C}$ , and a UV detector was used at a wavelength of 215 nm. Citrate was quantified by comparison with the retention time and peak areas of solutions prepared from reagent grade citric acid. Citrate sorption was calculated as the difference between the amount of citrate added and the amount of citrate measured in solution.

The solution concentration data of a number of solutions (selected as being representative of the different citrate, Al, Fe and P concentrations) were input into the computer program GEOCHEM-PC 2.0 (Parker *et al.* 1995) in order to obtain a first approximation of the dominant chemical species in solution (Dr. H. Percival, Landcare Research, pers. comm.). The equilibrium constants used are given in Appendix A.

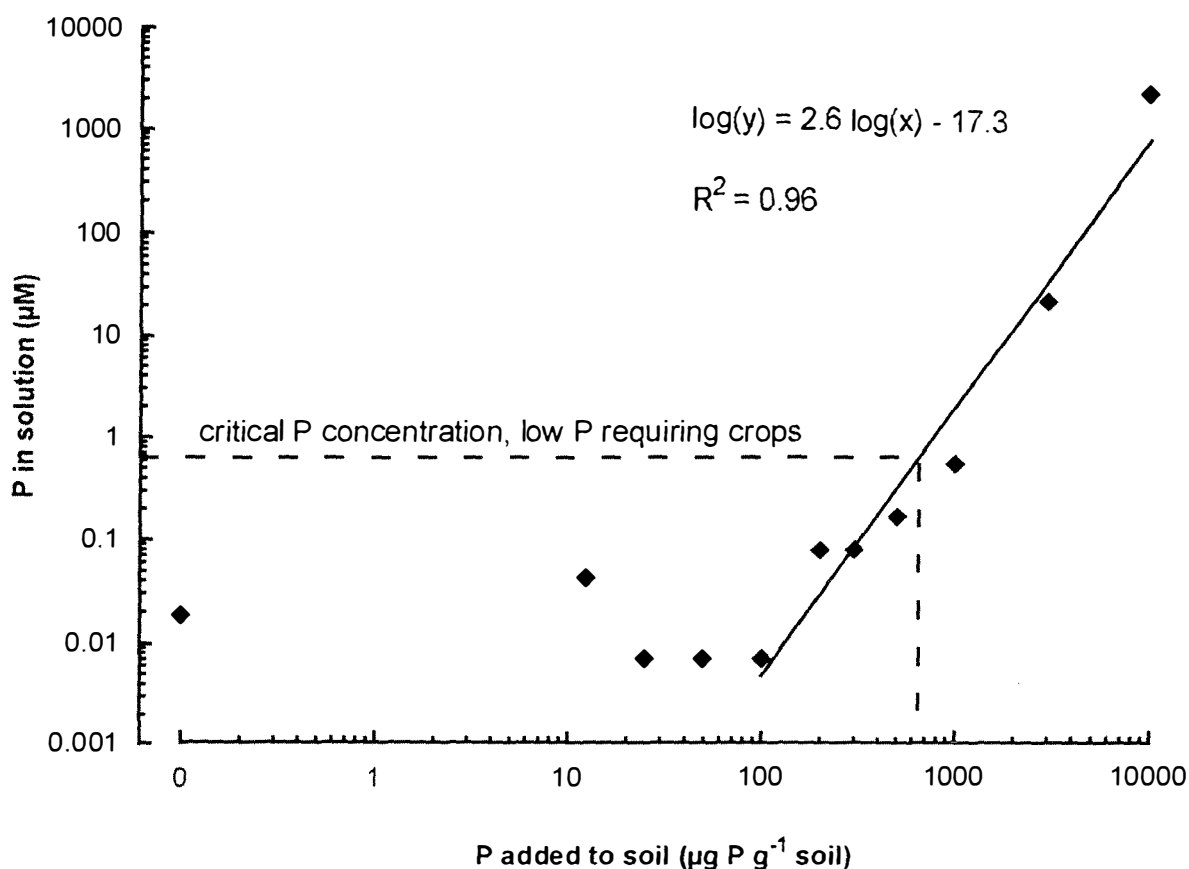
## 4.4 Results and Discussion

### ***4.4.1 Phosphate adsorption isotherm and the implications for fertiliser application***

The P concentration in unfertilised soil ( $0.02 \mu\text{M}$ ) was almost undetectable using the Murphy and Riley method and a 4 cm cuvette (Figure 4.1). There was no detectable increase in solution P until the amount of P added exceeded  $100 \mu\text{g P g}^{-1}$  soil. Phosphate additions above  $100 \mu\text{g P g}^{-1}$  soil gave a rapid increase in the amount of P in solution.

In order to increase the amount of P available to non-P-solubilising plants, the amount of P in solution must be increased. If this were done in the way of traditional temperate agriculture, by incorporating P fertiliser into the plough layer, a very large and expensive addition of P would be required. To get any observable increase in P in solution at least  $100 \mu\text{g P g}^{-1}$  soil would need to be applied, due to the very high P-fixation of Cavinti soil. Assuming a bulk density of  $1 \text{ Mg m}^{-3}$ , this calculates out at  $150 \text{ kg P ha}^{-1}$  in the plough layer (top 15 cm) of the soil. To supply adequate P ( $0.65 \mu\text{M P}$  in solution, Fox 1981) for low P-requiring plant growth (upland rice), then  $660 \mu\text{g P g}^{-1}$  soil would need to be applied, i.e.

990 kg P ha<sup>-1</sup>! Financially, this is impossible for the poor farmers of the harsh rainfed and upland rice environments.



**Figure 4.1.** P adsorption isotherm for 2 mm sieved Cavinti soil. P was added as MCP. A log-log line is fitted to the increasing portion of the curve. The critical concentration of P in solution for low P-requiring crops (Fox 1981) is shown as a dashed line.

Barry *et al.* (1986) compared the application of 100 kg P ha<sup>-1</sup> to a P deficient Oxisol (bicarbonate extractable P of 14 µg g<sup>-1</sup> soil) as a band occupying 2% or 0.5% of the soil volume (total pot volume was 5.5 L), or incorporated throughout the whole soil. They found that wheat yields in the banded treatments were twice that of the incorporated treatment, and that maximum yields can still be reached when P fertiliser occupied only 0.5 % of the soil volume. If 100 kg P ha<sup>-1</sup> (67 µg P g<sup>-1</sup> soil to 15 cm depth, assuming  $\rho = 1.0$ ) were applied to Cavinti soil, Figure 4.1 shows that there would be no increase in the concentration of P in solution (the source of P for plants). If, however, this P occupied



only 0.5% of the soil volume (i.e. the soil P concentration was increased by a factor of 200 times above  $67 \mu\text{g P g}^{-1}$  soil, to  $13,400 \mu\text{g P g}^{-1}$  soil) then, extrapolating using the equation for the line in Figure 4.1, this corresponds to an increase in plant-available solution P from 0.006 to 1600  $\mu\text{M}$ . The 270,000-fold increase in solution P concentration more than compensates for the 200-fold decrease in fertilised soil volume. Hence banding works because the P adsorption isotherm for Cavinti soil is non-linear: at low additions of fertiliser P (per gram of soil) there is no measurable increase soil solution P concentration, and at high fertiliser P additions, the relative increase in solution P concentration is much greater than the relative increase in soil P concentration.

#### 4.4.2 The effect of increasing P addition on the P buffer power

The P desorption isotherms (Figures 4.2 and 4.3) were best explained by Freundlich (log-log) equations, where the change in total P concentration in the whole soil ( $\Delta[\text{P}]$ ) is:

$$\Delta[\text{P}] = a[\text{P}_L]^b \quad (4.2)$$

The P buffer power ( $b_P$ , Equation 4.1) at any soil solution P concentration is given by the slope of the relationship between  $\Delta[\text{P}]$  and  $[\text{P}_L]$ . For Freundlich isotherms,  $b_P$  at any  $[\text{P}_L]$  is therefore:

$$b_P = ab[\text{P}_L]^{b-1} \quad (4.3)$$

The values for the constants  $a$  and  $b$  for each isotherm are given in Table 4.2. Since the values for the Freundlich constant  $b$  are always negative, Equation 4.3 shows that  $b_P$  decreases as  $[\text{P}_L]$  increases. The P buffer power also decreases as the amount of P fertiliser added to the soil increases, as shown in Table 4.2.

**Table 4.2.** Changes in soil solution P concentrations, Freundlich constants, and  $b_p$  when different rates of P fertiliser are added to Cavinti soil.

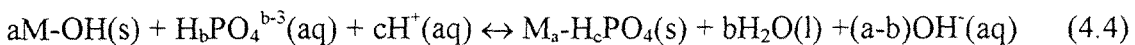
isotherm	$[P_L]_{initial}$	$[P_L]_{final}$	$a$	$b$	$b_p^†$
500 <sup>#</sup>	0.70	0.46	-3.760	-5.438	996
1000 <sup>#</sup>	2.1	1.8	$-4.590 \times 10^7$	-17.330	4135
2000	19.4	9.5	$-8.061 \times 10^7$	-4.625	240
4000	156	47	$-2.263 \times 10^5$	-0.744	70
5000	330	82	$-3.350 \times 10^5$	-0.626	50
6000	502	106	$-4.167 \times 10^5$	-0.523	51

<sup>†</sup> Since the values of  $\Delta[P]$  and  $[P_L]$  differ widely between the six isotherms,  $b_p$  was calculated from the Freundlich isotherms over the range of P desorbed by a constant sink, i.e. 500 mL of 0.02 M CaCl<sub>2</sub>

$$b_p \text{ was calculated using the formula } b_p = \frac{a([P_L]_2^b - [P_L]_1^b)}{[P_L]_2 - [P_L]_1}$$

<sup>#</sup> Note that when using least squares to fit curves, the errors increase as the slope of the curve becomes very steep (see Figure 4.3).

The changes in  $b_p$  with P addition are due to the effect of P sorption on soil surface charge. With increasing sorption, the surface negative charge increases, and a decreasing proportion of added P is sorbed on the soil solid. Some of the P sorbed displaces OH<sup>-</sup> ions from the soil solid (Bhatti *et al.* 1998), therefore the soil pH increases with increasing P addition (Figure 4.4). Goldberg and Sposito (1985) gave a generalised ligand exchange reaction for phosphate ions as follows:

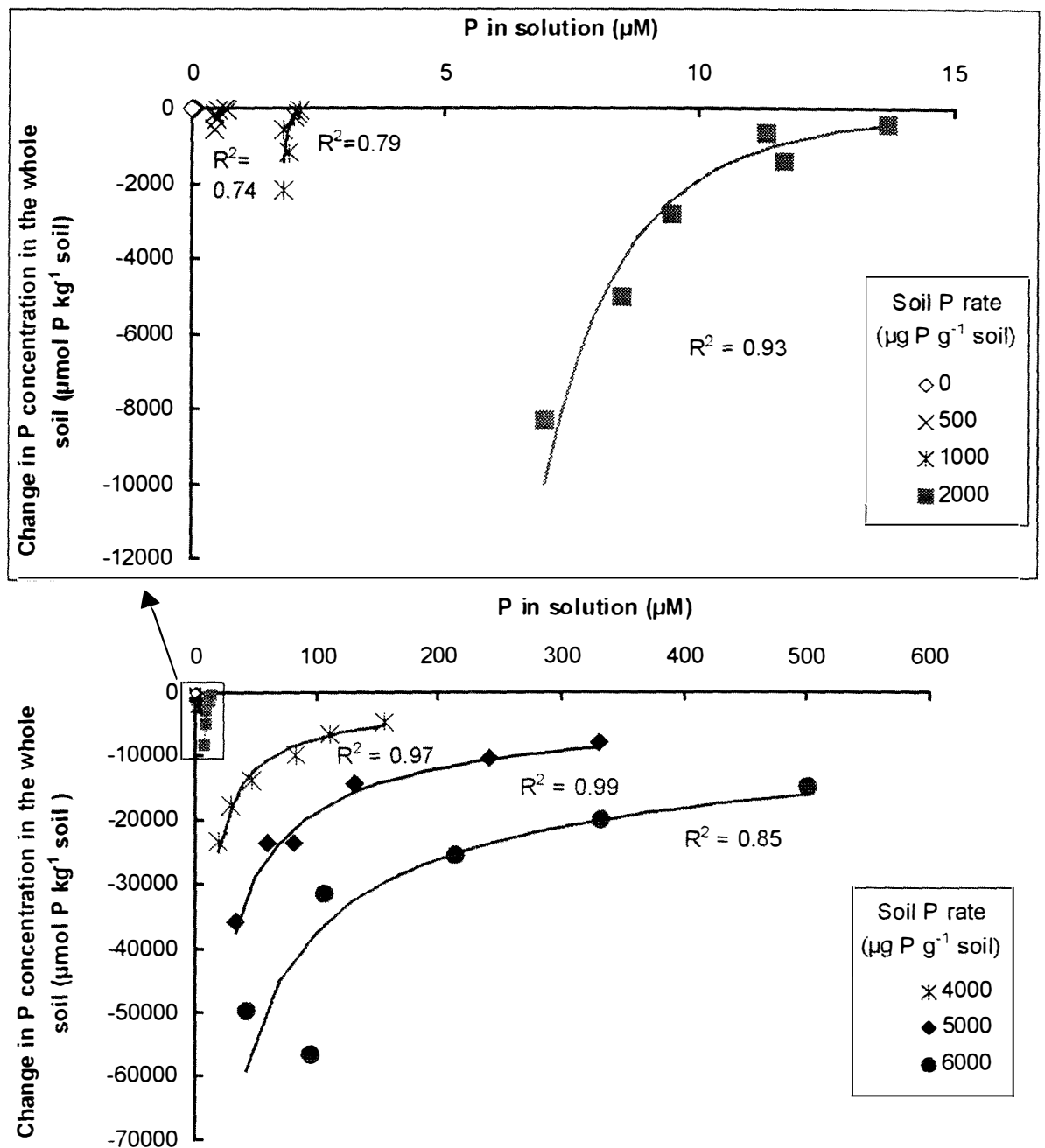


where: M refers to a metal ion in a hydroxylated mineral,

OH to a reactive surface hydroxyl,

$b \leq 3$  is the degree of protonation of the phosphate ion,

and (s), (aq) and (l) represent solid, aqueous and liquid, respectively.



**Figure 4.2.** Freundlich (log-log) P desorption isotherms for Cavinti soil. The numbers in the legend are the rates of P applied to the soil, in  $\mu\text{g P g}^{-1}$  soil. The volume of  $\text{CaCl}_2$  solution used to desorb P increases from right to left. The data have been split into two plots for clarity.

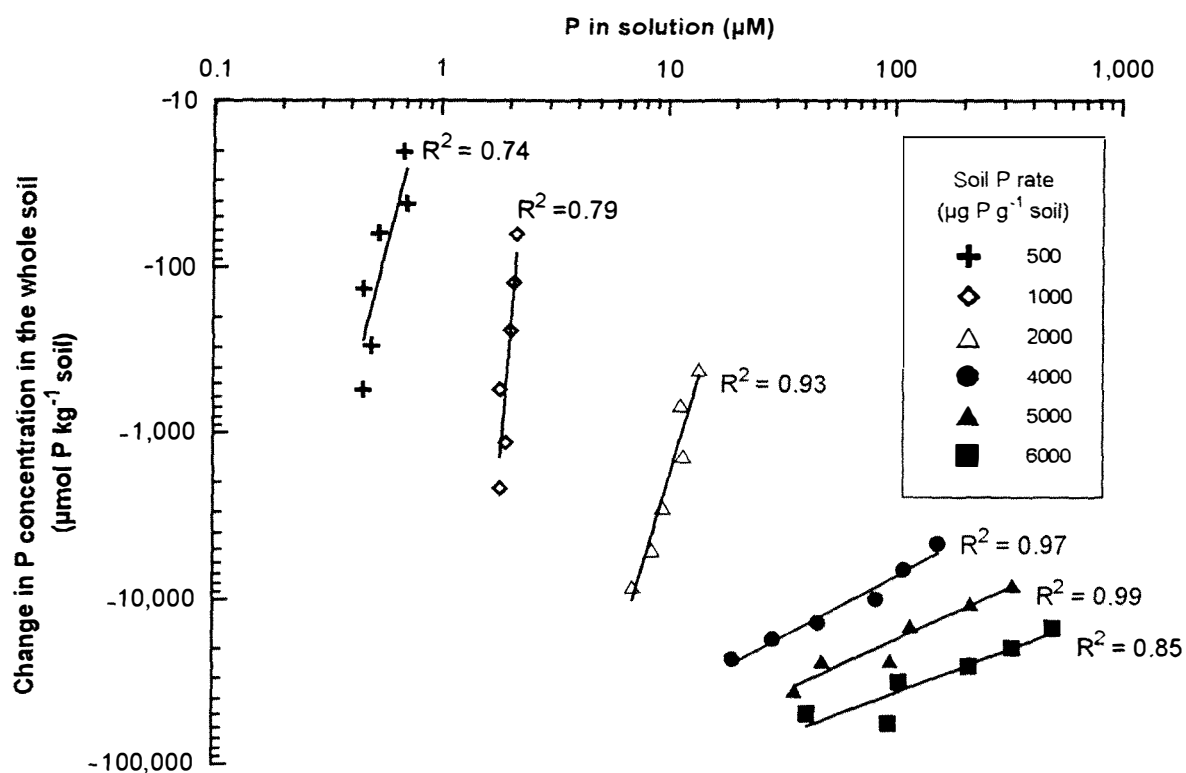


Figure 4.3. Log-log plots of P desorption isotherms for Cavinti soil. ‘Linear’ (log-log) regressions have been fitted for each soil P level.

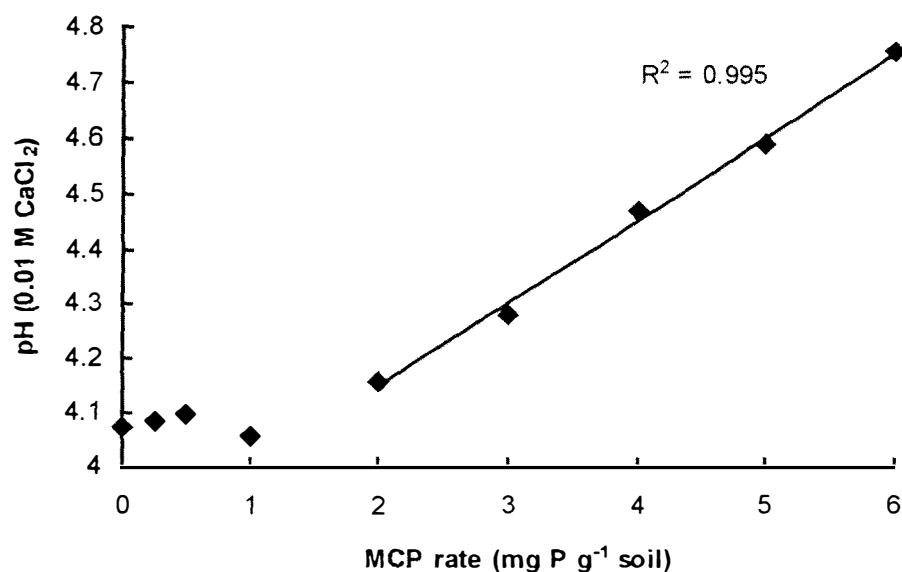


Figure 4.4. The effect of increasing soil MCP concentration on soil pH after 6 wk incubation. A linear regression has been fitted to describe the relationship above 2 mg P g<sup>-1</sup> soil.

### 4.4.3 Phosphate desorption by citrate

#### 4.4.3.1 Solution pH

The average pH of the citrate-CaCl<sub>2</sub> solutions after shaking was 4.35 (std dev. 0.22) for the P500 soil, and 4.54 (std dev. 0.19) for the P5000 soil. This relatively large variation in pH (shown by the large standard deviation) did not have an important effect on the amount of P released in this experiment, compared with the effect of citrate addition. For example, a difference in pH between two replicates, which was equivalent to an initial H<sup>+</sup> concentration of 150 μM H<sup>+</sup> in 250 mL,<sup>†</sup> resulted in a 0.7% difference in the amount of P in solution. For comparison, an increase in initial citrate concentration of 150 μM citrate in 250 mL gave a 160% increase in the concentration of P in solution. Further evidence that variation in pH had minimal effect on the amount of P desorbed was that there was no difference in the concentration of P in solution for P desorption isotherms conducted at initial pHs of 4.58 and 4.18 (in the absence of citrate) on P5000 Cavinti soil (Figure 9.9). Variation in pH appeared to only slightly affect the amount of Al, Mn and Fe desorbed. For example, the two replicates mentioned above that varied in initial H<sup>+</sup> concentration by 150 μM H<sup>+</sup> showed less than 4% variation in the concentration of Al, Mn and Fe in solution. Jones and Brassington (1998) found that citrate sorption by soil was not affected by solution pH over the range 4–8.

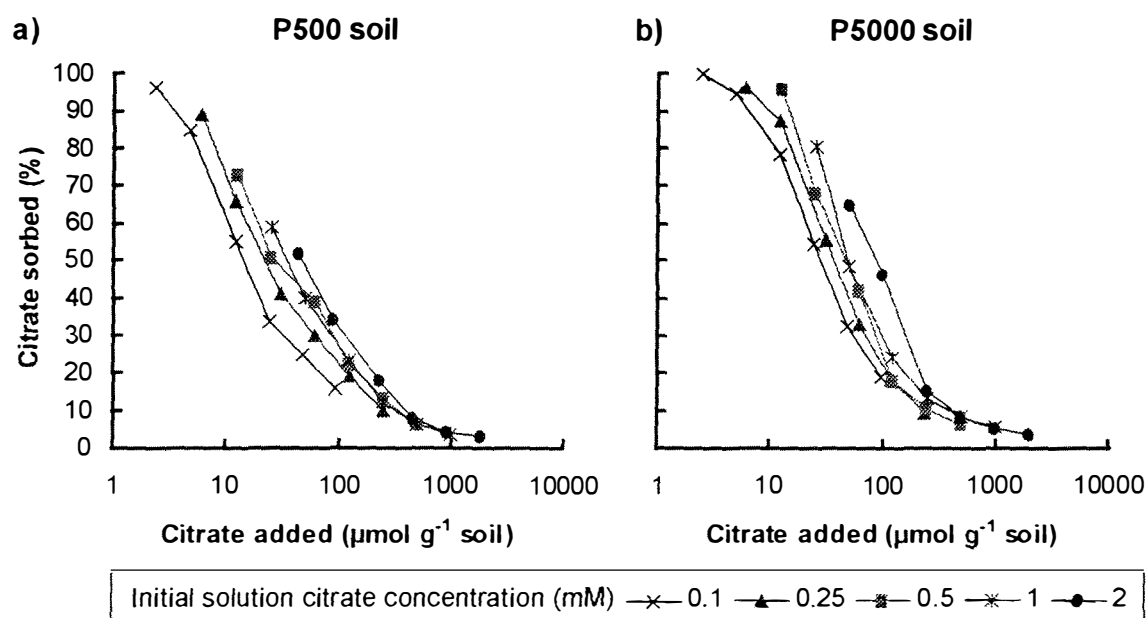
#### 4.4.3.2 Distribution of citrate between the soil surface and solution

At small amounts of citrate added, most of the citrate was sorbed by the soil (Figure 4.5a,b). This indicates that citrate has a stronger affinity for the solid phase, than for the Al in solution. GEOCHEM-PC 2.0 modelling confirmed that when most of the added citrate was bound to the soil surface (at small additions of citrate), Al in the soil solution existed as the free ion (Al<sup>3+</sup>). Jones and Brassington (1998) also found that most organic acids added to soil were rapidly sorbed to soil (> 80% of added citrate, oxalate, malate and acetate

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<sup>†</sup> Calculation: Two replicates for the 250 mL/ 0.5 mM citrate treatment from P500 soil had pH's of 4.49 and 4.77. From the buffer curve of Kirk *et al.* (1999b) for Cavinti soil this corresponds to a difference of 0.019 mmol H<sup>+</sup> g<sup>-1</sup> soil. For 2 g of soil and 250 mL, this corresponds to a difference in H<sup>+</sup> concentration of  $19 \times 2 \div 0.25 = 150 \mu\text{M}$ .

sorbed after 10 min, pH 4.5). They therefore suggested that this sorption would greatly reduce the effectiveness of organic acids to mobilise nutrients from the rhizosphere.



**Figure 4.5.** The percentage of citrate sorbed after a 16 h shake with Cavinti soil at two rates of P addition: 500  $\mu\text{g P g}^{-1}$  soil (P500) and 5000  $\mu\text{g P g}^{-1}$  soil (P5000).

#### 4.4.3.3 Amount of P, Fe, Mn or Al dissolved by citrate

The addition of citrate to Cavinti soil dramatically increased the dissolution of Al, Fe, and P but not Mn (Figures 4.6 and 4.7). Citrate solubilised approximately ten times more Al than Fe (Figure 4.7). Gerke (1992) also found greater solubilisation of Al than Fe when Na-citrate was added to a podsol (soil pH 5.3), or when citric acid was added to a luvisol (soil pH 7.3).

At small amounts of citrate added ( $< 250 \mu\text{mol g}^{-1}$  soil) there was a linear relationship between the number of moles of P, Fe and Al solubilised and the number of moles of citrate added (Figure 4.7). Above  $250 \mu\text{mol g}^{-1}$  soil this relationship reached a plateau suggesting that all citrate-soluble P, Fe or Al had been dissolved. Solubilisation of P (except in P500 soil), Al and Fe followed the same linear trend whether the amount of citrate was increased by raising the citrate concentration, or by increasing the volume of citrate solution

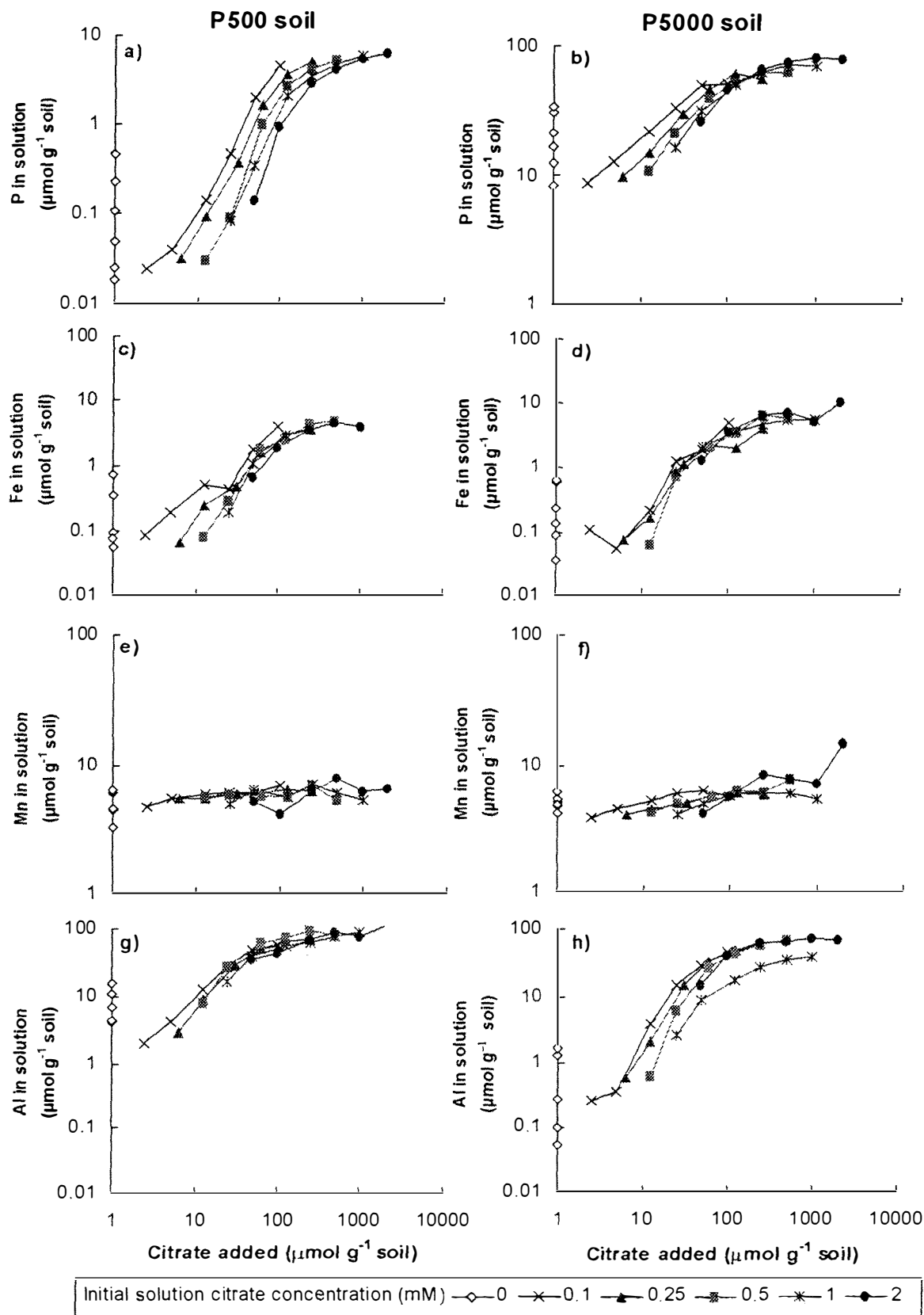
(Figures 4.7b-d,g,h). This indicates that the equilibrium for the solubilisation reaction is strongly in favour of the reaction products, even at low citrate concentrations. The reason for the exception in P500 soil, where the amount of P solubilised per mole of citrate added was lower at high soil:solution ratios, is presumably because more solubilised P is re-adsorbed in the strongly-buffered P500 soil, relative to the weaker buffered P5000 soil.

#### 4.4.3.4 Mechanism of dissolution of Al, Fe and P by citrate

The initial reaction of citrate with the soil was to bind to the soil surface, as discussed in Section 4.4.3.2. As the amount of citrate added to the soil increased, the negative charge on the soil surface would have increased (Nagarajah *et al.* 1968), further reducing the citrate-sorbing capacity of the soil. Calculations with the chemical speciation model GEOCHEM showed that in the pH range of the experiments, Al and Fe are expected to combine with citrate in a 1:1 ratio. This 1:1 ratio between the amount of citrate in solution and the amount of Al+Fe solubilised, is shown graphically in Figure 4.8a,b. This 1:1 ratio is maintained as more citrate is added, then reaches a plateau once all of the citrate-soluble Al and Fe is dissolved. Citrate did not solubilise Mn, presumably because Mn-citrate complexes are less stable than those of Fe or Al (Appendix A). GEOCHEM modelling indicated that <0.1% of the citrate was bound to Mn.

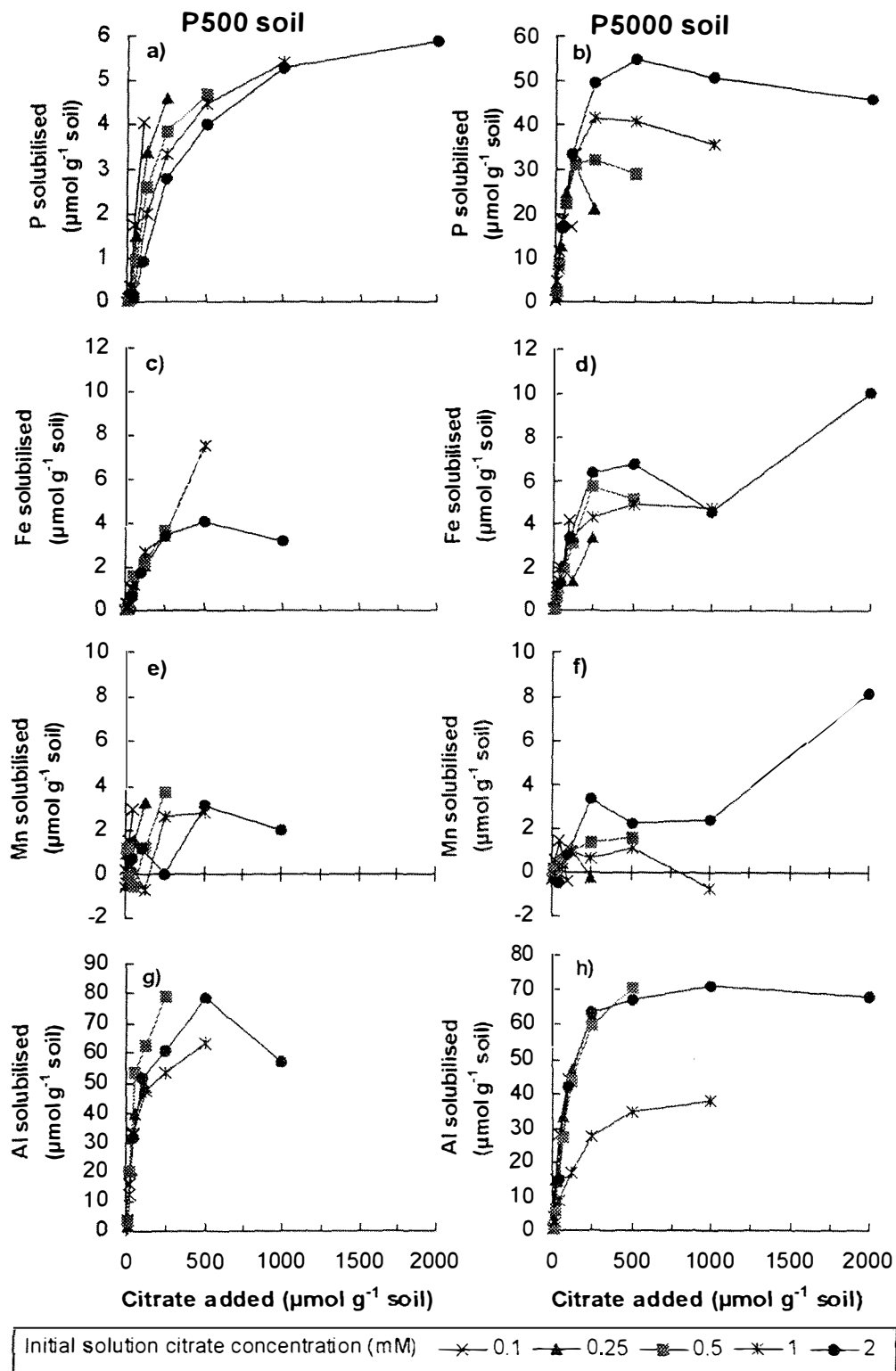
The release of P followed the same trend as the dissolution of Al and Fe (Figure 4.7). Presumably this indicates that P release by citrate is the result of the reduction of sorption sites due to the dissolution of Al and Fe hydrous oxides, as suggested by Earl *et al.* (1979). Some of the Fe, Al and P dissolved by citrate may also be associated with humic compounds (Gerke 1992, 1993b).

Given that Al is mobilised by citrate as an Al-citrate complex, this would allay fears that citric acid excretion by roots might exacerbate Al toxicity, since the Al-citrate complex is non-toxic to plants (Hue *et al.* 1986). In fact, citrate excretion by roots has been identified as a mechanism by which plants can tolerate high Al concentrations (Miyasaka *et al.* 1991, de la Fuente *et al.* 1997).

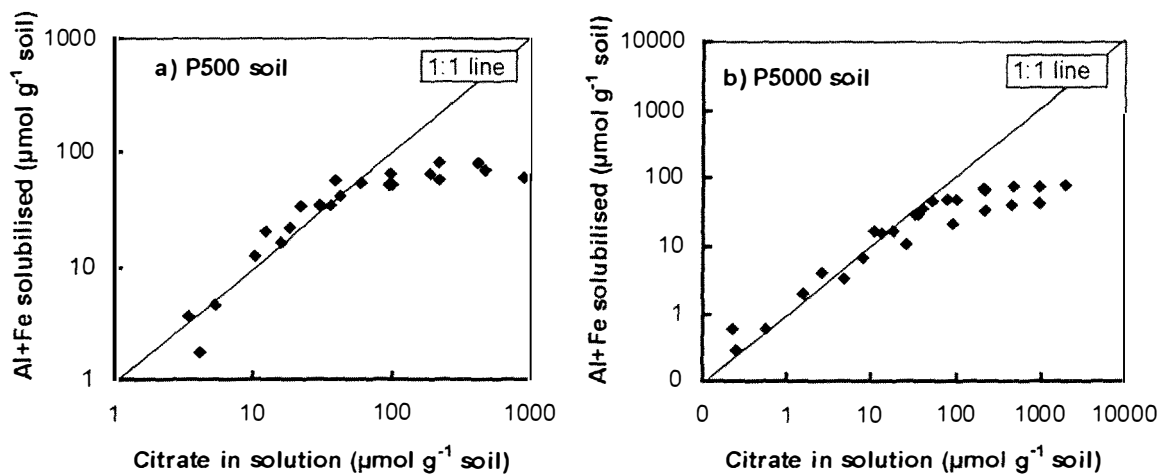


**Figure 4.6.** The amounts of P, Fe, Mn, and Al in solution for a given addition of citrate to P500 (left) and P5000 (right) Cavinti soil. The volume of  $\text{CaCl}_2$ -citrate solution used increases from left to right.





**Figure 4.7.** The amount of P, Fe, Mn, or Al solubilised for a given addition of citrate to P500 (left) and P5000 (right) Cavinti soil. Note: the amount solubilised = moles in solution in the presence of citrate - moles in solution in the zero citrate treatment. The volume of  $\text{CaCl}_2$ -citrate solution used increases from left to right.



**Figure 4.8.** The relationship between the amount of Al+Fe solubilised and the amount of citrate in solution for P500 and P5000 Cavinti soil.

#### 4.4.3.5 The recovery of fertiliser P by citrate, and the implications for soil P solubilisation by citrate exuded from rice roots

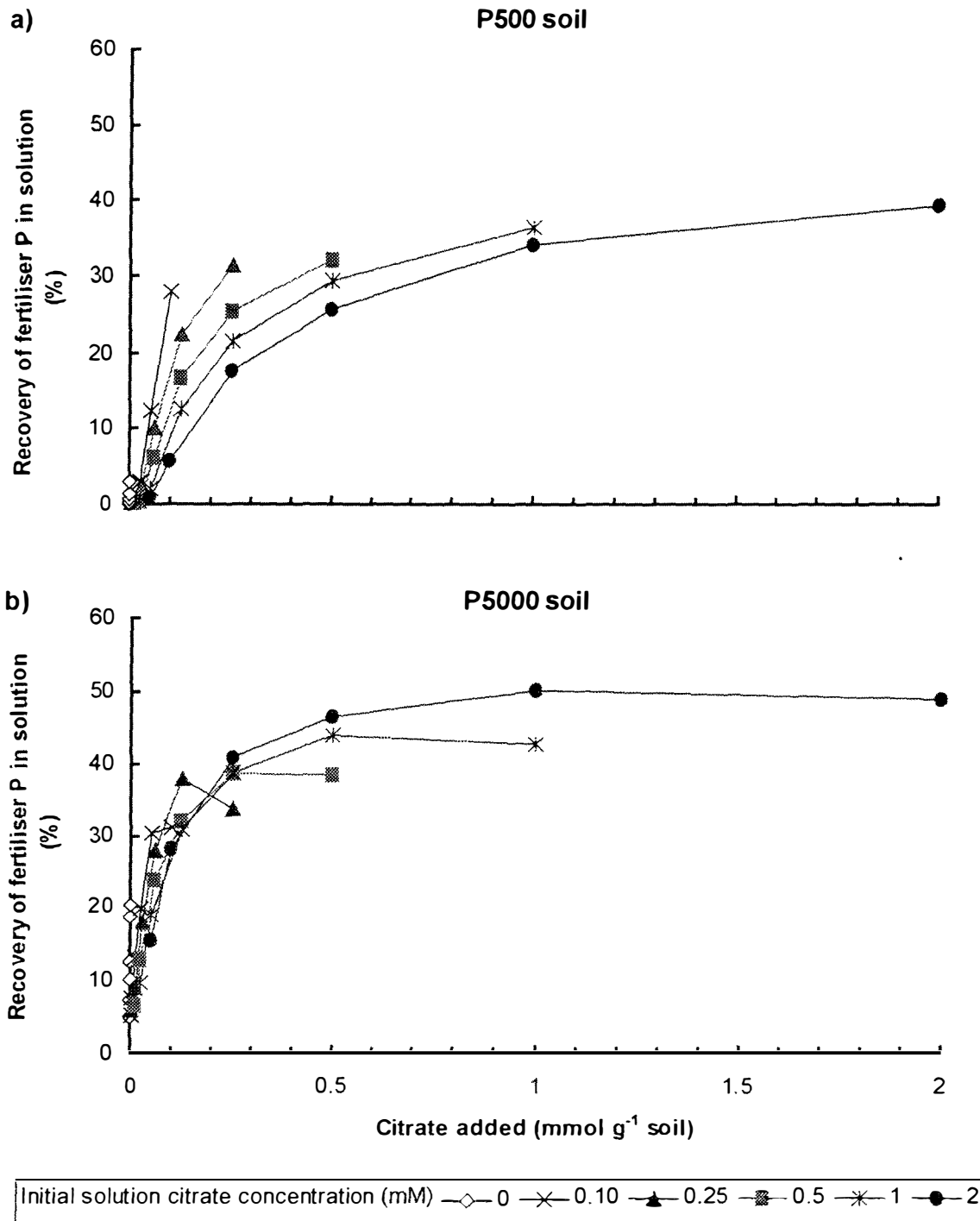
The addition of 0.1 mmol citrate  $\text{g}^{-1}$  soil recovered into solution approximately 30% of the fertiliser P from P500 and P5000 Cavinti soil (Figure 4.9). The addition of a further 1.9 mmol citrate  $\text{g}^{-1}$  soil recovered only an additional 11% or 19% of the added fertiliser P, from P500 or P5000 soil, respectively. One-tenth mmol citrate  $\text{g}^{-1}$  soil is a relatively high concentration in terms of observed citrate concentrations in rhizosphere soil. The highest citrate concentration measured in the rhizosphere soil of plants, 0.048 mmol citrate  $\text{g}^{-1}$  soil in the rhizosphere of white lupin (Dinkelaker *et al.* 1989), is half this value. This shows that it would be possible to increase the amount of P solubilised by plants by increasing citrate production, although more research would need to be done to assess whether increasing citrate release from roots was the most energy-efficient strategy.

Kirk *et al.* (1999b) reported citrate concentrations in the rhizosphere of moderately P stressed upland rice plants of around 3  $\mu\text{mol}$  citrate  $\text{g}^{-1}$  soil. Additions of 2.5  $\mu\text{mol}$  citrate  $\text{g}^{-1}$  soil to P500 soil gave a 30% increase in P solution concentration (Figure 4.6a). At low solution P concentrations this degree of increase can give rise to a significant increase in P uptake, as indicated by the modelling of Kirk *et al.* (1999b).

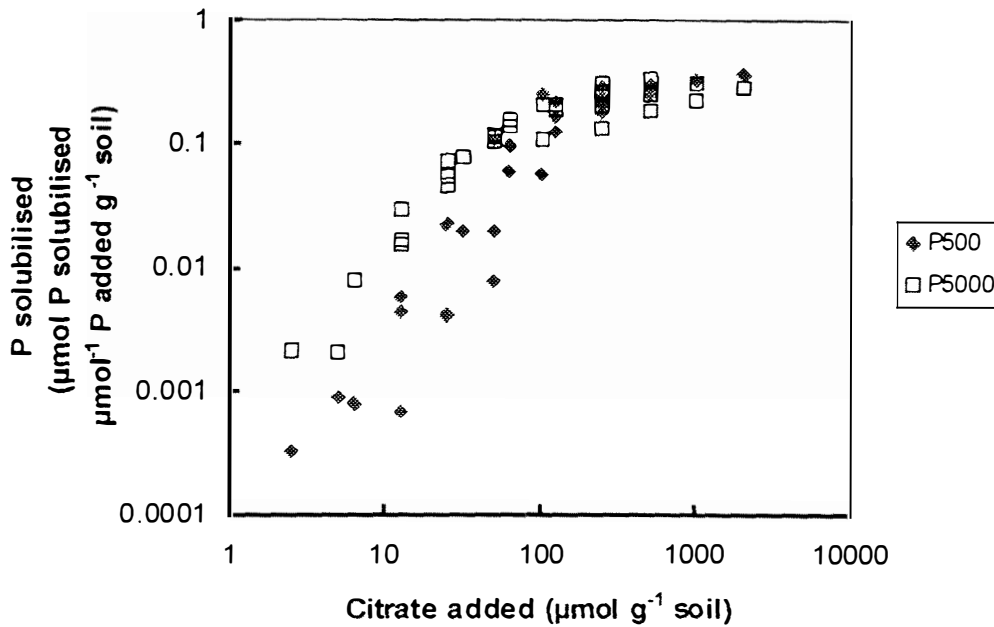
The plateauing of the P recovery curves (Figure 4.9) shows that only 40 to 50% of the added fertiliser P could be recovered from P500 and P5000 soils by a 16 h shake with citrate. This suggests that a portion of this unrecovered P became occluded inside soil aggregates formed during the 4 wk incubation period. Furthermore, P may have been sorbed to P-fixing minerals that are only slowly soluble in citrate. Gerke (1993a) found that just over 1% of the total Fe in a poorly crystalline Fe-oxide was solubilised by the addition of  $0.056 \text{ mmol citrate mmol}^{-1} \text{ Fe}$  (pH 4) after a 16 h shake.

#### **4.4.3.6 Enhanced dissolution of P by an interaction between high soil P concentration and citrate addition**

At small additions of citrate ( $< 100 \text{ } \mu\text{mol citrate g}^{-1} \text{ soil}$ ), the amount of P solubilised by citrate per mole of P added to the soil was increased notably by increasing the soil P concentration (Figure 4.10). A t-test was used to compare the amount of P solubilised per mole of P added, per mole of citrate added, in the P500 and the P5000 soil, for citrate additions of  $\leq 100 \text{ } \mu\text{mol citrate g}^{-1} \text{ soil}$ ; the null hypothesis being that the two treatment means were equal. The t-test showed that there was highly significantly ( $P < 0.0001$ ) more P solubilised  $\text{mol}^{-1} \text{ citrate mol}^{-1} \text{ P}$  added in the P5000 soil. The greater effectiveness of citrate at mobilising P from high P fertilised soils may have been due to an increase in negative charge on the soil surface of the high P fertilised soil (Hingston *et al.* 1967), which decreased the affinity of the soil surface for P. This is illustrated by a decrease in buffer power with increasing soil P concentration (Table 4.2). The positive interaction between the soil P concentration and amount of P solubilised by citrate shows that there is the potential to increase the efficiency of P mobilisation by root-released citrate, by banding of P in Cavinti soil.



**Figure 4.9.** The proportion of fertiliser P recovered into solution after shaking Cavinti soil for 16 h with different amounts of citrate. Both soils were fertilised with MCP: P500 soil at 500  $\mu\text{g P g}^{-1}$  soil and P5000 at 5000  $\mu\text{g P g}^{-1}$  soil.



**Figure 4.10.** The amount of P solubilised by citrate per  $\mu\text{mole}$  of P added to Cavinti soil plotted against the amount of citrate added. Note: the amount of P solubilised = moles of P in solution in the presence of citrate - moles of P in solution in the zero citrate treatment.

## 4.5 Conclusions

The Cavinti soil has a high P-fixation capacity and a highly non-linear P adsorption isotherm, making it a suitable soil to study the effects of banding and the interaction with citrate. The highly non-linear shape of the P adsorption isotherm makes it theoretically possible to considerably increase the amount of plant-available P in solution for upland rice by reducing the volume of soil fertilised, without needing to apply large amounts of P fertiliser, which would be required if P fertiliser was mixed through the plough layer.

The P buffer power of the Cavinti soil decreases as the soil solution P concentration increases, and as the amount of P added to the soil increases.

The addition of citrate to the soil increased the concentration of Al, Fe and P in solution, but had no effect on Mn. The mechanism by which citrate increased the P in solution was

therefore probably due to the dissolution of P-sorbing Al/Fe hydrous oxides and perhaps Al/Fe-humic-P complexes. The large increase in P solubilised from a modest addition of citrate ( $< 0.1$  mmol citrate  $\text{g}^{-1}$  soil) indicates that citrate release from plant roots has the potential to increase the amount of plant-available P in highly weathered, acidic tropical soils that contain large amounts of Fe and Al. Furthermore, there was a strong interaction between the soil P concentration and amount of P solubilised by citrate, indicating that there is the potential to increase the efficiency of P mobilisation by citrate, by banding of P in Cavinti soil.

## 5. Studies on P uptake mechanisms I. Observed and calculated uptake in banded and incorporated thin layer systems

### 5.1 Introduction

#### 5.1.1 *P uptake efficiency mechanisms: moving from theory to practice*

In Chapter 4, the P sorption curves for Cavinti soil were highly curvilinear, indicating that, by restricting the volume of soil in contact with P fertiliser by banding (or localised placement), the plant availability of P may be significantly increased. Experiments presented in Chapter 4 also demonstrated that a significant amount of P could be mobilised from Cavinti soil by citrate. Furthermore, evidence was provided for a positive interaction between these two strategies: significantly more P was solubilised (per mole of P added), by small additions of citrate, when the soil P concentration was increased – which could be achieved by banding of P.

Much research has been done on the strategy of banding of fertiliser P, and the circumstances under which banding is a beneficial strategy are beginning to be understood (Anghinoni and Barber, 1980b, Garrity *et al.* 1990). However, the quantitative role of organic anions in enhancing P uptake has only been studied relatively recently, and there is scant information on the interaction between banding of P and organic anion release. One pioneering study in this field was that by Hoffland *et al.* (1992). Hoffland *et al.*'s study showed that rape was able to alter its pattern of organic anion release and concentrate organic anion release in P-rich zones. One of the most P efficient plants studied, white lupin, has the ability to proliferate numerous fine roots and excrete large amounts of citrate (Dinkelaker *et al.* 1989). This enables it to extract acid-soluble P from otherwise very slowly-available sources of P.

Rice has been found to release organic anions, particularly citrate, when grown under aerobic conditions (Liu *et al.* 1990, Kirk *et al.* 1999b). The concentrations of citrate in the rhizosphere of rice in the study of Kirk *et al.* (1999b) ( $0.5 - 3 \mu\text{mol g}^{-1}$  soil) were much less than that found by Dinkelaker *et al.* (1989) for white lupin ( $48 \mu\text{mol g}^{-1}$  soil). However, modelling done by Kirk *et al.* (1999b) indicated that this rate of citrate release significantly enhanced the uptake of P from Cavinti soil. This chapter attempts to seek experimental evidence that banding and release of organic anions can combine to create efficient P uptake in the rhizosphere of upland rice.

### **5.1.2 Means of identifying P efficiency – justification for the methodology used**

In order to develop P efficient management strategies, and to breed P efficient plants, it is first important to identify the mechanisms that contribute to P efficiency (see Section 1.5.3.3) and to quantify their effects. It is extremely difficult to study P uptake mechanisms under field conditions, where soil spatial heterogeneity and complex root geometry greatly complicate experimental measurements. A better way of making progress is with controlled laboratory experiments that focus on particular processes, and are supplemented with mathematical modelling. In this chapter a thin-layer (TL) experimental system was used to study the processes involved in soil P mobilisation in the rhizosphere soil within a few millimetres of root surfaces. To obtain sufficient rhizosphere soil for detailed study, plants were grown with their roots ‘sandwiched’ between two 3-mm thick layers of soil. The roots were separated from the soil by fine mesh sheeting, but root hairs, water and solutes could pass through the mesh freely. Therefore all the soil in the TL systems could be analysed as rhizosphere soil.



The results were analysed with the help of a mathematical model (Kirk 1999 and Kirk *et al.* 1999b), outlined in Section 5.3.2.5. The model is used to first quantify the amount of P taken up by diffusion in the absence of solubilisation or other factors that might enhance P uptake. If the observed uptake is not explained by diffusion of readily-available P<sup>†</sup>, then additional factors that affect P uptake such as the release of organic anions or H<sup>+</sup> are incorporated into the model, until the observed P uptake is explained. This chapter describes a preliminary experiment (Section 5.2) followed by a main experiment (Sections 5.3–5.4).

## 5.2 Preliminary experiment

### 5.2.1 Objective of the preliminary experiment

The objective of this preliminary experiment was to identify a suitable soil P status for the TL systems (described in Section 5.2.2.2) used in the main experiment.

### 5.2.2 Method used for the preliminary experiment

#### 5.2.2.1 Soil

The soil used was the isohypothermic palehumult from Cavinti, Philippines, described in Chapter 4. Three hundred grams of 0.5 mm sieved, air-dried soil was hand-mixed with MCP at the following rates: 100, 200, 300, 500, 1000, 3000, 10000  $\mu\text{g P g}^{-1}$  soil (referred to as P100, P200 etc). These soils were moistened with 0.3  $\text{cm}^3$  water  $\text{cm}^{-3}$  soil then incubated at 40°C for 4 d. After incubation, the soils were dried at 60°C, re-sieved to < 2 mm and packed into TL systems (see Section 5.2.2.2) at 40 g of air-dried soil per side (Kirk *et al.* 1999b). There were three replicates of each treatment.

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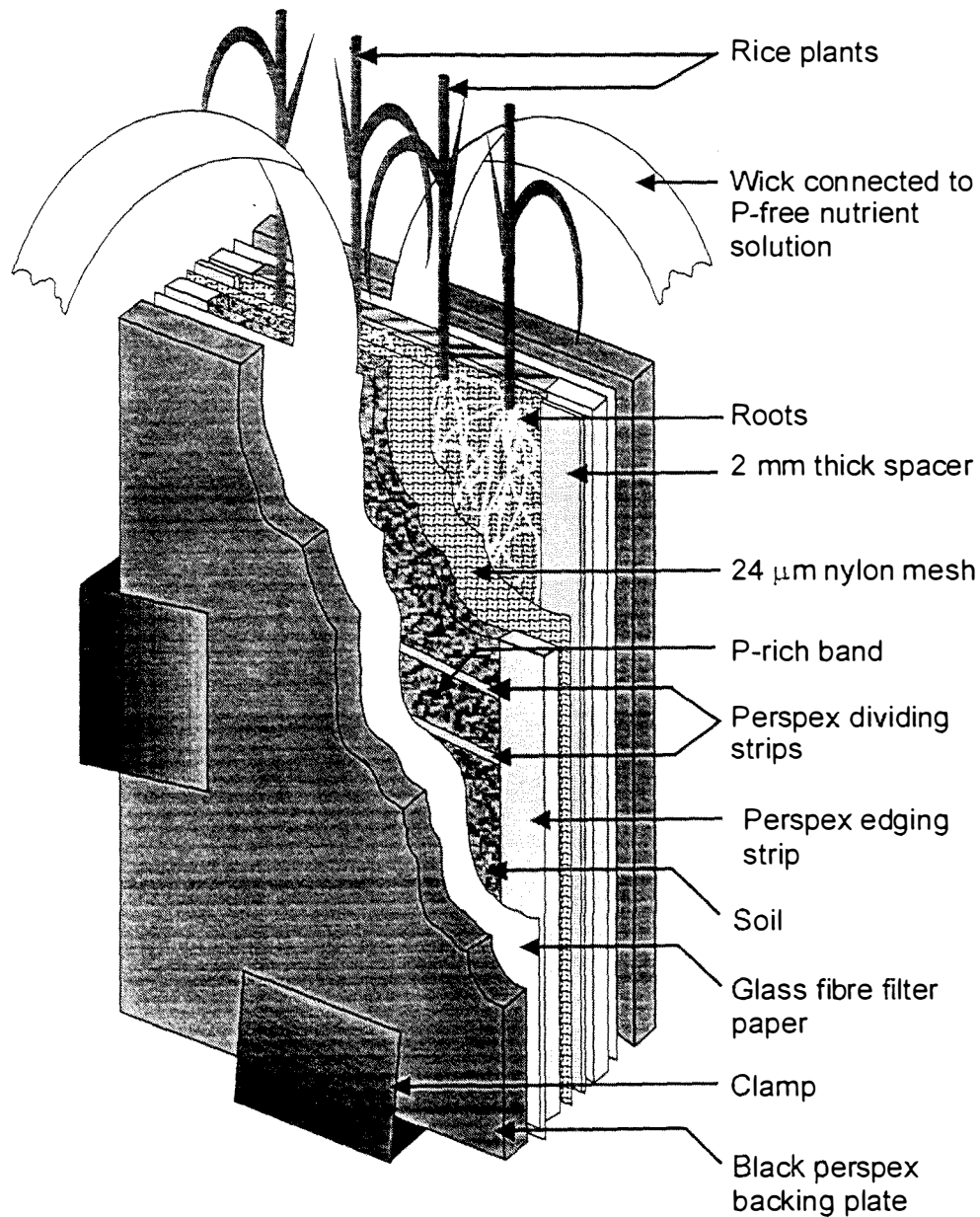
<sup>†</sup> readily-available P in these thin layer chapters refers to P that was desorbable by 0.02 M CaCl<sub>2</sub>

### 5.2.2.2 The thin layer system

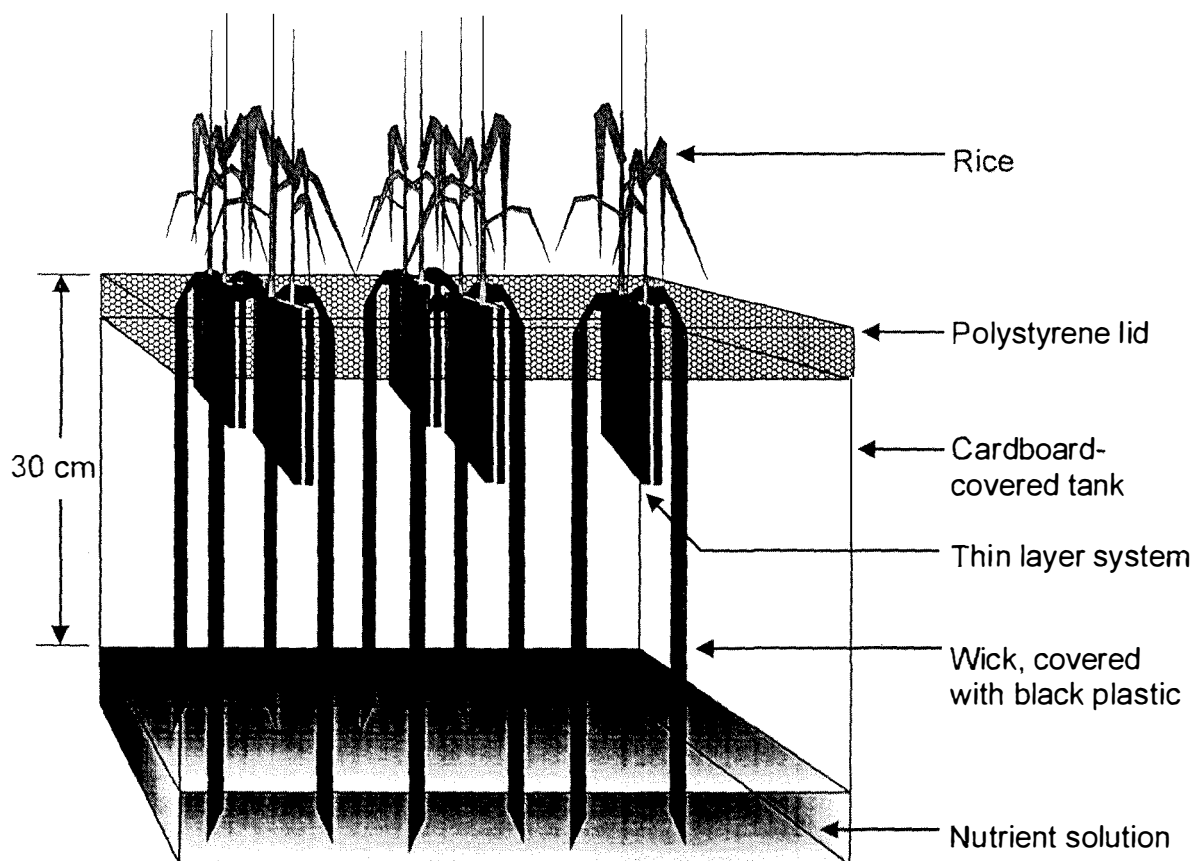
In brief, rice was grown in moist, aerobic soil in a TL system similar to that shown in Figure 5.1. The only difference was that there were no perspex dividing strips in the present experiment (these were used for the banded treatment, Section 5.3.2.1), as the P fertiliser was incorporated throughout the whole soil. The TL system comprised a mat of roots 'sandwiched' between two 3-mm thick layers of soil. The roots were kept separate from the soil by a 24  $\mu\text{m}$  pore-diameter nylon mesh sheet. The soil layers were connected to a nutrient solution reservoir via glass-fibre filter paper wicks. The wicks were covered with black plastic sheeting to reduce water loss and algal growth. The TL systems were suspended in cardboard-covered perspex tanks so that the soil surface was flush with the top of the tank, and the wicks dipped into the nutrient solution that was contained in the bottom of the tank (Figure 5.2). The tanks had polystyrene lids to reduce temperature changes in the nutrient solution and thin-layers.

### 5.2.2.3 Plants

Rice (cv. Azucena) seeds were pre-germinated in water for 24 h, then grown on a floating screen in a container of P-free nutrient solution (Yoshida *et al.* 1976, Appendix B) for 1 wk before being transplanted into the TL systems. Plants were harvested 4 wk after transplanting, dried at 60°C and weighed.



**Figure 5.1.** The TL system developed for growing a planar layer of roots between two small volumes of soil. Each thin layer of soil was 130 mm high, 80 mm wide, and 3 mm deep. The TL system for the banded treatment (shown above) had the same amount of P fertiliser as the incorporated treatment, concentrated in a band occupying one-tenth of the soil volume (13 mm high, 80 mm wide, and 3 mm deep) of the incorporated treatment.



**Figure 5.2.** The experimental set-up used to grow rice in the TL systems. The tank and lid have been drawn transparent for clarity, and only two rice plants per TL system are shown (in the experiment there were four plants per system).

### 5.2.3 Results of the preliminary experiment

#### 5.2.3.1 Plant growth

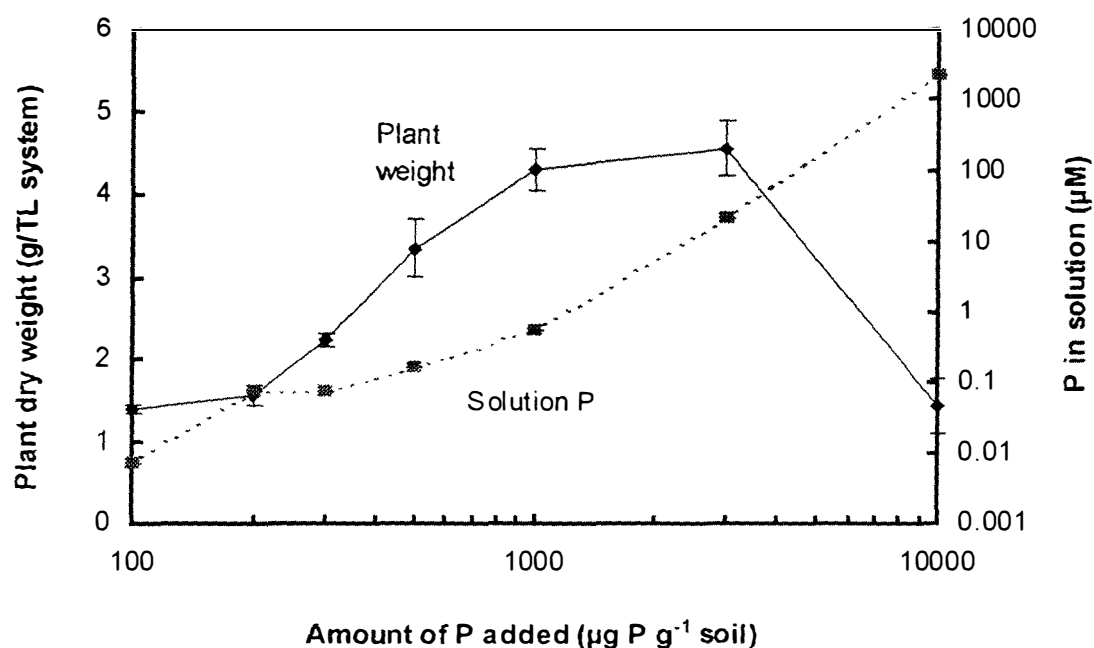
The leaves of rice from the P10000 treatment showed obvious toxicity symptoms, they appeared to bruise and turned brown almost immediately after transplanting. No toxicity symptoms were observed in plants from other treatments. Three weeks after transplanting the two oldest leaves in plants grown in the P100 and P200 treatments had turned yellow-brown and died, the oldest leaf died in the P300 and P500 treatments, but only the leaf-tips died in the P1000 and P3000 treatments. The two oldest leaves of plants left growing in

P-free nutrient solution also turned yellow-brown and died, suggesting that the senescence of the older leaves in the low-P TL systems was due to P deficiency.

Growth was most rapid in the P3000 treatment, which tillered first. Leaves of the P3000 treatment began to roll shortly after tillering, indicating water stress. This could not be rectified by watering from above once a day or by raising the level of nutrient solution in the reservoir. The P1000 treatment began to tiller about 1 wk after the P3000 treatment. It showed no signs of water stress and began to catch up to the P3000 treatment. These reasons suggest that the water stress observed in the P3000 treatment was due to the high ionic strength of the soil solution, however conductivities were not measured. By harvest time more leaves had died in all treatments, except the P10000, which was beginning to recover.

Plant dry weights showed a marked increase from 300 to 1,000  $\mu\text{g P g}^{-1}$  soil (Figure 5.3). Plant weights decreased above 3,000  $\mu\text{g P g}^{-1}$  soil. Based on the visual symptoms, and the P analyses of herbage from another almost identical experiment (data not shown), the shape of the response curve is best described as follows. The increasing growth rates correspond to increasing concentrations of plant-available P in the low fertiliser treatments, the plateau indicates adequate P in the P1000 and P3000 treatments, and the decline in growth is due to toxic conditions at high P concentration.

By comparing the P response curve to the P adsorption isotherm (from Chapter 4) for Cavinti soil (Figure 5.3), the optimum soil solution P concentration for rice growth in the TL system is around 6.5  $\mu\text{M}$ . Beckwith (1965) used P adsorption isotherms to estimate fertiliser requirements and suggested that a soil solution P concentration of 6.5  $\mu\text{M}$  was adequate for most crop species. However, Fox (1981) stated that low P-requiring crops (rice is a low P-requiring crop) could grow adequately at a soil solution P concentration  $> 0.65 \mu\text{M}$ . The reason why rice grown in the thin layers required high soil P concentrations is presumably because the root system was confined to a small volume of soil. Large rates of P had to be applied to the soil in order to obtain a soil solution P concentration of 6.5  $\mu\text{M}$  because of the low P status and high P-fixing capacity of Cavinti soil (Figure 4.1).



**Figure 5.3.** Plant dry weight after 4 wk growth in the TL systems with different additions of MCP. Also shown are the initial P concentrations in the soil solution estimated from the soil P adsorption isotherm (Figure 4.1). Dry weight data are means  $\pm$  SE,  $n=3$ .

#### 5.2.4 Conclusions of the preliminary experiment

Rice growth in the TL systems was responsive to increased soil P supply up to 1,000  $\mu\text{g P g}^{-1}$  soil. Soil P additions of 10,000  $\mu\text{g P g}^{-1}$  soil decrease plant growth. A suitable soil P concentration for a thin layer experiment appears to be 200–500  $\mu\text{g P g}^{-1}$  soil, as plant growth was very responsive to any increases in P availability over this range. This soil P concentration remains quite deficient, which hopefully would induce plants to exhibit any traits necessary to solubilise P, e.g. to release organic anions.

## 5.3 Main Experiment

### 5.3.1 Objectives

The objectives of this experiment were to identify and quantify mechanisms governing P uptake by rice in aerobic, highly weathered soils. Of particular interest, was the management strategy of banding fertiliser P, and the role of root-released organic acids in enhancing P uptake.

### 5.3.2 Materials and methods

#### 5.3.2.1 Preparation of thin layers

Based on the results of the preliminary experiment, MCP was mixed with air-dried, 0.5 mm sieved Cavinti soil at a rate of 200  $\mu\text{g P g}^{-1}$  soil. Cavinti soil fertilised at 2000  $\mu\text{g P g}^{-1}$  soil was also prepared for use in a banded thin layer treatment, where the same amount of P (as the incorporated treatment) would be concentrated in one-tenth of the volume of the thin layer. The soil was moistened with 0.3  $\text{cm}^3$  water  $\text{cm}^{-3}$  soil, incubated for 4 wk at 23°C (lab. temperature), then dried at 60°C, re-sieved to < 2 mm and mixed thoroughly by end-over-end shaking overnight.

Portions (40 g) of air-dried soil were packed uniformly into thin-layers (described in Section 5.2.2.2), giving a bulk density of 1.16 kg oven-dry soil  $\text{dm}^{-3}$ . For the banded treatment, 4 g of 2000  $\mu\text{g P g}^{-1}$  soil was packed between two thin (2 mm) perspex strips glued 1.3 cm apart, i.e. one-tenth of the height of the thin layer (Figure 5.1). Unfertilised soil (17.38 g) was then packed into equal-sized compartments either side of the band.

#### 5.3.2.2 Plant growth system

The TL systems were wet-up to 0.45  $\text{cm}^3$  water  $\text{cm}^{-3}$  soil via filter paper wicks that dipped into water-filled reservoirs (Figure 5.2). The water level was approximately 30 cm below the top of the TL systems. The TL systems were equilibrated in the reservoirs in a controlled-environment glasshouse for 4–5 wk, then 4 d before planting the water in the

reservoirs was replaced with P-free, full strength nutrient solution (as written in Yoshida *et al.* (1976) except that the 36  $\mu\text{M}$  Fe as Fe-citrate was replaced with 89  $\mu\text{M}$  Fe as Fe-EDTA, see Appendix B). The volume of solution in the reservoirs was adjusted as necessary over the course of plant growth to maintain the volumetric soil moisture content at 0.45.

A large number of upland rice (cv. Azucena) seeds were surface sterilised with 1%  $\text{HgCl}_2$  and then germinated in distilled water for 2 d. They were then grown in low P (32  $\mu\text{M}$ ) but otherwise full strength nutrient solution (as above) for 12 d. Four seedlings (selected for similar root lengths) were transplanted into each TL system by spreading the roots across the mesh-covered face of one of the thin layers then placing the other thin layer on top. There were twenty planted and ten unplanted (control) TL systems of each treatment. The plants were grown in the temperature-and-humidity controlled glasshouse (day/night temperatures of 29/21°C, 70% relative humidity) with ambient light (c. 1000  $\mu\text{E m}^{-2} \text{s}^{-1}$ , 12 h photoperiod). The nutrient solution (full strength P-free Yoshida) in the reservoirs was changed approximately every 4 d and the pH was adjusted every 1–2 d to 5.0.

### 5.3.2.3 Plant analysis

Two unplanted and four planted TL systems were harvested for each treatment at 2, 3, 4, 5 and 6 wk after transplanting. At harvest, the systems were dismantled. The roots covering the P-rich band were carefully cut out from the roots that had been covering the unfertilised soil, then all roots were soaked in ethanol containing methyl violet for 48 h and analysed for coarse, medium, and fine root length using a delta-T root scanner (Webb and Potschul 1990). A subsample of 30 – 60 roots from each P treatment (P0, P200, and P2000), at 5 and 6 wk after transplanting were examined for mycorrhizal infection using the staining technique of Koske and Gemma (1989), except that a 0.525% sodium hypochlorite solution (used by Bevege 1968) was used to remove the methyl violet stain. (Note that in subsequent experiments root length was not determined, so no hypochlorite was necessary.) The percentage of root length infected was assessed by observing between thirty and ninety 1.8 mm lengths of root and then recording each length as infected, or non-infected.



The roots and shoots were then dried at 67°C for 48 h, weighed, and duplicate subsamples of 500 mg were digested with concentrated nitric and perchloric acid (Jones and Case 1990), then analysed for P (Murphy and Riley 1962). Representative seedlings at the transplanting stage (from a separate experiment using identical conditions) were also dried, weighed and their P contents determined.

To obtain an estimate of organic anion release rates from the rice roots, one side of the TL system was removed and a filter paper, moistened with 4 mL of deionised water, was pressed against the root mat for 4.5 h immediately prior to the sixth week harvest. During this time plants remained in the controlled-environment glasshouse and one wick remained in the nutrient solution. Filter papers were then immediately cut into sections to separate paper that had been exposed to the roots from the fertilised band from paper exposed to roots covering the unfertilised soil of the banded treatment. Filter papers were immediately placed in centrifuge tubes and frozen for 2 months. The filter papers were then extracted with 20 mL (3 mL for band) of 18 mM  $\text{KH}_2\text{PO}_4$  and analysed for organic acids by HPLC, as described in Section 4.3.3.

To determine the effect of mass flow on P uptake, an additional experiment was conducted to estimate the amount of water transpired. A known volume of nutrient solution was poured into a reservoir holding three TL systems containing plants that had been grown for 3½ wk post-transplanting. The lid and the soil surface of the TL systems were sealed with plastic tape and the plants grown on for 5 d in the climate-controlled glasshouse under the same conditions as those in the experiment. The difference between the initial and final volume of the nutrient solution was used to calculate transpiration per gram of plant dry weight. Phosphorus uptake by mass flow was calculated by multiplying the average concentration of P in the soil solution by the amount of water used by the plant for transpiration.

#### 5.3.2.4 Soil analysis

At each of the five harvests, the soil removed from each TL system was thoroughly mixed. For the banded treatment, the band of fertilised soil was kept separate from the unfertilised soil. Duplicate 13.8 g samples (a single 6.7 g sample was taken from the band soil) of moist

soil were immediately extracted with 0.1 M HCl at a 1:1 soil (dry-weight basis): solution ratio (Bolan *et al.* 1997), and then frozen, awaiting organic acid analysis by HPLC (described in Section 4.3.3). Soil solution P was estimated by adding 20 mL of P-free Yoshida nutrient solution (Section 5.3.2.2) containing  $70 \text{ mg L}^{-1} \text{ HgCl}_2$  to duplicate 10.9 g samples of moist soil (8.0 g of soil on an air-dry basis, assuming a water content of 57% for the TL systems and water content of 15% for air-dried soil). For the soil from the P-rich band, a single sample of 2.3 g of moist soil was extracted with Yoshida nutrient solution at the same soil:solution ratio. (Note that the EDTA was found to interfere with the Murphy and Riley P assay, particularly in the blank treatments, which did not contain soil, so in later thin layer experiments 0.01 M  $\text{CaCl}_2$  was used in place of Yoshida nutrient solution). The soil and solution was shaken end-over-end (30 rpm) for 30 min, centrifuged at 17,200 g for 5 min, then filtered (Whatman #5), and the P content determined (Murphy and Riley 1962).

The remaining soil was dried at  $40^\circ\text{C}$ , then extracted with 0.1 M NaOH to gain an estimate of the depletion of plant available P, since Hedley *et al.* (1994) had found that approximately 80% of the P taken up by upland rice from MCP-fertilised Cavinti soil was from a fraction extracted by 0.1 M NaOH without digestion (NaOH- $P_i$ ). Duplicate 0.5 g samples were shaken with 30 mL of 0.1 M NaOH for 16 h on an end-over-end shaker, centrifuged at 17,200 g, filtered (Whatman #5), and the supernatant analysed to determine total P. A 5 mL aliquot of the supernatant was taken and digested with  $\text{H}_2\text{SO}_4$  and  $\text{H}_2\text{O}_2$  for the determination of total 0.1 M NaOH-extractable P. Subtraction of the NaOH- $P_i$  from the total NaOH-P then gave a measure of the organic P extracted by NaOH (NaOH- $P_o$ ). The 0.5 g soil sample was then digested with  $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$  for the determination of total soil P.

### 5.3.2.5 Modelling P uptake

Measured plant P uptake was compared with P uptake predicted by mathematical models. Two models were used. The first calculates uptake assuming that the roots behave as a planar sink absorbing P but not otherwise influencing conditions in the thin layer soil. The second model calculates the additional uptake that would occur if the roots were excreting a P-solubilising organic anion, such as citrate, based on Nye's (1983) theory for the diffusion of two interacting solutes in soil. Full details are given in Kirk (1999) and Kirk *et*

al. (1999b). An outline is given below. The symbols used are defined at the front of this thesis.

*Model 1: Uptake without solubilisation*

In the absence of solubilisation and mycorrhizal infection, established theory for the movement of P to the planar layer of roots by diffusion gives:

$$b_p \frac{\partial [P_L]}{\partial t} = \frac{\partial}{\partial x} \left( D_{LP} \theta f \frac{\partial [P_L]}{\partial x} \right) \quad (5.1)$$

The following boundary conditions are applied to solve Equation 5.1. The root-plane ( $x = 0$ ) is extended into the soil by root hairs ( $x = l_h$ ), and P depletion across the root hair zone will be uniform. Therefore the effective absorption surface is the outer edge of the root hair zone,  $x = l_h$ . Following Tinker and Nye (2000), the flux,  $F$ , of P across  $x = l_h$  is related to the concentration of P in the soil solution at  $x = l_h$ ,  $[P_L]_0$ , by a Michaelis-Menten equation:

$$F = \frac{F_{\max} [P_L]_0}{K_m + [P_L]_0} \quad (5.2)$$

where  $F_{\max}$  = maximum influx that the roots can achieve and  $K_m$  = Michaelis-Menten constant = P concentration in solution when P uptake by roots is half of the maximum P uptake. (Note that the values of  $F_{\max}$  and  $K_m$  vary with plant P status and other conditions.) The boundary condition at  $x = l_h$  is therefore

$$D_{LP} \theta f \frac{d[P]_L}{dx} = - \frac{F_{\max} [P_L]_0}{K_m + [P_L]_0} \quad x = l_h \quad t \geq 0 \quad (5.3).$$

Note that if  $[P_L]_0 \ll K_m$ , equation (5.2) simplifies to

$$F = \frac{F_{\max} [P_L]_0}{K_m} = \alpha [P_L]_0$$

where  $\alpha$  = 'root absorbing power' for P (Tinker and Nye 2000) =  $F_{\max}/K_m$ , and

$$D_{LP} \theta f \frac{d[P]_L}{dx} = -\alpha [P_L]_0 \quad x = l_h \quad t \geq 0 \quad (5.4).$$

At the outer boundary ( $x = L$ ), there will be no transfer of P. Therefore

$$D_{LP} \theta f \frac{d[P_L]}{dx} = 0 \quad x=L \quad t \geq 0. \quad (5.5)$$

*Model 2: Uptake with solubilisation by organic anions*

The following two equations describe the diffusion and interaction of P and organic anion, C in the soil (Kirk 1999, equations 15 and 16)

$$b_{P^*} \frac{\partial}{\partial t} ([P_L^*] - \lambda_C [C_L]) = \frac{\partial}{\partial x} \left( D_{LP^*} \theta f \frac{\partial [P_L^*]}{\partial x} \right) \quad (5.6)$$

$$b_C \frac{\partial [C_L]}{\partial t} = \frac{\partial}{\partial x} \left( D_{LC} \theta f \frac{\partial [C_L]}{\partial x} \right) - \theta k_C [C_L]. \quad (5.7)$$

In deriving these equations it is assumed that the diffusion of C is not affected by the diffusion of P; this is reasonable because  $[C_L] \gg [P_L^*]$ . It is also assumed that the decomposition of C adsorbed on the soil solid is slow and that the decomposition of C in solution follows first order kinetics.

The following boundary conditions apply, analogous to those for equation (5.1):

$$D_{LP} \theta f \frac{d[P_L]}{dx} = -\alpha [P_L^*], \quad D_{LC} \theta f \frac{d[C_L]}{dx} = F_C, \quad x = l_h \quad t \geq 0 \quad (5.8)$$

$$D_{LP} \theta f \frac{d[P_L^*]}{dx} = 0, \quad D_{LC} \theta f \frac{d[C_L]}{dx} = 0 \quad x = L \quad t \geq 0. \quad (5.9)$$

*Solution to the equations*

The diffusion equations and boundary conditions were expressed in finite-difference form using Crank-Nicholson approximations and solved using standard numerical methods (Smith 1985).

The amount of P taken up by the root system after a particular time (mol/TL system) is found from the relation

$$\text{Uptake} = \sum \left[ \left\{ \alpha [\overline{P_L}] \Delta t - b_p (\Delta[P_L] - \lambda_c \Delta[C_L]) l_h \right\}_{\text{Band}} A_{\text{Band}} + \left\{ \alpha [\overline{P_L}] \Delta t - b_p (\Delta[P_L] - \lambda_c \Delta[C_L]) l_h \right\}_{\text{NonBand}} A_{\text{NonBand}} \right] \quad (5.8)$$

where  $[\overline{P_L}]$  is the mean  $[P_L]$  value at the root surface over time step  $\Delta t$ ,  $\Delta[P_L]$  and  $\Delta[C_L]$  are the changes in  $[P_L]$  and  $[C_L]$  at the root surface over  $\Delta t$ ,  $A$  is the root-plane surface area ( $\text{dm}^2/\text{TL system}$ ), and subscripts  $\text{Band}$  and  $\text{NonBand}$  refer to the banded and non-banded regions of the TL system, for which Equations 5.1–5.9 are solved separately with the appropriate parameter values. The sum is taken over all time steps. Time steps of 1 h and distance steps of 0.1 mm were used. Mass balances for all reactants were conserved within 1% for simulations up to 42 d.

The models assume that the roots in the thin-layers behave as a continuous planar layer. In the banded TL systems, the roots completely covered the P-fertilised soil about 3 wk after planting, whilst in the incorporated TL systems complete coverage (except at the top of the thin layer) occurred about 4 wk after planting. The incomplete coverage of the soil in the incorporated treatment at the end of the experiment was determined by photocopying the root mat, overlaying the photocopy with an acetate sheet, and shading the area not covered by roots. The shaded area was determined using a leaf area machine, and the area not covered by roots was subtracted from the total area of the thin layers for use in the model. It is also assumed that there was no P uptake for the first week following transplanting because the plants suffered a ‘transplanting shock’.

### *Measurement of parameter values used in the models*

#### i) Root hair length

The root hair length ( $l_h$ ) was measured as follows: halves of TL systems were packed as for the main TL experiment (Section 5.3.2.1), except that there were no perspex strips dividing the unfertilised soil from the  $2000 \mu\text{g P g}^{-1}$  soil in the banded treatment, and no mesh was used. Each half of the TL system was covered with a perspex plate and then wetted up for 2 d in P-free Yoshida nutrient solution. Four seeds of germinated upland rice (cv. Azucena) were sown directly on each thin layer. These were then placed in boxes containing nutrient

solution with the thin layers set at an angle of approximately  $60^\circ$  from horizontal in order that the roots would grow along the surface of the perspex. After 10 d, root hair lengths were measured by viewing the thin layer under a microscope using a graticulated eyepiece at  $40\times$  magnification and an external light source. Forty root hair measurements were made on roots grown in each soil (P0, P200 and P2000).

## ii) Phosphorus buffer power

The P buffer power was derived from a linear fit to the data in Figure 4.2. Although the data were better described by a Freundlich relation over the concentration range measured in the desorption study (Figure 4.2), at concentrations below this range the Freundlich relation predicts unrealistically large buffer powers. For example, the Freundlich isotherm predicts that when all of the labile  $P^\dagger$  has been desorbed, the P concentration in the soil solution is still very high ( $4.9 \mu\text{M}$ ). According to the desorption data in Figure 4.2,  $[P_L]$  falls to about  $11 \mu\text{M}$  with negligible desorption of P relative to the amount desorbed in the thin layers. For  $[P_L] < 11 \mu\text{M}$ , a linear fit is reasonable (Figure 5.4), giving  $b_P = 2150$  and a maximum P desorption of  $23000 \mu\text{mol kg}^{-1}$ , i.e. about half the NaOH- $P_i$  and one-third of the P added.

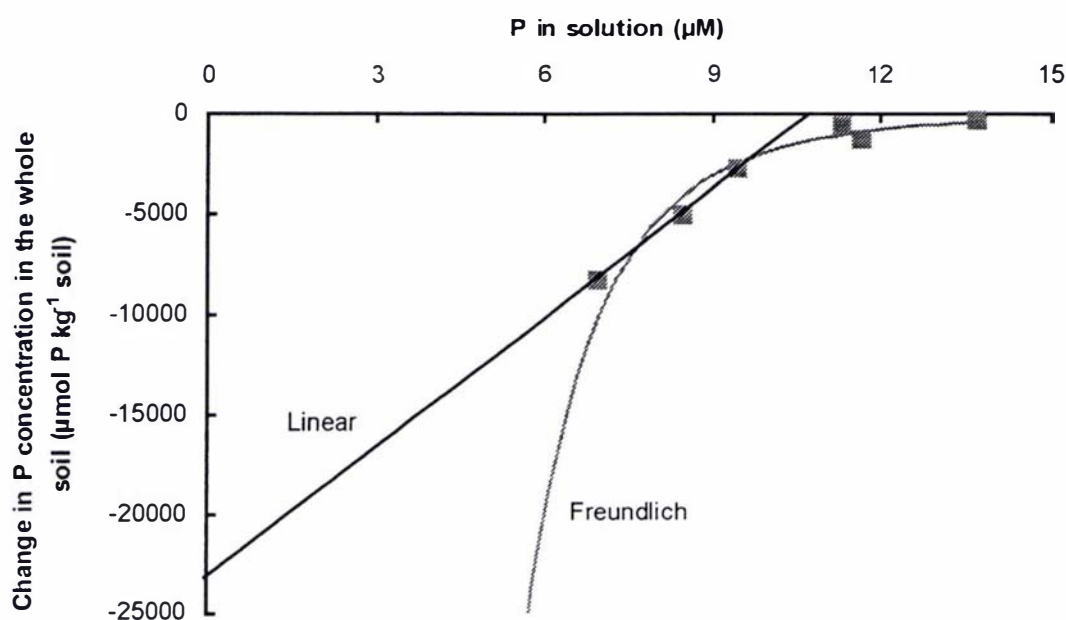


Figure 5.4. A comparison of two curves for predicting  $b_P$  over the range of desorbable P for the P2000 soil.

<sup>†</sup> the amount of labile P is indicated by the NaOH- $P_i$  (Table 5.2) =  $51,300 \mu\text{mol P kg}^{-1}$  soil.

### iii) Solution P concentration

The discussion above indicates that it is appropriate to use a single linear  $b_p$  with an initial  $[P_L]$  value of 11  $\mu\text{M}$  rather than a more complicated expression for  $b_p$  with the measured initial  $[P_L]$  of 14  $\mu\text{M}$ . The  $[P_L]$  value decreases from 14 to 11  $\mu\text{M}$  with negligible P desorption.

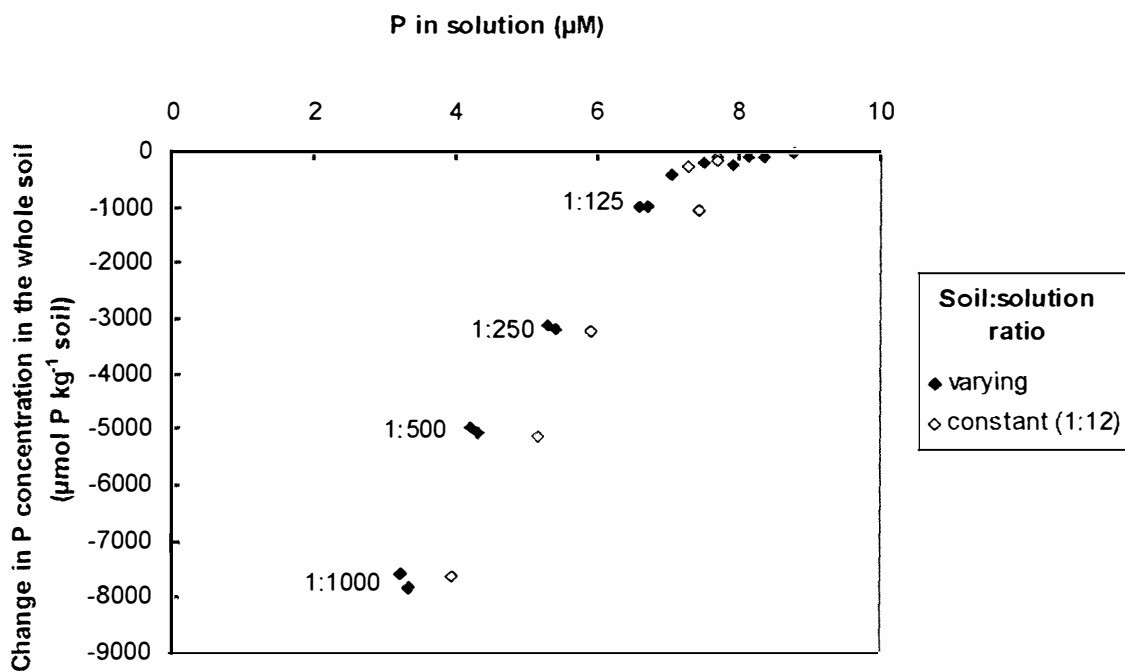
The mean  $[P_L]$  value measured in the thin layer control soils by extracting the soil with P-free Yoshida's solution at a soil:solution ratio of 1:2.5 was 19  $\mu\text{M}$ . The true soil pore solution concentration was probably a little larger than this – the soil:solution ratio in the thin layers being only 1:0.35 and the desorption isotherm indicating that  $[P_L]$  was poorly buffered in the initial soil so that differences in soil:solution ratio would cause differences in  $[P_L]$ . But since very little P is desorbed at high  $[P_L]$ , the exact value of the initial  $[P_L]$  in the thin layers is not critical. What is important is that the relationship between  $[P_L]$  and  $[P]$  over the range of P desorbed by the plants is described accurately. Therefore it is important to know the effect of soil:solution ratio on  $b_p$  over the relevant part of the desorption isotherm.

To this end, another desorption isotherm was measured using the methodology described in Section 4.3.3 for the zero citrate isotherm, except that the soil had been sieved to  $<0.5$  mm not  $<2$  mm. Aliquots from each solution of varying volumes (0.25 – 2 L) were removed, centrifuged, filtered and their P concentration determined as before. The remaining solution was then removed by decanting and filtration (0.45  $\mu\text{m}$ ), the soil collected, and all soils re-suspended in 24 mL of 0.01 M  $\text{CaCl}_2$  (i.e. soils with a range of P desorbed were all extracted at the same soil:solution ratio). These solutions were shaken for 16 hours, centrifuged, filtered, and analysed as for the other isotherms. Only one replicate from each treatment was analysed.

These data (Figure 5.5) showed that large decreases in soil:solution ratio did not change the slope of the isotherm ( $b_p$ ) and gave only a small increase in  $[P_L]$  ( $\approx 0.7$   $\mu\text{M}$ ) for a given amount of P desorbed. Thus, in case the  $[P_L]$  may have increased further at a 0.35:1 solution:soil ratio, and in order to be conservative, the estimate of initial  $[P_L]$  was increased from 11 to 13  $\mu\text{M}$ .

Note that Figure 5.5 gives much lower  $[P_L]$  values than Figure 5.4. This discrepancy is due to the fact that the soil in Figure 5.5 was fertilised then stored for a much longer time than the soil in Figure 5.4, and even though both soils were air-dried, P-fixation reactions would have continued. Also, the greater amount of P-sorbing surface exposed in the 0.5 mm sieved soil may have lowered the P concentration in solution relative to that of 2 mm sieved soil. Therefore while the relative differences between the two isotherms presented in Figure 5.5 are important, the actual values of  $[P_L]$  have no meaning for this study.

The initial solution P concentration for the P200 soil was taken from a P200 isotherm (data not shown).



**Figure 5.5.** The effect of varying the soil:solution ratio on the solution P concentration and the change in soil P concentration.

#### iv) Other parameters

The volumetric water content ( $\theta$ ) was measured by oven drying a known weight of soil at 105°C and converting to a volume using the bulk density. The values for the diffusion



impedance factor ( $f$ ) for Cavinti soil at this moisture content, and for the root absorbing power ( $\alpha$ ), were taken from (Kirk *et al.* 1999b). The amount of labile P at the start of the experiment ( $[P]$  initial) was taken as the amount of 0.1 M NaOH-Pi. The values of the solubilisation parameters for Model 2 were obtained as described in Section 5.4.5.5.

### 5.3.2.6 Statistics

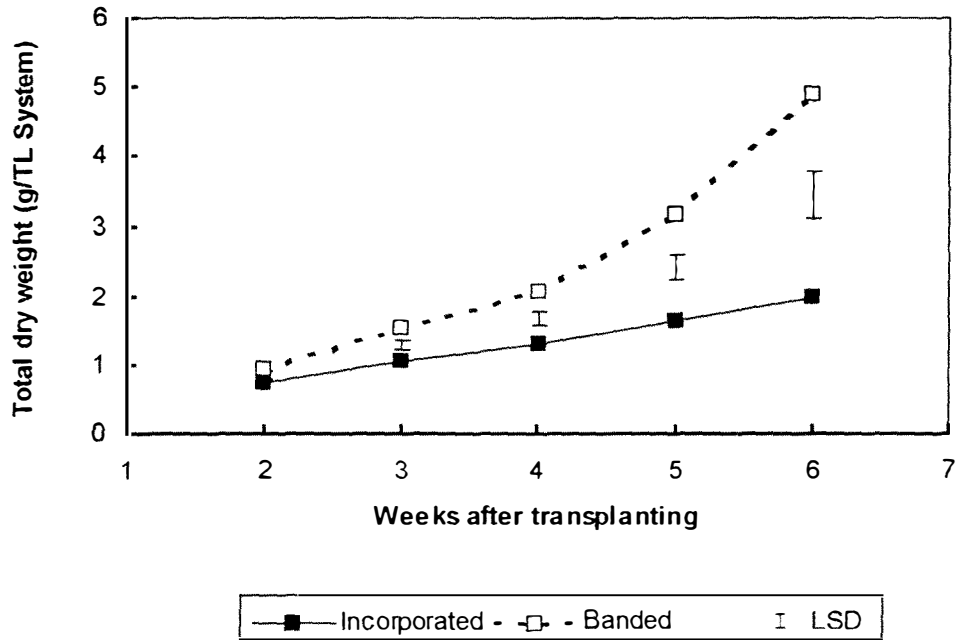
Treatment means were tested for significant differences by ANOVA followed by DMRT (where the differences were found to be significant) using the SAS<sup>®</sup> System. Differences stated as significant are significant at ( $P < 0.05$ ) unless another P value is given.

## 5.4 Results and discussion

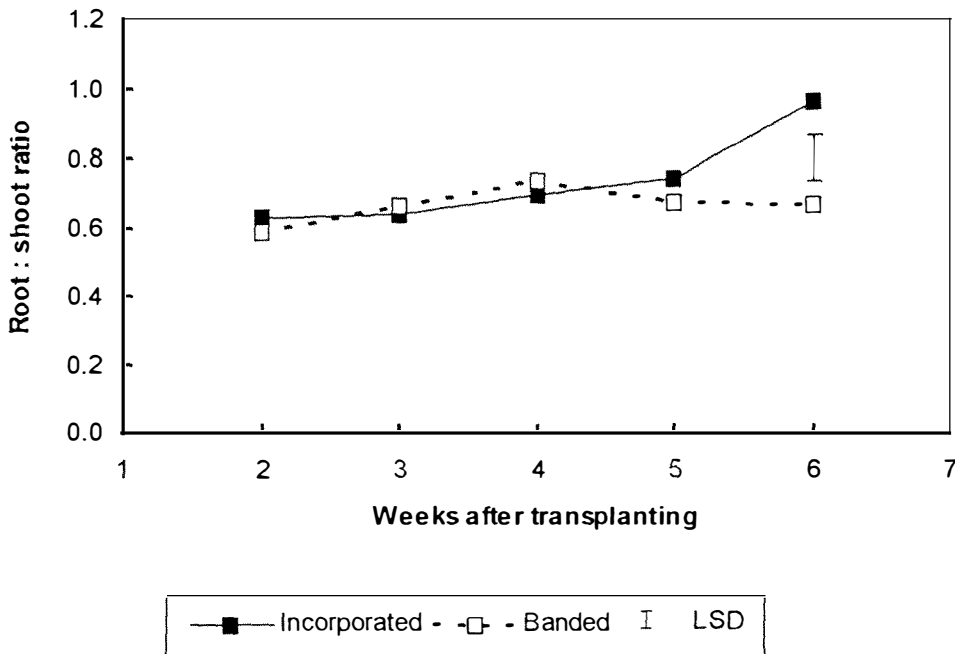
### 5.4.1 Plant growth

After 2 wk growth the rice in the banded treatment was significantly larger (by 27%) than rice in the incorporated treatment (Figure 5.6). This difference continued to increase throughout the course of the experiment and after 6 wk growth the banded treatment had produced more than twice as much dry matter as the incorporated treatment. The root:shoot ratio of the incorporated treatment increased after 6 wk growth (Figure 5.7), a response that is typical of P stressed plants (Plaxton and Carswell 1999), whereas the root:shoot ratio of the banded treatment remained roughly constant. None of the plants tillered after 6 wk, suggesting that herbage P concentrations were low in both treatments.

Roots were observed to penetrate the 24  $\mu\text{m}$  mesh in some replicates. This usually resulted in significantly more growth, so these replicates were omitted from the analysis. Root penetration was thought to be due to fine holes in the mesh as the mesh had been washed and reused from a previous experiment (but see Chapter 7).



**Figure 5.6.** Root + shoot dry weight of four rice plants grown in TL systems. P fertiliser was either applied as a band occupying 10% of the soil volume or incorporated throughout the entire soil volume.



**Figure 5.7.** Root:shoot ratio for rice grown in the TL systems.

### 5.4.2 Plant P concentration

The average shoot P concentrations after 6 wk of growth were very low, being 0.05% and 0.11% P for the incorporated and banded treatments, respectively. According to Tanaka and Yoshida (1970), the critical herbage P concentration for deficiency is 0.1%, meaning that the banded treatment was bordering on deficiency and that the incorporated treatment was very deficient.

### 5.4.3 Soil P concentration

The measured concentration of solution P in the control soil of the banded treatment (21  $\mu\text{M}$ ) was two orders of magnitude greater than the P in solution for the incorporated soil (0.15  $\mu\text{M}$ ), as predicted from the P adsorption isotherm (Figure 4.1). Soils were also extracted with 0.1 M NaOH (Table 5.1). The difference in the amount of NaOH- $P_i$  between the control and the planted soils shows a general agreement with the amount of P taken out of the soil by the plants (Table 5.2), although the standard errors are very large. These could be reduced by repeating the analysis, then averaging the results, or by grinding the soil with a mortar and pestle which would enable better mixing, although this would expose much more soil surface area during the extraction.

**Table 5.1.** The distribution of P between various pools in planted and unplanted Cavinti soil for the thin layer experiment, 6 wk after transplanting. The SE of the mean is in parenthesis. The values obtained by Hedley *et al.* (1994) are included for comparison.

Treatment	NaOH- $P_i$	NaOH- $P_o$	Residual P
	( $\mu\text{mol g}^{-1}$ soil)		
Banded (control)	53.1 (2.8)	9.1 (0.5)	17.4 (1.2)
Banded (planted)	33.1 (2.0)	8.5 (1.5)	17.0 (0.8)
Incorporated (control)	7.2 (0.3)	8.1 (0.8)	11.7 (1.2)
Incorporated (planted)	6.8 (0.2)	8.0 (0.4)	11.8 (0.4)
Unfertilised (control)	2.0 (0.01)	7.4 (0.05)	11.4 (0.7)
Unfertilised (planted)	2.1 (0.1)	7.1 (0.1)	11.0 (0.6)
Unfertilised (Hedley)	2.5 (0.1)	7.7 (0.5)	13.2

**Table 5.2.** A comparison of total plant P uptake with total P depleted from the NaOH-Pi fraction after 6 wk of plant growth. The SE of the mean is in parenthesis.

Treatment	Plant P uptake ( $\mu\text{mol/TL system}$ )	NaOH-Pi depleted from soil ( $\mu\text{mol/TL system}$ )
Banded	150 (12)	175 (44)
Incorporated	13 (9)	35 (41)

#### 5.4.4 Modelling P uptake in the absence of solubilisation effects (Model 1)

##### 5.4.4.1 Discussion of parameters used in the model

The parameters used for the P model are shown in Table 5.3. Sensitivity analysis found that the predicted uptake was relatively insensitive<sup>†</sup> to increases in  $\alpha$  at values greater than  $2.5 \times 10^{-5}$  (which would have been unrealistically low for this experiment, see discussion on  $F_{max}$  and  $K_m$  values in Section 9.4.2.1). The insensitivity of predicted uptake to  $\alpha$  indicates that the rate of diffusion of P through the soil was the main factor limiting P uptake, rather than the rate of P absorption by the root.

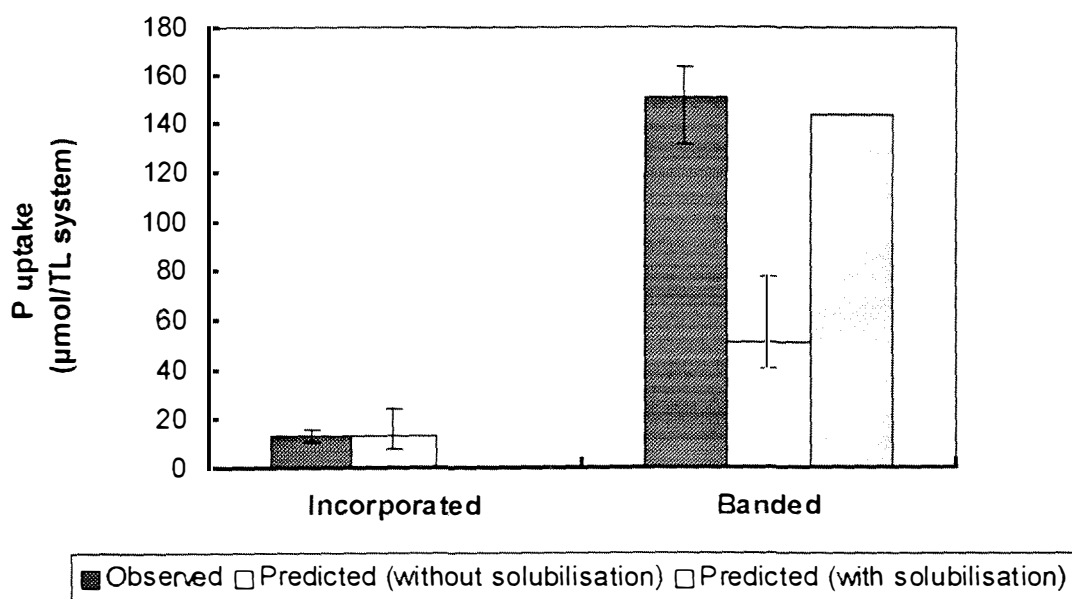
**Table 5.3.** Parameters and values used in Model 1 to predict P uptake from P200 and P2000 soil (see Section 5.4.4). Minimum and maximum values are given in brackets.

Parameter	Units	P200 soil (Incorporated)		P2000 soil (Banded)	
		Value	(Min. – Max.)	Value	(Min. – Max.)
[P <sub>L</sub> ] initial	$\text{mol dm}^{-3}$	$0.22 \times 10^{-6}$	$(0.13 \times 10^{-6} - 0.26 \times 10^{-6})$	$13 \times 10^{-6}$	$(11 \times 10^{-6} - 15 \times 10^{-6})$
[P] initial	$\text{mol dm}^{-3}$ soil	0.009	(–)	0.067	(–)
$b_P$		4490	(3490–5490)	2150	(2000–2300)
$\theta$		0.44	(0.44–0.5)	0.47	(0.47–0.50)
$A$	$\text{dm}^2$	2.08	(–)	0.208	(–)
$f$		0.4	(0.39–0.45)	0.4	(0.4–0.45)
$l_h$	dm	$3.6 \times 10^{-3}$	$(3.6 \times 10^{-3} - 5.3 \times 10^{-3})$	$3.6 \times 10^{-3}$	$(3.6 \times 10^{-3} - 5.3 \times 10^{-3})$
$\alpha$	$\text{dm s}^{-1}$	$10^{-4}$	(–)	$10^{-4}$	(–)
$D_{LP}$	$\text{dm}^2 \text{s}^{-1}$	$8.9 \times 10^{-8}$	(–)	$8.9 \times 10^{-8}$	(–)

<sup>†</sup> there was only a 2% difference in predicted uptake between  $\alpha = 1 \times 10^{-4}$  and  $\alpha = 2.5 \times 10^{-5}$

#### 5.4.4.2 Comparing P uptake predicted by the model with observed P uptake

Model 1 showed that the P uptake predicted by diffusion in the absence of solubilisation showed good agreement with the observed P uptake in the incorporated treatment (Figure 5.8). In the banded treatment, however, diffusion of readily-available P explained only 34% of the observed P uptake. This indicates that rice plants have some mechanism of enhancing P uptake. Kirk *et al.* (1999b) also observed that rice took up more P than could be explained by diffusion of readily-available P, and found that this P uptake could be explained by root release of citrate. Ae *et al.* (1995) observed that rice was markedly superior to nine other common crop plants in its ability to take up P from a high P-fixing Andosol, and in utilising sparingly soluble  $\text{FePO}_4$ . Ae *et al.* (1995) found no evidence that organic anions were responsible for the enhanced P uptake, but were unable to identify the mechanism. In the present experiment, it is therefore necessary to examine a range of mechanisms that might explain the P uptake in the banded treatment.



**Figure 5.8.** Observed and predicted P uptake without solubilisation (Model 1) for rice grown in the TL systems after 6 wk of growth. The bars indicate maximum and minimum values for each treatment; for the predicted treatment these are based on the errors given in Table 5.3. Also shown is an estimate of P uptake with solubilisation (Model 2, Section 5.4.5.5) for the banded treatment, based on the flux of citrate observed by Kirk *et al.* (1999b) and parameters in Table 5.5.

## 5.4.5 Factors governing P uptake

### 5.4.5.1 Water content

Modelling showed that the small difference in water content between the banded and incorporated treatments (0.03, Table 5.3) had a minimal ( $\approx 2\%$ ) effect on P uptake. Calculations, based on  $\theta$  of 0.44 – 0.47 and a soil particle volume of 0.41, showed that 12 – 15% of the soil in the TL system was air-filled. The low air-filled porosity is likely to have resulted in some localised reduction. A few small black spots (covering  $< 1\%$  of the area), presumably  $\text{MnO}_2$ , were visible in the control soil of the incorporated treatment by 5 wk after planting. This indicates that some  $\text{Mn}^{2+}$  was being mobilised and moving to the soil surface where it was being oxidised upon contact with air. The control treatment was also slightly darker in colour than the planted treatments. No black spots were observed in the planted treatments. The planted TL systems would have been slightly drier than the control TL systems during peak evapotranspiration, which would have allowed more oxygen into the soil.

Manganese reduction occurs at a fairly high  $E_h$ , especially under acidic conditions. At pH 5, the  $E_h$  for  $\text{MnO}_2$  reduction is 634 mV, whereas that for  $\text{NO}_3^-$  is 539 mV, and that for  $\text{Fe}(\text{OH})_3$  172 mV. Kirk *et al.* (1999b), using the same TL system, were still able to measure  $\text{NO}_3^-$  in their planted TL systems, which also indicates that the amount of reduction occurring in planted TL systems is minimal. To get a significant increase in solution P, the  $E_h$  needs to drop low enough to reduce Fe(III) oxides (Section 1.3.2.2) – far below that at which  $\text{NO}_3^-$  is reduced. Localised reduction is therefore not likely to have caused a three-fold increase in soil solution P, which would be necessary in order to explain the observed P uptake in the banded treatment.

### 5.4.5.2 Mass flow

The TL systems in the transpiration experiment (Section 5.3.2.3) transpired an average of 22.7 mL of water per TL system per day, i.e.  $11 \text{ mL g}^{-1} \text{ DM d}^{-1}$  assuming the plant dry weight would have been about 2 g (4 wk-old plants grown in P500 soil were 2.3 g). This rate ( $11 \text{ mL g}^{-1} \text{ DM d}^{-1}$ ) compares well with  $12 \text{ mL g}^{-1} \text{ DM d}^{-1}$  found by Horiguchi (1988) for rice grown in culture solution. Multiplying the transpiration rate by the average soil

solution P concentration gave P uptake values (due to mass flow) of less than 2% of the observed P uptake. Hence mass flow was deemed unimportant as a means of explaining P uptake in this experiment.

#### 5.4.5.3 Root studies

##### *Root hairs*

The average root hair length measured using the perspex plate technique (Section 5.3.2.5) was 0.36 mm, with a standard deviation of 0.17 mm. There was no significant effect of soil P concentration on root hair length. The variability in root hair length was large, and is allowed for in the error bars in Figure 5.8. However, even when a high estimate for average root hair length was used in the calculation of the maximum P uptake, (0.36 plus one standard deviation, i.e. 0.53 mm), this only increased the predicted P uptake by 15%, which is still a long way short of accounting for the 200% increase in predicted P uptake needed to explain the observed P uptake.

##### *Root length*

Root geometry only affected the amount of P uptake from the TL systems in-so-far as it affected the coverage of the nylon mesh. After 6 wk growth the roots of the incorporated treatment covered 89% of the thin layer, but no adjustment to the predicted P uptake was necessary for the banded treatment, as the root coverage was 100%. Root characteristics were still investigated, however, since an understanding of how these are affected by fertiliser management practices is important for P uptake in the field. The average root length per unit area in the incorporated treatment and the unfertilised soil of the banded treatment, 2 wk after transplanting, was not significantly different (Table 5.4). In the third week the unfertilised soil of the banded treatment had a slightly greater root length per unit area than the incorporated treatment (P200), which may have been due to the better growth of the plants in the banded treatment (Figure 5.6). The main point to note from Table 5.4 is that the root length per unit area over the MCP-fertilised band was significantly ( $P < 0.0001$ ) longer than over the soil outside the band throughout the course of the experiment. This multiplication of roots was mostly medium and fine roots, which are the feeding roots. The multiplication of feeding roots may have been a response to P (Anghinoni and Barber

1980a), or a response to Ca (Alva *et al.* 1986), as the concentrations of both nutrients were severely deficient in this soil. The increase in root length per unit area is more likely a response to P, as Ca was supplied via the nutrient solution to the whole TL system, whereas P was only supplied in the fertilised band.

The proportions of coarse, medium and fine roots covering the unfertilised soil outside the band were approximately equal from the third week after transplanting onwards. However, both the incorporated treatment and the roots growing over the P-rich band showed a marked increase in the percentage of fine and medium roots relative to coarse (Table 5.4). The decrease in root diameter in the incorporated treatment (P200 soil) was most likely a response to P stress (Garcia and Ascencio 1992). In contrast the increase in the amount of roots in the P-rich band (P2000 soil) is likely to be a response to nutrient enrichment, as discussed in the preceding paragraph.

**Table 5.4.** Root parameters of upland rice 2, 3 and 5 wk after transplanting, in the soils of the incorporated treatment (P200), the unfertilised soil outside the band (P0), and the P-fertilised band (P2000). Significant differences in the means for each parameter, within each week, are indicated by different capital letters below the number. Significant differences between % fine, medium and coarse, within each week, are indicated by a different lower case letter.

Parameter	Week 2			Week 3			Week 5		
	P0	P200	P2000	P0	P200	P2000	P0	P200	P2000
Root length ( $\frac{m}{g}$ )	29	41	96	102	124	132	99	115	188
Dry weight ( $\frac{g}{cm^2}$ )	C	B	A	B	A	A	B	B	A
Root length ( $\frac{cm}{cm^2}$ )	9	11	28	54	43	66	107	89	153
Area of TL ( $\frac{cm^2}{cm^2}$ )	B	B	A	B	C	A	B	B	A
Coarse roots (%) (diam. >300 $\mu m$ )	46 a A	36 B	37 a B	32 A	28 b AB	26 b B	32 A	30 b A	24 b B
Medium roots (%) (diam. 150-300 $\mu m$ )	29 b B	31 B	38 a A	32	31 b	36 a	34 B	29 b C	38 a A
Fine roots (%) (diam. 50-150 $\mu m$ )	25 b B	33 A	25 b B	35	40 a	39 a	34	41 a	37 a



#### 5.4.5.4 Mycorrhiza

No mycorrhizal infection was found. The absence of mycorrhizae is presumably due to the soil having been stored for a long period of time in a humid environment (Black and Tinker 1979, Hall 1979).

#### 5.4.5.5 Release of organic anions (Model 2)

No citrate, and only small quantities of other organic anions, were present in several soil extracts that were analysed in a preliminary run soon after extraction. The remaining solutions were frozen awaiting analysis several weeks later. Upon thawing the soil extracts appeared cloudy. The pH was checked and was found to be 2.9, thus a large amount of the acidity had been absorbed by the soil during extraction. The reasons for the cloudy appearance of the solutions was unclear but it may have resulted from bacterial growth or precipitation. Therefore the solutions were discarded as they were no longer representative of the organic anions that were released by the plants.

Organic acid analysis of the filter papers that had been pressed against the root mat, showed a large citric acid peak in all treatments, plus there were two additional unidentified smaller peaks that appeared in the more P deficient incorporated treatments, but not in the banded treatments. The rate of citrate release was  $1.3 \times 10^{-10} \text{ mol dm}^{-2} \text{ s}^{-1}$  – approximately one-third of the flux of citrate calculated by Kirk *et al.* (1999b). The amount of P solubilised by this flux of citrate could be estimated using Model 2. Other parameters required for the citrate solubilisation model (Table 5.5) were either taken from Kirk *et al.* (1999b) (since they used the same soil), or were estimated as shown in Appendix C. The remaining parameters were the same as given for Model 1 (Table 5.3).

Model 2 indicated that this rate of citrate release would result in a P uptake of 97  $\mu\text{mol}$  per TL system, which would be sufficient to explain 65% of the observed P uptake. A problem with the filter paper technique, however, is that root hairs were broken when the roots were separated from the mesh. Ayers and Thornton (1969) showed that even slight abrasion of roots, such as gently drawing roots across filter paper, caused a large increase in the amount of amino acids released from plant roots. However, organic anion release rates into filter papers might also be underestimated, because the soil is a much greater sink (see

Section 1.5.3.2.3e) for organic anions (due to sorption and microbial degradation) than filter paper. Furthermore, it is also possible that the citrate was generated during the two months the sample was frozen awaiting analysis (see Chapter 6).

**Table 5.5.** Additional parameter values required for Model 2 to explain P uptake from the banded treatment. Other parameters, which were the same as those used in Model 1, are given in Table 5.3.

Parameter	Source	Value	Units
$b_C$	Kirk <i>et al.</i> (1999b)	5.1	–
$b_{P^*}$	Appendix C	150	–
$[P_L^*]$	Appendix C	$9 \times 10^{-5}$	$\text{mol dm}^{-3}$
$\lambda_C$	Appendix C	0.17	–
$F_C$	Kirk <i>et al.</i> (1999b)	$3.3 \times 10^{-10}$	$\text{mol dm}^{-2} \text{s}^{-1}$
$k_C$	Kirk <i>et al.</i> (1999b)	$4.16 \times 10^{-5}$	$\text{s}^{-1}$
$D_{LP^*}$	Kirk <i>et al.</i> (1999b)	$8.9 \times 10^{-8}$	$\text{dm}^2 \text{s}^{-1}$
$D_{LC}$	Kirk (1999)	$6.9 \times 10^{-8}$	$\text{dm}^2 \text{s}^{-1}$

Kirk *et al.* (1999b) grew the same cultivar of rice, in the same soil (although fertilised at  $1000 \mu\text{g P g}^{-1}$  soil), also using the TL system. The net flux of citrate released from roots in the P1000 soil from Kirk *et al.*'s experiment ( $3.3 \times 10^{-10} \text{ mol dm}^{-2} \text{ s}^{-1}$ ) was almost three times greater than that measured on the filter papers, and would have been sufficient to explain the observed P uptake in the present experiment within the experimental error (Figure 5.8). It was concluded therefore, that organic anion release might have been the reason for the greater P uptake than that predicted by Model 1, but that further research is needed to improve methods of extracting, storing and quantifying organic anions in Cavinti soil, to verify this hypothesis.

If organic anions are invoked as the mechanism for explaining P uptake that could not be explained by diffusion of readily-available P, why then did organic anion release not result in more P uptake in the incorporated TL systems? The data of Kirk *et al.* (1999b) show that very little organic anions are released from highly P stressed plants (P100 soil).

Vancura (1988) also stressed that the amount of organic anions released is proportional to the root biomass, therefore with less biomass we would expect less organic anion release from the P200 plants. It is likely therefore, that very P stressed plants release small amounts of organic anions. In contrast, Lipton *et al.* (1987) found that the rate of citrate release from alfalfa roots increased under P stress. Kirk *et al.* (1999a) found a large variation between rice cultivars as to whether the amount of organic anions increased or decreased under P stress. The apparent disagreement in the literature indicates that more research still needs to be done to determine the factors that affect organic anion release.

## 5.5 Conclusions

Banding of P fertiliser can enhance the availability of P to rice grown in TL systems, compared with the incorporation of P fertiliser. The P uptake in the banded treatment was approximately three times more than could be explained by diffusion of readily-available P. Mycorrhizal infection, mass flow or small pockets of reducing conditions, could not explain the extra P uptake. A possible explanation was the presence of organic anions. The experiment should be repeated, using an improved procedure for extracting and storing organic anions, and a slightly lower water content in the TL systems.

Rice can proliferate medium and fine (feeding) roots in P-rich zones. This P efficient mechanism would greatly enhance P uptake from P-rich zones. However it was not possible to quantify this mechanism using numbers that would be relevant to rice grown in the field, as the roots were grown in an extremely restricted volume between nylon mesh.

## 6. The extraction, storage, and analysis of organic acids from highly weathered soils

The discussion at the end of Chapter 5 suggested that the unexplained P uptake by rice plants in the thin layer experiment might have been due to solubilisation of soil P by organic acids. Despite this, organic acids were not detected in the few samples that were analysed. There are four possible reasons why no organic acids were detected:

- 1 – no organic acids were released
- 2 – organic acids were released in undetectable concentrations
- 3 – organic acids were released but were not recovered by the extraction procedure
- 4 – organic acids were extracted but were degraded during storage.

Reasons 1 – 3 indicate the need to trial extraction procedures on soil samples spiked with different concentrations of citrate to determine the detection limit for the extraction procedure, and to compare the efficiencies of different procedures. Reason 4 shows the need to investigate the effects of sample storage on the composition of an organic acid extract.

### 6.1 Extraction of organic acids

#### 6.1.1 Introduction

There has been little work done on extracting organic acids from soils with a high anion retention capacity. Due to the difficulties in extracting organic acids from soils most research on root exudation of organic acids has been conducted using solution culture (e.g. Pellet *et al.* 1995), inert materials such as silica sand (Hoffland *et al.* 1992), or agar (Hoffland *et al.* 1989). The problem with such soil-less experiments is that it is difficult to know how to extrapolate from these results to the field situation, because the quantity and composition of root exudates depends on the composition of the surrounding solution, the concentration gradient of the

released substances, and the need for mechanical force to push the root between solid particles (Vancura 1988).

Hue *et al.* (1986) extracted soil solution from an Ultisol by centrifugation and were able to detect a range of organic acids (including citric and oxalic) at concentrations as low as 0.75  $\mu\text{M}$  (oxalic acid). This was achieved by concentrating the sample (freeze-drying 5 mL of solution and resuspending it in 0.5 mL of 5 mM  $\text{H}_2\text{SO}_4$ ). The samples were then analysed by high performance liquid chromatography (HPLC) in a sulphonated polystyrene-divinylbenzene copolymer based column. The soil solution extraction technique of Hue *et al.* (1986) was not practical for the thin layer experiments because there was not enough soil in the band of the banded treatment to obtain sufficient soil solution for analysis.

The research most relevant to the problem of extracting organic acids from Cavinti soil was conducted by Kirk *et al.* (1999b), who used the same soil, plant growth system, and HPLC system as this research, although the HPLC column used was an OA HY (not a RP8) column. To summarise, Kirk *et al.* (1999b) and Kirk (pers. comm.) found that:

- ⇒ citrate was the most abundant organic anion released from roots of upland rice
- ⇒ there was a large peak of unidentified soil compounds, presumably largely  $\text{NO}_3^-$ , which eluted in the void volume of an OA HY HPLC column.
- ⇒ the stronger the concentration of extractant used, the more organic acids recovered, but this also increased the recovery of interfering compounds. Five millimolar  $\text{H}_2\text{SO}_4$  at a 1:4 soil:solution ratio was the best compromise between the amount of organic acids extracted, and the amount of interfering compounds extracted.
- ⇒ the proportion of organic acids recovered by the extraction process increased as the concentration of organic acids in the soil increased.

Kirk *et al.* (1999b) obtained a low recovery of citrate from Cavinti soil (< 20%) using 5 mM  $\text{H}_2\text{SO}_4$ . Bolan *et al.* (1994) recovered 75% of added organic acids from an allophanic soil, which has an extremely high anion retention (95%), using a 30 min extraction with either

deionised water or 0.1 M HCl at a 1:1 solid:solution ratio. However the methodology outlining how the extraction efficiency was determined, was not mentioned. It was therefore of interest to compare H<sub>2</sub>SO<sub>4</sub> with HCl as extractants for citrate from Cavinti soil. Phosphate was included because P is strongly adsorbed by soil and may therefore be effective in displacing citrate that is bound to soil anion exchange sites. All three acids were compared at 0.1 mol H<sup>+</sup> L<sup>-1</sup>; 5 mM H<sub>2</sub>SO<sub>4</sub> was also included, as this was the concentration used by Kirk *et al.* (1999b).

### 6.1.2 Objective

This chapter is divided into three sections: the extraction (Section 6.1), storage (Section 6.2), and analysis (Section 6.3) of organic acids. The objective of this first section (Section 6.1) was to compare different extractants (H<sub>2</sub>SO<sub>4</sub>, HCl, H<sub>3</sub>PO<sub>4</sub> and alkaline KH<sub>2</sub>PO<sub>4</sub>) in their effectiveness at extracting citrate from soil. Once the best extractant had been identified, experiments were conducted to determine the most appropriate extractant concentration and soil:solution ratio.

### 6.1.3 Method

#### *Soil preparation (conditioning)*

Phosphorus fertilised Cavinti soil was prepared by mixing soil, MCP (at 240 µg P g<sup>-1</sup> soil) and 0.6 g water g<sup>-1</sup> soil, shaking the mixture slowly on an end-over-end shaker overnight, then incubating for 4 d at 40°C. The soil was then air-dried, sieved (< 0.5 mm), and incubated at 0.3 g water g<sup>-1</sup> air-dried soil in a moisture saturated atmosphere for 4 wk. After this the soil was air-dried and 0.5 mm sieved.

#### 6.1.3.1 Effectiveness of different extractants

Two grams of air-dried soil was weighed into 30 mL test tubes and then shaken on an end-over-end shaker with 3 mL of solution containing 50 mg HgCl<sub>2</sub> L<sup>-1</sup> and either 3330 µM or

833  $\mu\text{M}$  citric acid (analytical grade) for 30 min. This allowed sufficient time for citrate to react with the soil without significant degradation by micro-organisms – Jones and Brassington (1998) showed that  $> 80\%$  of added citrate, malate or oxalate became sorbed on the soil exchange complex within 10 min. After 30 min, 1 mL of either 20 mM  $\text{H}_2\text{SO}_4$ , 200 mM  $\text{H}_2\text{SO}_4$ , 133 mM  $\text{H}_3\text{PO}_4$ , or 400 mM  $\text{HCl}$ , was added to the soil/organic acid mixture, resulting in acid concentrations of either 0.01 or 0.1 mol  $\text{H}^+$   $\text{L}^{-1}$ , and a soil:solution ratio of 1:2. The main experiment was therefore a two-factor design: having two citrate concentrations, four extractants and two replicates of each treatment. In addition, two concentrations of  $\text{KH}_2\text{PO}_4$  (0.1 and 0.5 M, adjusted to pH 8.0 with  $\text{NaOH}$ ) at a 1:2 soil:solution ratio, were evaluated as extractants. The solutions were immediately shaken on a reciprocating shaker (200 cycles  $\text{min}^{-1}$ ) for 30 min, centrifuged at 17,000  $g$  for 5 min, filtered (Whatman #5), acidified with 0.02 mL of 2.5 M  $\text{H}_3\text{PO}_4$ , then 0.45  $\mu\text{m}$  nucleopore filtered (Acrodisk<sup>®</sup> LC PVDF) and frozen. Citric acid<sup>†</sup> was determined in the filtered samples by HPLC as described in Section 4.3.3.

### 6.1.3.2 Effect of extractant concentration

Citrate-loaded soil was prepared by shaking 2 g of soil for 30 min with 3 mL of 833  $\mu\text{M}$  citric acid containing 50 mg  $\text{HgCl}_2$   $\text{L}^{-1}$ . Immediately after shaking, a 1 mL aliquot of  $\text{H}_2\text{SO}_4$  (of varying concentration) was added to give the following  $\text{H}_2\text{SO}_4$  concentrations: 12.5, 25, 50, 125 and 250 mM  $\text{H}_2\text{SO}_4$ , at a 1:2 soil:solution ratio. The suspensions were shaken for 30 min and analysed for citrate as above. Each treatment was replicated twice.

### 6.1.3.3 Refining the procedure

This experiment was conducted a few months later than the first two, and used a different batch of soil, although the soil was taken from exactly the same location (Section 4.3) and prepared in the same way. The standard extraction procedure (30 min shake with 50 mM  $\text{H}_2\text{SO}_4$  at a 1:2 soil:solution ratio, followed by 1:9 dilution) and HPLC analysis, did not separate citric acid from the nearest interfering peak. In fact the interfering peak, which eluted 0.2 min before citric acid, was larger than the citric acid peak, which meant that it was impossible to separate the

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<sup>†</sup> Since the mobile phase is buffered at pH 2.1, citrate is measured as citric acid by the HPLC.

peaks by diluting. To quantitatively determine concentrations of citrate down to  $1.25 \mu\text{mol citrate g}^{-1}$  soil in this batch of soil, a weaker  $\text{H}_2\text{SO}_4$  solution was tried, in attempt to extract less of the interfering compound relative to citrate. Accordingly, 25 mM  $\text{H}_2\text{SO}_4$  was compared with 50 mM  $\text{H}_2\text{SO}_4$  at 1:2 and 1:4 soil:solution ratios, using the same citrate-loading, shaking and analysis procedures as above (Section 6.1.3.2).

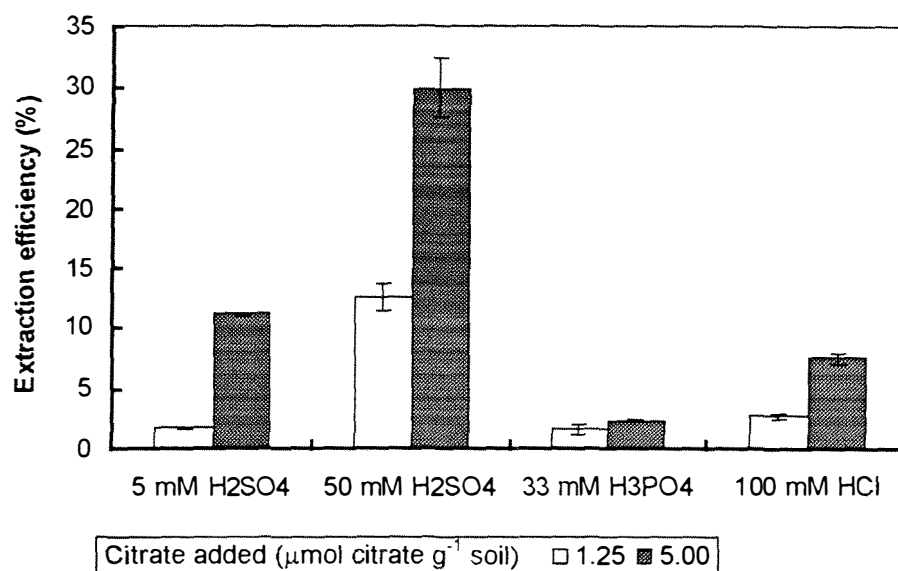
## 6.1.4 Results

### 6.1.4.1 Comparing the effectiveness of different extractants

Sulphuric acid was clearly a better extractant of soil citrate than either HCl or  $\text{H}_3\text{PO}_4$  (Figure 6.1), when compared at the same normality. It was therefore decided to use  $\text{H}_2\text{SO}_4$  for future extractions. Figure 6.1 also showed that the proportion of citrate recovered increased as the amount of citric acid added to the soil increased. This might be due to the binding of citrate increasing the amount of negative charge on the soil particle, which would reduce further binding of citrate to the soil. Also, soil microbes might consume a certain *amount* of the citrate during the mixing and extracting procedure ( $50 \text{ mg HgCl}_2 \text{ L}^{-1}$  does not completely inhibit microbial activity (Kirk, unpublished data)). This amount would represent a greater proportion of the total citrate when less is added to the soil.

Alkaline  $\text{KH}_2\text{PO}_4$  extracted many compounds from the soil in addition to citrate (Table 6.1). In particular  $\text{KH}_2\text{PO}_4$  recovered a large amount of a compound that eluted within 0.3 min of citric acid. This could not be removed by dilution and with the equipment available it was impossible to quantitatively measure citric acid. Therefore  $\text{KH}_2\text{PO}_4$  was not used as an extractant for citrate from soil.





**Figure 6.1.** The efficiency of different acids in recovering citrate from Cavinti soil. The data are means  $\pm$  SE,  $n=2$ .

**Table 6.1.** Compounds extracted from Cavinti soil by  $\text{KH}_2\text{PO}_4$ , the area of the citric acid peak, and that of the nearest interfering compound, which eluted 0.3 min before citric acid.

Extractant	Dilution factor	Number of peaks*	Area of interfering peak	Area of citric acid peak	Comment
0.1M $\text{KH}_2\text{PO}_4$	2	12	35650V	35826V <sup>#</sup>	Unresolvable <sup>†</sup>
0.1M $\text{KH}_2\text{PO}_4$	8	12	10950V	12131V	Unresolvable
0.5M $\text{KH}_2\text{PO}_4$	50	13	2609V	2648V	Unresolvable

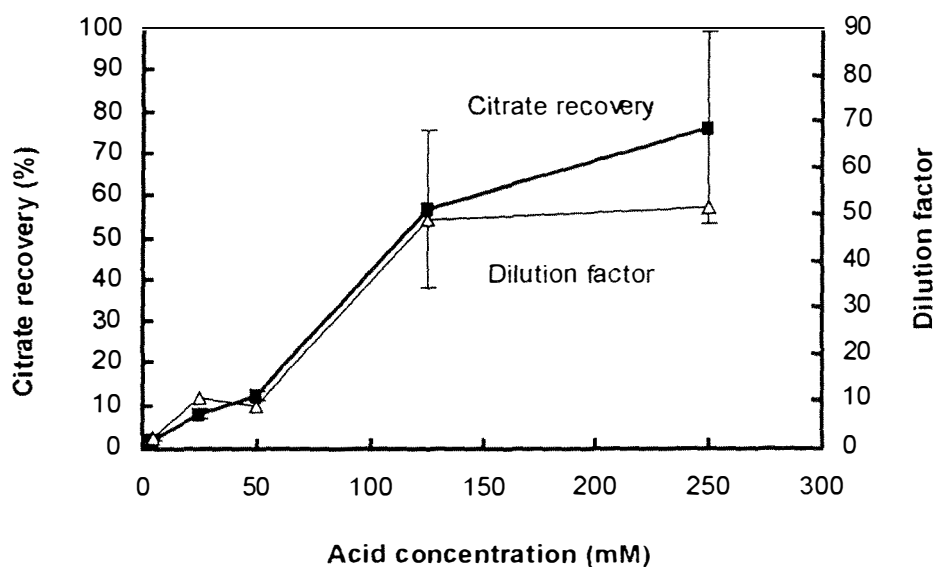
\* retention time  $\leq$  10 min.

<sup>#</sup> V symbolises that the peak was unresolved by the integrator.

<sup>†</sup> Very close peaks can only be resolved by dilution if the interfering peak is smaller than the peak of interest.

#### 6.1.4.2 The effect of extractant concentration

Increasing the concentration of  $\text{H}_2\text{SO}_4$  from 5 to 250 mM markedly increased the recovery of citrate – from 2% to 76% (Figure 6.2). However, more concentrated  $\text{H}_2\text{SO}_4$  also extracted more interfering compounds from the soil. Consequently, the dilution factor required to separate the peaks of the interfering compounds from the citric acid peak greatly increased as the extractant  $\text{H}_2\text{SO}_4$  concentration increased (Figure 6.2). The reason for diluting the extracts is illustrated by the chromatograms in Figure 6.3. When the peaks are large their bases overlap, which means that the integrator is unable to accurately quantify the two peaks, indicated by a ‘V’ printed after the area (Figure 6.3a). By diluting the sample (Figure 6.3b) the whole peak becomes smaller – the bases of the peaks become narrower and no longer overlap, so the peak area can be quantified. At  $\text{H}_2\text{SO}_4$  concentrations above 100 mM, samples had to be diluted to such an extent that their peak areas were significantly affected by baseline noise. When the measured area was multiplied by the large dilution factor (50 times) the measurement errors



**Figure 6.2.** The effect of increasing the concentration of  $\text{H}_2\text{SO}_4$  on the recovery of citrate, and on the dilution factor needed to separate citrate from other compounds extracted from Cavinti soil. The data are means  $\pm$  SE,  $n=2$ .

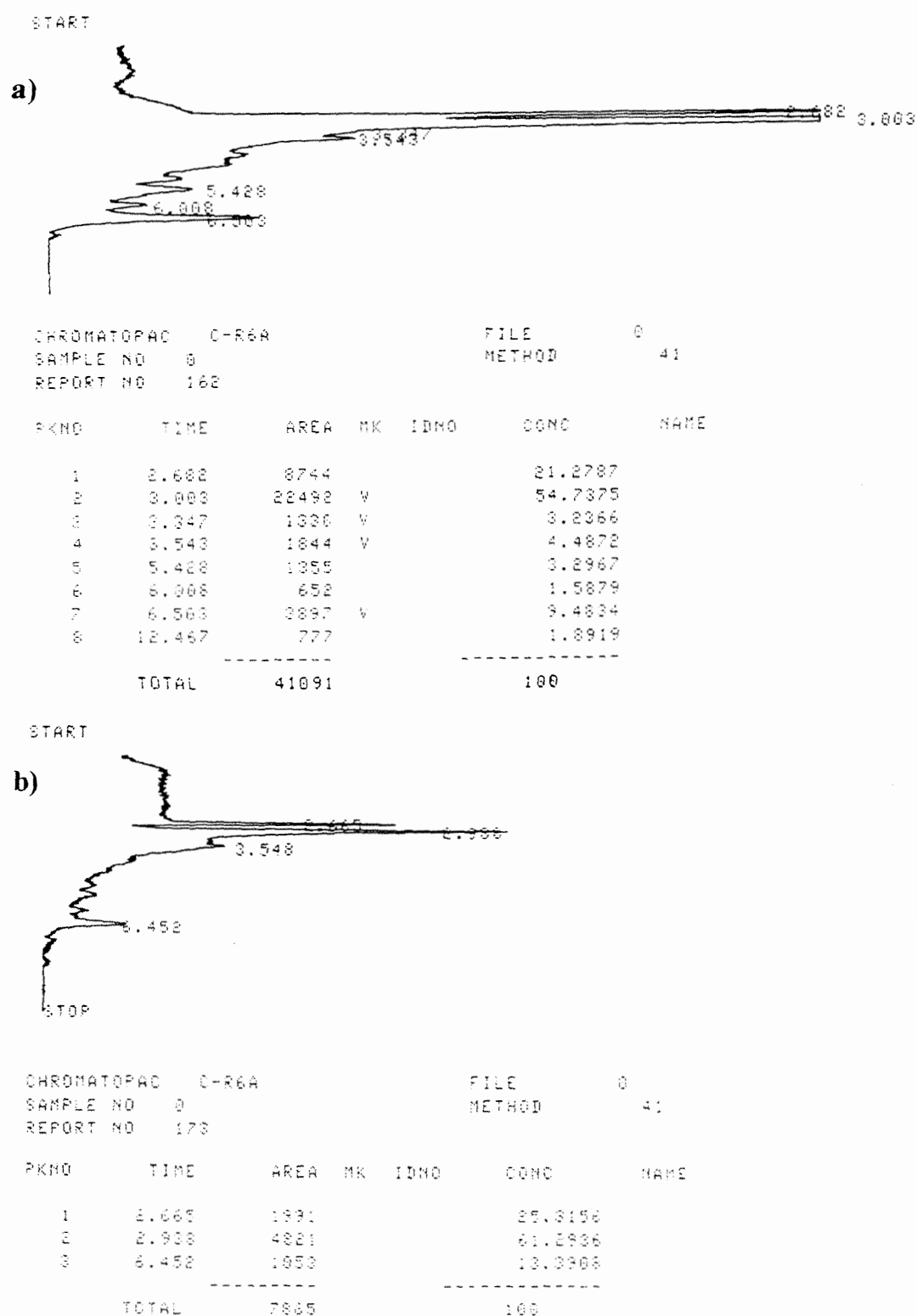
became quite large (Figure 6.2). The need to dilute samples almost to the limit of detection also meant that several attempts at diluting were necessary before the sample could be quantified. If the dilution factor was too small, then the two peaks would not be resolved, if the dilution factor was too large, no peak would be detected. The trial and error process of diluting, injecting, then diluting again was time consuming (each sample took 12 – 25 min, depending on the concentration of extractant used) and the errors were quite large. It was therefore decided to use a weaker acid (50 mM H<sub>2</sub>SO<sub>4</sub>), which had a low but repeatable recovery (12 – 14% in both Parts I and II), followed by a 1:9 dilution to separate citric acid from nearby interfering peaks. Note that although this recovery is low, the recovery efficiency increases as the amount of citric acid added to the soil increases (Figure 6.1). The minimum quantifiable recovery of citrate, using 50 mM H<sub>2</sub>SO<sub>4</sub>, would be approximately 0.2 μmol citrate g<sup>-1</sup> air-dried soil<sup>†</sup>. This recovery was assumed to be adequate for the purposes of the thin layer experiments, as 0.2 μmol citrate g<sup>-1</sup> air-dried soil is 2 – 16 times lower than the rates of citrate production found by Kirk *et al.* (1999b) for upland rice plants growing in thin layer systems.

#### 6.1.4.3 Refining the procedure

Fifty millimolar H<sub>2</sub>SO<sub>4</sub> extracted a large amount of an interfering compound that was eluted 0.3 min before citric acid and prevented quantifiable recovery of 1.25 μmol of citric acid added per gram of soil from the new batch of Cavinti soil (Table 6.2). However, good peak separation was obtained using 25 mM H<sub>2</sub>SO<sub>4</sub>, although the recovery of citrate dropped to 8% (Table 6.2). A 1:4 soil:solution ratio of 5 mM H<sub>2</sub>SO<sub>4</sub> would also have been suitable, although the recovery was lower (6%). This experiment highlights the fact that the composition of organic acids in the soil varies with different soil batches.

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<sup>†</sup> A 1:9 dilution of the 50 mM H<sub>2</sub>SO<sub>4</sub> extract gave a peak area of 2280 units. The minimum area detected by the integrator was set to 300 units i.e. 7.6-fold lower than 2280. Therefore the minimum quantifiable amount of citrate will be approximately 7.6-fold less than the amount of citrate added in the experiment (1.25 μmol g<sup>-1</sup> soil).  $1.25 \div 7.6 = 0.2 \mu\text{mol citrate g}^{-1} \text{ soil}$ .



**Figure 6.3.** HPLC chromatograms of rhizosphere extracts soil spiked with  $1.25 \mu\text{g}$  citric acid  $\text{g}^{-1}$  soil. **a)** A  $1/5$  dilution of the extract, which shows that the citric acid peak (retention time 6.5 min) could not be accurately quantified (indicated by the symbol 'V'), due to an interfering peak at 6.0 min. **b)** The same sample after a  $1/12$  dilution, the 6.0 min peak has been diluted out and the peak can be quantified.

**Table 6.2.** Comparing different soil:solution ratios and concentrations of H<sub>2</sub>SO<sub>4</sub> for extracting citrate from the batch of soil used for the thin layer experiment in Chapter 9, n=2.

Soil:Solution ratio	Acid concentration (mM)	Dilution factor	Extract citrate concentration (μM)	Recovery (%)
2:1	50	9	Unresolvable	?
2:1	25	11	50.8	8.1
2:1	5	2	*12.4	2.0
4:1	25	7	Unresolvable	?
4:1	5	4	20.0	6.4

\* 1 peak was not resolved.

### 6.1.5 Discussion

There are a number of factors that have scope for significant improvement.

#### a) *The low recovery*

If better methods of separation were found it might be possible to use much stronger extractants, reducing the errors associated with a low recovery. Inorganic anions, particularly NO<sub>3</sub><sup>-</sup>, are a major source of interference in analysis of soil samples by HPLC. Where this is critical, an alternative might be to use gas chromatography (GC) rather than HPLC. With assay by GC, organic acids must first be derivatised to make them volatile. As a consequence, interference by inorganic anions does not arise (Szmigielska *et al.* 1997), and it may therefore be possible to use stronger extractants to remove the acids from the soil. However, the derivatisation step introduces problems of its own, such as incomplete recovery of the organic acids during extraction from the aqueous phase into ethyl acetate prior to derivatisation (Szmigielska *et al.* 1997).

*b) Poor separation between compounds*

With most chromatographic procedures it is impossible to separate out all the compounds from a soil extract in one run. With the current HPLC set-up, it was not possible to distinguish oxalic acid from the large peak (mostly  $\text{NO}_3^-$ ) that elutes in the void space of the column. Also lactic and shikimic acid could not be adequately separated (Kirk, G.J.D. pers. comm.). Procedures that would enhance the separation include having an additional in-line column, or in-line detector (set at a different wavelength or measuring a different property). Perhaps the least expensive option would be to have a fraction collector, collect the fraction of interest, and reinject it into the column using different operating conditions. However this would double the analysis time. Separation is much better with GC analysis (Szmigielska *et al.* 1997). Mingxin Guo (pers. comm.) obtained good separation of organic acids obtained from sewage sludge using ion exchange chromatography (IC). Current research using IC at IRRI suggests that two injections, using different operating conditions, may be necessary to separate the organic acids commonly found in Cavinti soil (Kirk and Guingab, unpublished).

*c) Cleanliness of sample*

Analysis could be continued for 2 d before the baseline became erratic, which indicated that it was necessary to clean the column. Furthermore, the retention time of citric acid was initially 8 min, but dropped within a few runs to 6.5 min (e.g. Figure 6.3), then more slowly to 5.9 min where it stabilised. The shortening of retention times reduces the ability of the column to separate organic acids, and might have been caused by the binding of nonpolar compounds to the nonpolar stationary phase of the RP-8 column. Nonpolar compounds are probably unimportant in enhancing the amount of P in solution, and take a long time to pass through the RP-8 column. Removing the nonpolar compounds by pretreatment with a disposable RP18 filter would reduce the frequency of cleaning and also enable the next sample to be reinjected sooner. It is also likely to lengthen the life of the column.

#### *d) Sample preparation time*

The time taken to harvest the soil, weigh, extract, centrifuge and then filter was generally 2 h, during which time approximately one quarter of the original sample may be lost, based on the half life of 4.6 h for citrate measured by Kirk *et al.* (1999b). This might be improved by using Rhizon soil solution samplers, which would enable soil solution to be sampled within a few minutes and immediately acidified to prevent further degradation. The disadvantage with this procedure is that the concentration of organic acids in the soil solution is likely to be much lower than when 50 mM H<sub>2</sub>SO<sub>4</sub> is used as the extractant. However the organic acids could be concentrated by freeze-drying, and then resuspended in a very small volume of solution, as only 20 µL is used for injection.

#### *e) Sample analysis time*

Each run took approximately 25 min before the next sample could be reinjected. Even then the baseline was not smooth, but by this time the small, later peaks had become so wide that they simply appeared as a drifting baseline and did not significantly affect the accuracy of the measurements in the following run. An autoinjector would double the number of samples that could be done in a day as the samples could be run overnight. Gas chromatography is much quicker than HPLC, however overall there is no time saved as more time is taken to prepare (derivatise) the samples (Szmigielska *et al.* 1997).

## **6.2 Storage of organic acids**

### **6.2.1 Introduction**

After harvesting an experiment there are many analyses to do, so it is often necessary to store the organic acid extracts. Due to the large microbial population in the rhizosphere, organic acids are quickly consumed, and new microbial compounds are released. Therefore, it is necessary to store the samples in such a way as to prevent microbial activity. It is also important that any microbicide added does not damage the HPLC column, or interfere with the

organic acid analysis. Methods used by other researchers included freezing (Szmigielska *et al.* 1997), freezing and lyophilising (Pellet *et al.* 1995), acidifying and freezing (Kirk *et al.* 1999b), and the addition of sodium azide (Laurenson and Nouws 1989).

There was also some evidence in an early experiment (data not shown) that the concentration of citrate in samples decreased when stored in the light. Some citrate compounds, e.g. ammonium ferric citrate, degrade when exposed to light (Budavari 1989).

### **6.2.2 Objectives**

There were two objectives:

- 1) To investigate the effectiveness of freezing and acidification in maintaining the organic acid composition of a rhizosphere extract.
- 2) To determine if degradation occurred in samples stored under light conditions, the rate that it occurs (if samples do degrade), and whether degradation occurred in both acidified and non-acidified samples.

### **6.2.3 Method**

#### **6.2.3.1 The effect of acidification and freezing**

Rhizosphere soil was generated by growing rice plants in 0.5 mm sieved Cavinti soil for 6 wk, using the thin layer system described in Chapter 5. Immediately after harvest, a 60 g soil sample was extracted with 120 mL of 50 mM H<sub>2</sub>SO<sub>4</sub> using the method developed in Section 6.1.4.2. The supernatant was filtered (Whatman #5) then thoroughly mixed. Twelve millilitre aliquots of filtrate were taken and subjected to various treatments:

1. No treatment (pH of sample 2.4).
2. Acidified to pH 1.7 (0.12 mL of 2.5 M H<sub>2</sub>SO<sub>4</sub> per 12 mL).
3. Acidified to pH 1.7 (0.12 mL of 2.5 M H<sub>2</sub>SO<sub>4</sub> per 12 mL) then frozen.



There were three replicates of each treatment. Each replicate was then split into four 3-mL samples to examine the effects of storage over time. All samples were stored at room temperature (25°C) except for the frozen samples ( $\approx -18^\circ\text{C}$ ). Samples were analysed immediately, after 1 d, and 15 wk. [Note: samples were also going to be analysed after 1 wk of storage, however the HPLC broke down and took 3 months to repair.]

All samples were diluted by one-sixth and 0.45  $\mu\text{m}$  filtered immediately prior to injection into the HPLC. Dilution raised the pH of the acidified samples to 2.3, and the pH of the no-treatment samples to 3.0. One millilitre of the acidified samples was taken and one drop (approximately 0.02 mL) of 1 M NaOH was added, to test the effect of adding concentrated hydroxide to acidified samples. Addition of  $\text{OH}^-$  would be necessary for acidified, undiluted samples, because it is important to keep the pH of the eluent above 2.0 to avoid damaging the RP-8 column.

The HPLC operating conditions were the same as described in Section 4.3.3.

### 6.2.3.2 The effect of light

Solutions were prepared containing 50  $\mu\text{M}$  citrate and 36  $\mu\text{M}$  Fe (as  $\text{FeCl}_3$ ), in a matrix of  $\text{NO}_3^-$ -free Yoshida nutrient solution. Half of the 25 mL solutions were acidified to  $\text{pH} < 2$  with 0.5 mL of 2.5 M  $\text{H}_2\text{SO}_4$ . An aliquot of these solutions was 0.45  $\mu\text{m}$  filtered and immediately analysed by HPLC (Section 4.3.3). The solutions were then further split into light and dark treatments. The tubes for the dark treatments were wrapped with aluminium foil and placed in a closed cupboard. The light treatment was kept on the bench and the laboratory fluorescent lights left on overnight. The non-acidified solutions were filtered and injected after 7 and 23 h, the acidified solutions were injected only after 23 h. Each treatment was replicated twice. Differences between the treatments after 23 h were analysed by one-way analysis of variance.

## 6.2.4 Results

### 6.2.4.1 Storage of organic acid samples

#### *The effect of room temperature storage on a non-acidified sample*

The differences between immediately analysed samples and those kept for 1 d were small and of a similar magnitude to the differences between replicates (compare Figures 6.4a and 6.4b). However large changes occurred in sample composition during 15 wk of storage (compare Figures 6.4a and 6.4c). The majority of compounds disappeared but a large amount of one compound (which has the same retention time as citric acid – 6.4 min) was synthesised. Note that the scale of Figure 6.4c is seven times larger than that of 6.4a and 6.4b.

#### *The effect of acid or alkali addition*

There was little change in the composition of an immediately analysed sample as a result of acidification, except for an increase in the height of the 4.7 min peak (Figure 6.4a). Adjusting the pH of an acidified sample with NaOH to  $\text{pH} \geq 4.4$  immediately prior to injection caused a considerable change in the composition of the sample (Figure 6.4a).

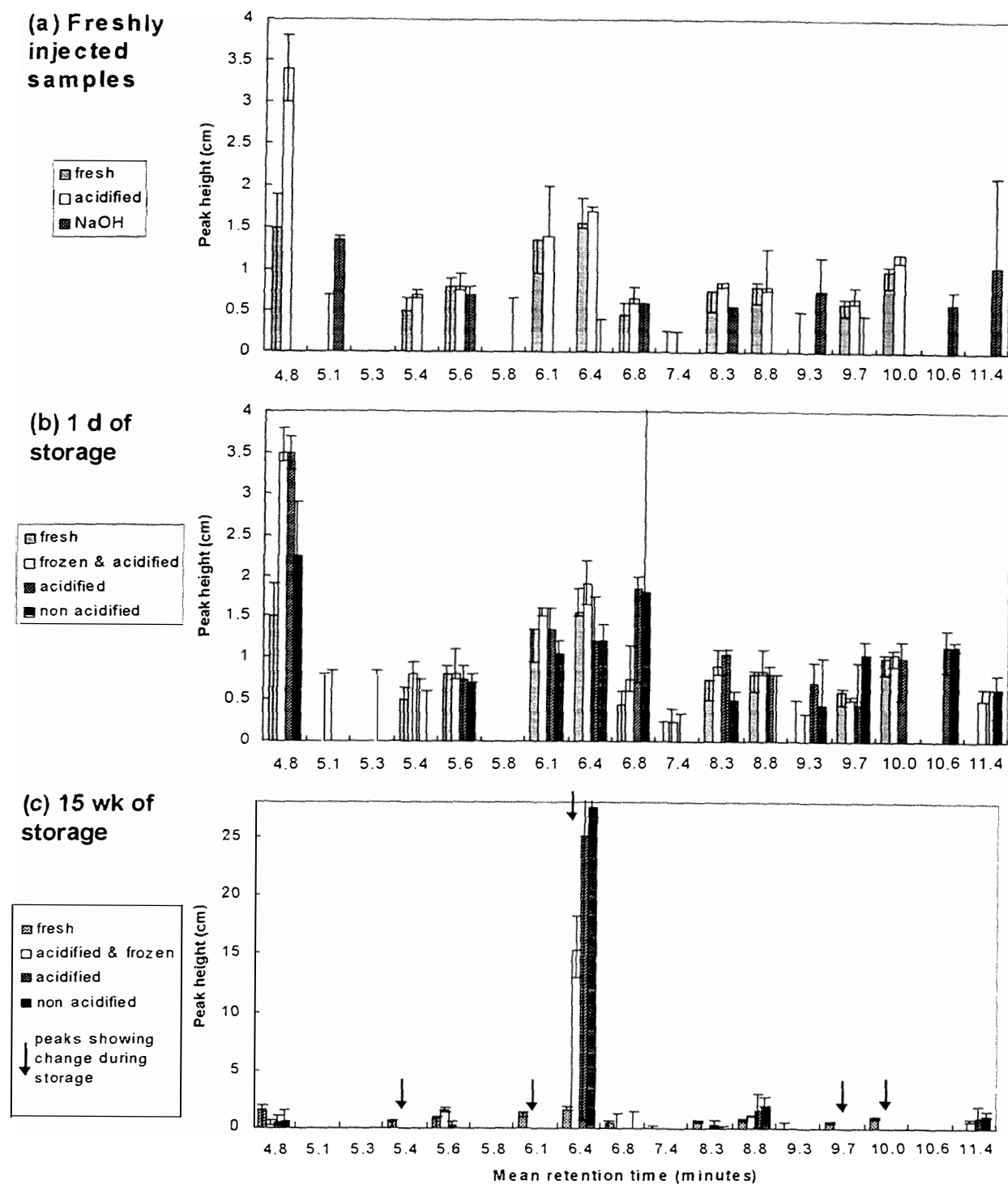
The acidified samples showed the same pattern as the non-acidified samples – there was little change in sample composition after 1 d, and a large change in composition over a period of 15 wk.

#### *The effect of freezing*

There was little difference in composition between a fresh sample and one that had been acidified and frozen for 1 d (compare Figure 6.4a with 6.4b). Samples frozen for 15 wk (Figure 6.4c), however, showed a large change in composition, although they only contained half the amount of ‘citric acid’<sup>†</sup> of the unfrozen samples.

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<sup>†</sup> ‘citric acid’ is placed in quotation marks as the exact identity of this compound was unknown, although it had the same retention time as analytical grade citric acid.



**Figure 6.4.** The effects of different storage procedures on the composition of a rhizosphere soil extract. (a) Sample acidified to pH 1.7, or acidified then readjusted to pH > 4 with NaOH, compared to a fresh, non-pH-adjusted sample. (b) The non-acidified and acidified treatments from (a) stored for 1 d, compared to the fresh sample. (c) As for (b) but samples were stored for 15 wk. Note that the scale for (c) is seven times that of (a) and (b). Median values are plotted, and the maximum and minimum values are represented by the extent of the error bar,  $n=3$ .

## 6.2.5 Discussion

### 6.2.5.1 Storage of organic acid samples

The experiment clearly shows two things that cause large changes in the organic acid composition of soil extracts: the addition of concentrated NaOH, and storage for over 3 months.

#### *Addition of concentrated NaOH*

The change in retention times of almost every peak (including citric acid, retention time of 6.4 min) after addition of 1 M NaOH is likely to be due to a change in the charge on the organic acids at the higher pH. This is supported by the facts that the dissociation constants of many common organic acids found in soils are between 3 and 4.4 (Table 6.3), and that the pH of the rhizosphere extracts showed strong buffering over this pH range (buffer power,  $b_H = 1.5 \times 10^{-2} \text{ mol H}^+ \text{ L}^{-1} \text{ pH}^{-1}$ ). This buffer power is much greater than that of the 50 mM citrate solution in Experiment 6.3 ( $b_H = 1 \times 10^{-5} \text{ mol H}^+ \text{ L}^{-1} \text{ pH}^{-1}$ ), which showed no change in retention time with pH (Table 6.4). The change in retention times indicates that the pH of an injected sample will need to be monitored closely, and adjusted to pH 2.1 if it is significantly different from this value. This is particularly true for undiluted samples, which will have a greater buffer capacity than more dilute solutions.

**Table 6.3.** Dissociation constants ( $pK_a$ ) of organic acids commonly found in soil (25°C) (Dean 1985).

Organic acid	Formula	$pK_1$	$pK_2$	$pK_3$
Acetic	CH <sub>3</sub> COOH	4.76	–	–
Citric	HOOCCH <sub>2</sub> COHCOOHCH <sub>2</sub> COOH	3.12	4.76	6.40
Formic	HCOOH	3.75	–	–
Lactic	CH <sub>3</sub> CHOHCOOH	3.86	–	–
Malic	HOOCCH <sub>2</sub> CHOHCOOH	3.46	5.10	–
Oxalic	HOOC <sup>2</sup> COOH	1.27	4.27	–
Succinic	HOOCCH <sub>2</sub> CH <sub>2</sub> COOH	4.21	5.64	–

### *Changes over 15 wk*

Changes in sample composition during freezing are unlikely to be due to microbial activity. The samples did not defrost while in the freezer as they were frozen in a vertical position and then laid horizontally a few days after freezing. If the samples had defrosted then the solution would have re-frozen in a horizontal position.

The disappearance of some of the smaller peaks (Figure 6.4c) may have been due to volatilisation of compounds (e.g. acetic acid) during freezing, since the vapour pressure of water and volatile gases (with a freezing point of  $> -20^{\circ}\text{C}$ ), is very low inside a freezer.

Some of the large 'citric acid' peak might have originated from the cytoplasm of bacterial cells. El-Kest and Marth (1992) observed that the plasma membrane of *Listeria monocytogenes* strain California began to rupture after 2 wk of freezing, with progressively more loss of cytoplasm at 4 wk. This would explain why no change was observed in the citrate concentrations of solutions with just 1 d of freezing, but large changes were observed over 15 wk. Approximate calculations (Appendix D) however, estimate that only around 44% of the observed increase in citrate concentration in the frozen treatment of the present experiment could have originated from inside bacteria extracted from the rhizosphere soil during the organic acid extraction. Moreover, cell lysis is likely to release other organic acids besides citrate, as microbial cells may also contain high concentrations of malate (Gallmetzer *et al.* 1998). Figure 6.4c shows that only 'citric acid' concentrations were greatly increased. However the composition of organic acids in the cell does vary according to the microbial species and the substrate (Evans and Ratledge 1984, Hossain *et al.* 1984). A large increase in sample citrate concentration during freezing has also been recorded by Gonzales-Castro *et al.* (1997) where the citrate concentration in blanched beans first dropped, then rose to double the original concentration after 6–8 months of freezing. No explanation was given regarding the mechanism by which these changes occurred, but the studies of El-Kest and Marth (1992) illustrate that continued cell degradation does occur when bacteria are frozen at  $-18^{\circ}\text{C}$ , then

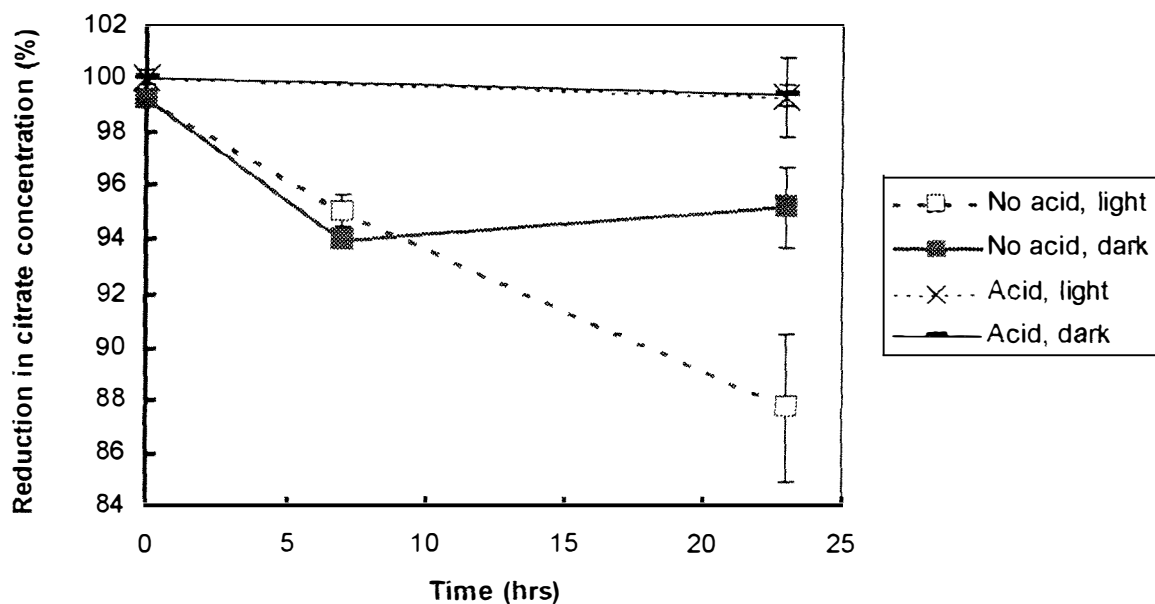
subsequently thawed. Additional citrate may have been synthesised by micro-organisms in the non-acidified, room temperature samples (Kucey *et al.* 1989).

Given that the reason for the change in sample composition during storage is likely to involve micro-organisms, a suggested improvement to the storage technique is to 0.2  $\mu\text{m}$  filter acidified samples into sterile tubes before freezing (as well as prior to injection). It is recommended to change to 0.2  $\mu\text{m}$  filters, because the coarser 0.45  $\mu\text{m}$  filters are not guaranteed to remove all of the micro-organisms (Harrigan and McCance 1976). Micropore (0.2  $\mu\text{m}$ ) filtration would also remove the possibility of sample contamination due to microbial cell lysis during freezing. The only drawback in this method is that it requires twice as many filters, which are expensive. Due to the changes in sample composition during long-term storage it was decided to analyse all future samples immediately after extraction.

#### **6.2.5.2 Storage: the effect of light**

There was no change in citric acid concentration during 23 h of storage in the acidified treatments (Figure 6.5). This agrees with the data in Figure 6.4b. The non-acidified treatments showed a decrease in citrate concentration of 5% after 7 h, whether stored under dark or light conditions. Under light conditions, this decrease continued. However no further decrease in citrate concentration was observed under dark storage. The non-acidified light treatment was highly significantly different ( $P < 0.01$ ) to the other three treatments after 23 h. The non-acidified dark treatment was not significantly different to the acidified treatments.

One possible explanation for these results is that the decrease in citrate concentration observed in the non-acidified light treatment is due to photodegradation. It must then be assumed that photodegradation transforms citrate to some other compound. The fact that no degradation was observed in the acidified treatment might then be due to the fact that photodegradable compounds were not formed in an acidified sample. Whatever the reason for the degradation of citrate in the light, if the sample is acidified to  $\text{pH} < 2$  there is no further degradation in the short term. Thus all future samples taken for citrate analysis were acidified.



**Figure 6.5.** The effect of light, and acid addition, on the short-term storage of samples containing citrate. The bar is the SE of the mean,  $n=2$ ,  $LSD = 4.9$ .

### 6.3 Analysis: The effect of different ions on the measured concentration and retention time of citrate

#### 6.3.1 Introduction

Citrate forms stable compounds with Al, Fe, (Mortvedt *et al.* 1972) and other ions (Budavari 1989) under acid conditions. Significant amounts of exchangeable Al and EDTA-extractable Fe are present in Cavinti soil (Table 4.1). Many other ions are supplied to the thin layer in the Yoshida *et al.* (1976) nutrient solution. It is therefore possible that citrate in the soil solution may be complexed with ions. These complexes may have a different retention time in the RP8 HPLC column than citric acid.

### 6.3.2 Objective

To ascertain whether the presence of  $\text{Al}^{3+}$ ,  $\text{Fe}^{3+}$ , or ions supplied in Yoshida *et al.* (1976) nutrient solution affected the HPLC analysis of citric acid under the established operating conditions (Section 4.3.3).

### 6.3.3 Method

Solutions were prepared as shown in Table 6.4. These were 0.45  $\mu\text{m}$  nucleopore filtered and immediately injected into the HPLC (same operating conditions as in Section 4.3.3). Duplicate injections were made of each solution, except those of pH 0.9, 1.8 and 5.4 – 7.2 where one injection was made.

### 6.3.4 Results

The presence of a range of salts, acid and alkali had no effect on the retention time of citrate (Table 6.4). The only exception was non-acidified solutions containing  $\text{FeCl}_3$ , which may be due to photodegradation of citrate (see Section 6.2.5.2).

### 6.3.5 Discussion

The absence of any change in retention time or concentration of citric acid measured by HPLC indicates that it should be possible to measure the citric acid concentration under a wide range of solution conditions. The absence of any effect of solution pH on citric acid retention time is due to the high pH buffering capacity of the 18 mM  $\text{KH}_2\text{PO}_4$  mobile phase.



**Table 6.4.** Measured citric acid concentration (relative to standard 50  $\mu\text{M}$  citric acid) and retention time for solutions of different compositions, as determined by HPLC. The presence of a salt, acid or alkali is indicated by a 'Y'.

50 $\mu\text{M}$ citric acid	Ions/compounds in the test solution						pH	% of 50 $\mu\text{M}$ citrate	Retention time (min)
	Yoshida (NO <sub>3</sub> <sup>-</sup> , P- and Fe- free)	Fe 36 $\mu\text{M}$	Al 0.2mM	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> 0.32mM	5M H <sub>2</sub> SO <sub>4</sub>	NaOH			
Y							3.4	100	5.9
Y							3.4	100	6.0
Y	Y						3.7	100	5.9
Y	Y	Y					–	92	6.0
Y	Y	Y		Y			–	104	6.0
Y	Y	Y		Y	Y		–	105	6.0
Y	Y		Y	Y	Y		–	102	6.0
Y	Y	Y			Y		0.9	101	5.9
Y	Y	Y			Y		1.8	104	5.9
Y	Y	Y		Y			3.6	92	5.9
Y	Y	Y		Y		Y	4.8	84	5.9
Y	Y	Y		Y		Y	5.4	89	5.9
Y	Y	Y		Y		Y	6.0	93	5.9
Y	Y	Y		Y		Y	7.2	92	5.9

#### 6.4 General discussion on the experimental technique as a means for determining the role of root released organic anions in P uptake

With the improvements made to the thin layer technique, it should be possible to determine whether or not most of the organic anions commonly found in soils are important in enhancing the P uptake of aerobically grown rice. However, using the current HPLC procedure, not all of the potential P-solubilising compounds can be identified, for example oxalic acid elutes at the same time as NO<sub>3</sub><sup>-</sup>, making quantification impossible. Further improvements are required to enable an even wider range of organic compounds that may be important in P solubilisation to be quantified. Work is currently being done at IRRI to see if the separation can be improved using ion chromatography or gas chromatography. An alternative strategy is to use NO<sub>3</sub><sup>-</sup>-free nutrient solution and a nitrification inhibitor to reduce the amount of NO<sub>3</sub><sup>-</sup> in the soil sample.

However, some  $\text{HCO}_3^-$  may have to be added to the nutrient solution to ensure that the soil pH did not become too acid as this is likely to affect plant growth (Kirk *et al.* 1999b).

## 6.5 Conclusions

The method developed for extracting citrate by shaking soil and 50 mM  $\text{H}_2\text{SO}_4$  at a 1:2 soil:solution ratio for 30 min extracted more citrate than the 5 mM  $\text{H}_2\text{SO}_4$  method of Kirk *et al.* (1999b). It is recommended for organic acid extraction from similar highly weathered soils. Other acids extract less citrate than  $\text{H}_2\text{SO}_4$  at the same normality. However, there was a large variability in the composition of organic acids in each batch of soil, so it is recommended that trial extractions with  $\text{H}_2\text{SO}_4$  be done with each batch of soil to determine the best concentration to use.

The composition of rhizosphere extracts changes dramatically during prolonged storage. It is therefore recommended that samples be 0.2- $\mu\text{m}$ -filtered, acidified to  $\text{pH} \leq 2$  and preferably analysed immediately, otherwise within 24 h. Acidification to  $\text{pH} \leq 2$  also removed the possibility of Fe interference in the measurement of citric acid by HPLC. Other inorganic ions likely to be present in the thin layer soil extracts (Al and those in the modified Yoshida's nutrient solution) do not interfere with the citric acid analysis. Note that the pH of highly buffered solutions, e.g. undiluted rhizosphere extracts, should be increased to 2.1 immediately prior to injection to avoid damaging the HPLC column.

More research needs to be done to improve the recovery of organic acids from soils, and to separate the recovered acids from interfering compounds. It is also recommended that research be done on how to clean up the samples and remove nonpolar compounds, as this should improve the life of the HPLC column used to assay the acids.

## **7. Studies on P uptake mechanisms II. Factors affecting root penetration through fine pores**

### **7.0 Introduction**

This chapter is divided into two parts. The first part describes a thin layer experiment; which was a repeat of the experiment in Chapter 5, following the development of improved procedures for extracting and analysing organic acids from Cavinti soil (Chapter 6). In the course of the thin layer experiment it was found that fine roots were able to penetrate the nylon mesh, so the second part of this chapter outlines experiments designed to elucidate the reason for the root penetration.

### **7.1 PART I: The second thin layer experiment**

#### **7.1.1 Objectives**

The objectives of this experiment were the same as those outlined in Section 5.3.1, i.e. to identify and quantify mechanisms governing P uptake by rice in aerobic, highly weathered soils. Of particular interest, was the management strategy of banding fertiliser P, and the role of root-released organic anions in enhancing P uptake.

#### **7.1.2 Materials and methods**

The methods used were the same as the first thin layer experiment (Section 5.3.2), with a few minor changes that are outlined below.

##### **7.1.2.1 Soil**

Half millimetre sieved Cavinti soil was packed into the thin layers instead of 2 mm sieved soil. This was because 0.5 mm particle size packs more uniformly into the thin layer. Also, since 0.5 mm sieved soil is a more even particle size than 2 mm sieved soil, this should reduce errors associated with mixing and sampling soil and reduce the large standard error

in the simplified soil P fractionation. The soil used for the incorporated treatment was fertilised with MCP at  $240 \mu\text{g P g}^{-1}$  soil ( $200 \mu\text{g P g}^{-1}$  soil was used in Chapter 5) as this was the only soil available. Therefore the width of the fertilised band (containing  $2000 \mu\text{g P g}^{-1}$  soil) in the comparative banded treatment was enlarged to 15.5 mm wide in order to provide the same amount of P in both treatments.

The wicks of the control TL systems (unplanted) were removed from the nutrient solution for approximately two days out of three in order to prevent them from becoming too moist, as water was not being lost by transpiration as for the planted TL systems. This should lessen the potential for localised reduction to occur in the control soils. To ensure that the control TL systems were maintained at a water content similar to the planted TL systems, extra TL systems were prepared and sampled in order to monitor the water content.

#### **7.1.2.2 Plant growth**

Plants were transplanted into the TL systems on the 4<sup>th</sup> of November 1997, and harvested on the 11<sup>th</sup> of December. There were six replicates of each treatment and one harvest, 37 d after transplanting.

#### **7.1.2.3 Measurements**

At harvest, the TL systems were dismantled. The roots were rinsed with deionised water then dried, along with shoots, at  $67^\circ\text{C}$  for 48 h and then weighed. Subsamples (500 mg) of the dried roots and shoots were digested with concentrated  $\text{H}_2\text{SO}_4$  and  $\text{H}_2\text{O}_2$  (Jones and Case 1990) and analysed for P (Murphy and Riley 1962).

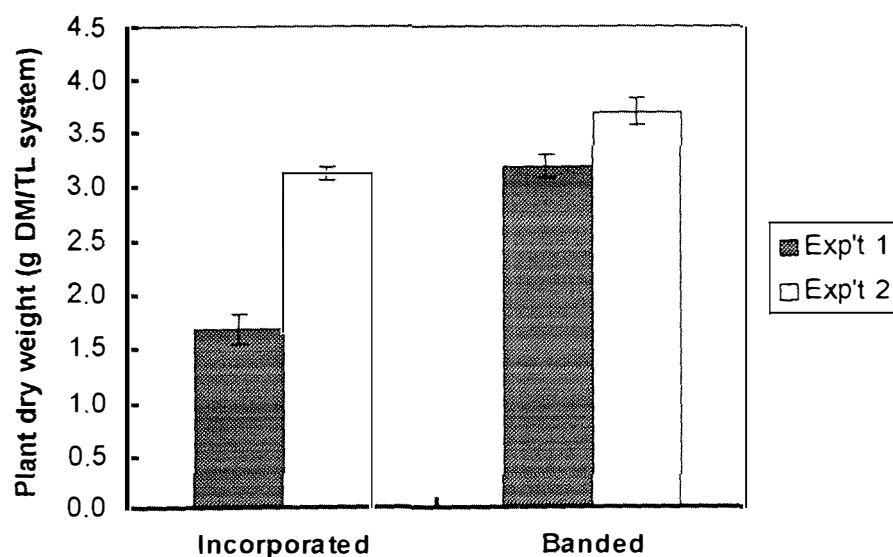
### **7.1.3 Results**

#### **7.1.3.1 Growth and P uptake**

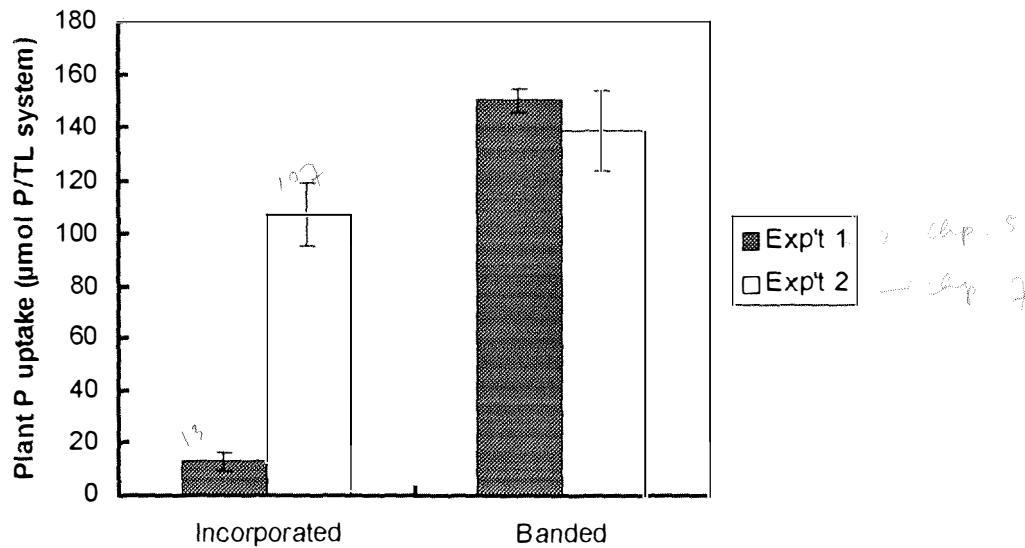
The plant dry weights were very different to those obtained in the first experiment (Figure 7.1). Although this second experiment supplied 20% more P ( $3.2 \text{ mg P/TL system}$ , see Section 7.1.2.1), rice in the incorporated treatment grew 80% more dry matter than in the first experiment for the same growth period of 5 wk. (The 20% extra P supplied explained

the 16% extra growth observed in the banded treatment). The increased growth in the incorporated treatment was due to increased P uptake (Figure 7.2). Many fine roots, covering an area of about one-quarter of the thin layer, had penetrated the nylon mesh and were growing directly in the soil. This greatly reduced the P diffusion path length, making significantly more P available to the plant. Plants in the incorporated treatment took up  $107 \mu\text{mol}$  after 5 wk growth in the current experiment, compared with only  $13 \mu\text{mol}$  after 6 wk growth in the first thin layer experiment (Figure 7.2). The shoot P concentration was above the critical concentration of 0.1% for rice (Tanaka and Yoshida 1970) for both the incorporated (0.13%) and the banded treatment (0.15%).

The penetration of the  $24 \mu\text{m}$  mesh by the plant roots meant that the simple geometry of the root-plane system was violated and the models developed in Chapter 5 were no longer applicable. It was therefore important to ascertain the reasons for the roots penetrating the mesh so as to avoid this happening in the future.



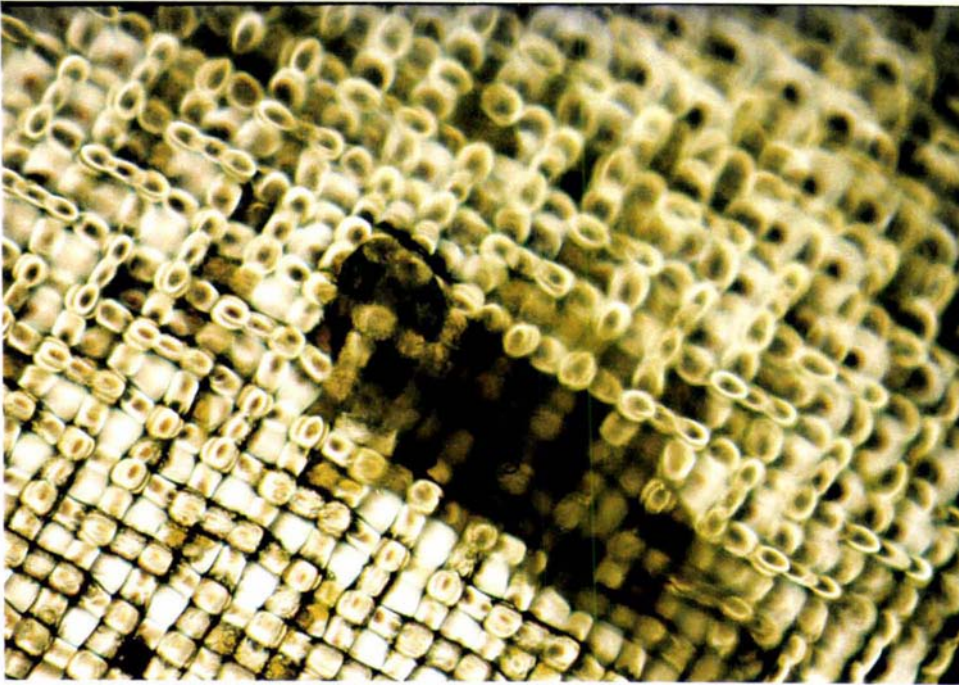
**Figure 7.1.** Total plant dry weight of upland rice grown in the second thin layer experiment compared with those of the first experiment, 5 wk after transplanting. P fertiliser was incorporated throughout the thin layer soil, or mixed in a band occupying 12% of the thin layer soil. Data are means  $\pm$  SE.



**Figure 7.2.** P uptake by rice plants grown in Experiment 1, where no roots penetrated the mesh (6 wk of growth), compared with Experiment 2, where roots penetrated the mesh (5 wk of growth). Data are means  $\pm$  SE.

### 7.1.3.2 Microscopic examination of roots

A number of roots that had penetrated the  $24 \mu\text{m}$  nylon mesh were examined under a microscope. All of these roots had diameters of  $\geq 90 \mu\text{m}$ . Therefore these roots were almost four times too large to have 'fitted' through the mesh. Several points where roots had penetrated the nylon mesh were examined (Figure 7.3). The mesh was not damaged, nor had it been pushed apart by the root. It appeared that the root had been able to grow through the mesh pore then had re-expanded to its original size and continued to grow normally (Figure 7.3)



**Figure 7.3.** A fine root penetrating 24  $\mu\text{m}$  nylon mesh, viewed from above. The circular portion of the root at the top, slightly left of centre, is above the mesh, and the shaded portion bending towards the lower right, is below the mesh. The point of penetration (top, slightly left of centre) is in focus. Note that the mesh is not being forced apart by the root. (This root came from Experiment 7.2). Mag. 350 $\times$ .

#### **7.1.4 Discussion**

##### *Identifying the reason why the roots penetrated the mesh*

The ability of roots to explore the soil is crucial for the uptake of water and nutrients. Restriction of root development by physical barriers (Boone and Veen 1982) or chemical toxicities (Shainberg *et al.* 1989, and references cited therein) results in reduced nutrient and water uptake, leading to decreased plant growth. The results presented in Section 7.1.3 provide clear evidence of the large impact of root length on the growth and P uptake of upland rice. It is therefore important in studies on the mineral nutrition of plants, to understand the factors that affect root penetration.

Wiersum (1957) concluded that elongating roots were unable to decrease in thickness to penetrate small rigid pores. In contrast, Aubertin and Kardos (1965) and Scholefield and

Hall (1985) found that roots had some ability to decrease in thickness to penetrate rigid pores. Research by Scholefield and Hall (1985) indicated that the diameter of the root cap and the diameter of the stele govern the size of pores that can be penetrated by a root. They also observed that oxygen was important in order for roots to grow into constricted pores.

A few fine roots had penetrated 24  $\mu\text{m}$  nylon mesh in the first thin layer experiment (Section 5.4.1): these were found mostly at the very top of the TL system, where the soil was drier due to evaporation. The second thin layer experiment (Section 7.1) was conducted at the beginning of the dry season (November – December), so there was very little cloud cover during the day, compared with Experiment I (Chapter 5), which was conducted during the wet season (July). The amount of transpiration from rice plants grown in the phytotron increased with the increasing amount of solar radiation, even though the temperature (measured in the shade) and humidity were tightly controlled. The soil was somewhat drier in the second thin layer experiment than the first (average moisture content 0.47  $\text{g g}^{-1}$  dry soil compared with 0.52). It was therefore hypothesised that the moisture content of the soil affected the ability of roots to penetrate 24  $\mu\text{m}$  nylon mesh.

## **7.2 PART II: Investigating why the root hairs penetrated the nylon mesh**

### **7.2.1 Objectives**

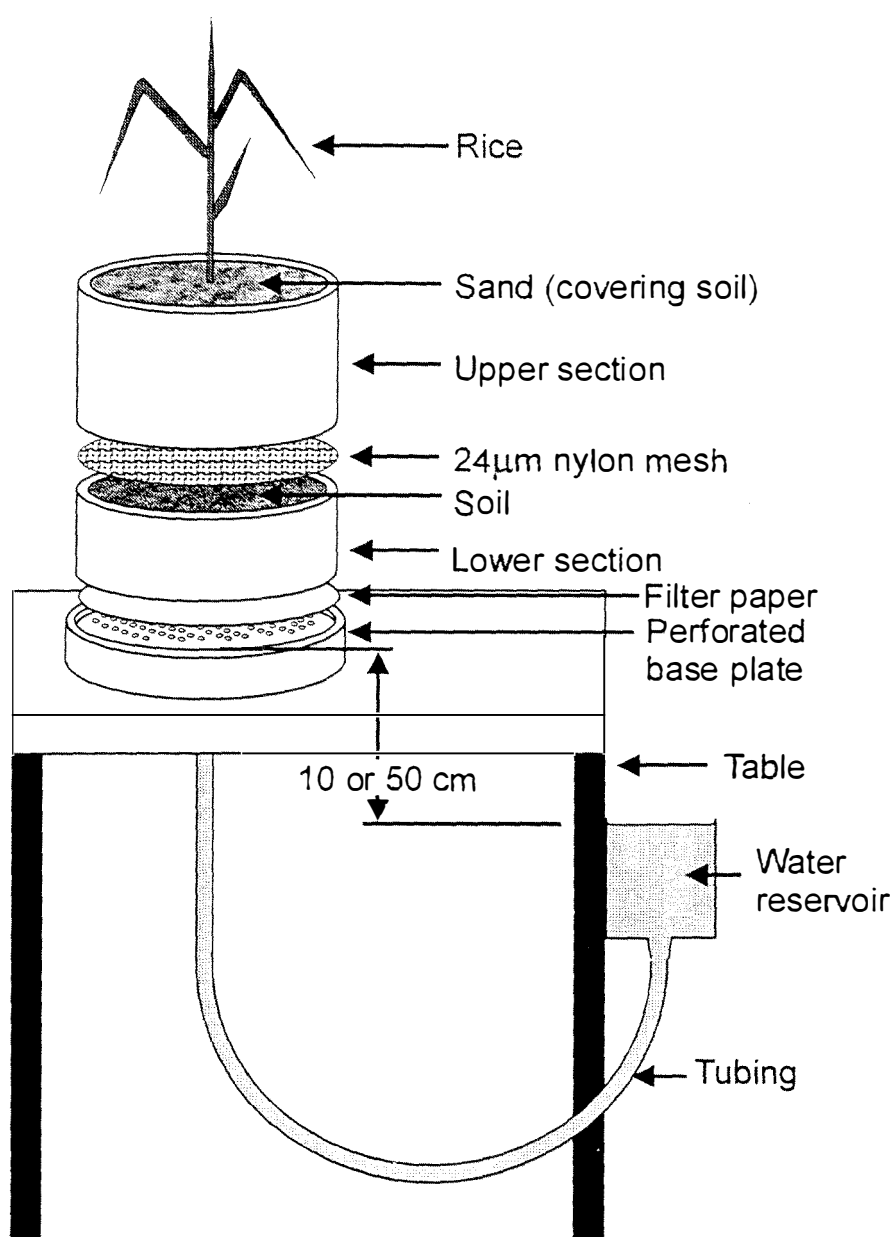
The objectives of this study were twofold: firstly; to determine whether the ability of upland rice to penetrate 24  $\mu\text{m}$  nylon mesh was affected by the moisture content of the soil. And secondly, to investigate the relationship between the diameter of the root cap and stele in determining whether roots can penetrate 24  $\mu\text{m}$  nylon mesh.

### **7.2.2 Materials and methods**

The soil used was air-dried, 2 mm sieved Cavinti. To this soil, N, P and K were added at respective rates of 188, 200 and 200  $\mu\text{g g}^{-1}$  soil, as urea, MCP and potassium sulphate. This



soil was packed into eight PVC tubes (66 mm internal diameter) (Figure 7.4). The tube was divided into an upper and lower section separated by new 24  $\mu\text{m}$  (pore size) nylon mesh. The lower section (30 mm deep) was fitted with a 24  $\mu\text{m}$  nylon mesh base (to prevent the soil from falling out of the bottom of the tube) then packed with 103 g of air-dried soil, and the upper section (6 cm deep) with 206 g of soil.



**Figure 7.4.** Exploded view of the pressure plate apparatus used to control the moisture content for Experiment 7.2. (Modified from Huguenin 2000. Not to scale).

The tubes of soil were then placed on a suction-plate-like apparatus (Figure 7.4) developed by Huguenin (2000). The soil was wet-up at 10 cm suction (corresponding to a volumetric moisture content of 0.5). Unbroken water contact between the soil and the plate was ensured by placing the tubes of soil onto the filter paper at zero suction (while there was water covering the filter paper) and then lowering the reservoir.

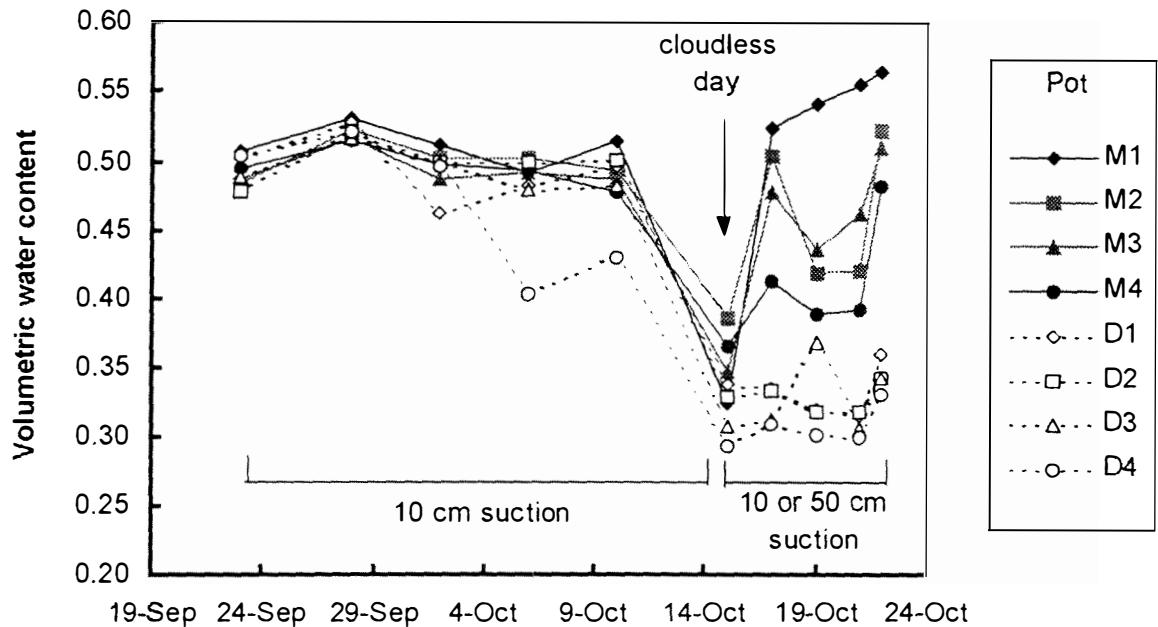
One 3-wk-old seedling of Azucena was then transplanted into each pot and the surface covered with 80 g of silica sand in order to reduce evaporative water loss. The sides of the pots were protected from the sun by a cardboard shield as direct sunlight on the sides of the pot can cause soil temperatures of 30–40°C. Plants were grown for 3 wk, then the water content of four out of the eight pots was lowered to 50 cm suction. At this moisture content plants wilted during the middle of the day.

After 10 d of this water regime the lower section was removed and the number of roots that had penetrated the mesh in each of the eight pots was counted. Root, root cap and stele diameters were also measured using a microscope. The stele diameter was measured by clearing the roots with NaOH; staining with trypan blue (Koske and Gemma 1989) and then measuring the diameter of the dark-blue stained central core. Thus the roots were not cut – the stele was measured using a graticular microscope and viewing from above. Transverse sections of one coarse and one fine root were taken in order to verify that the dark staining core was in fact the stele.

### **7.2.3 Results and discussion**

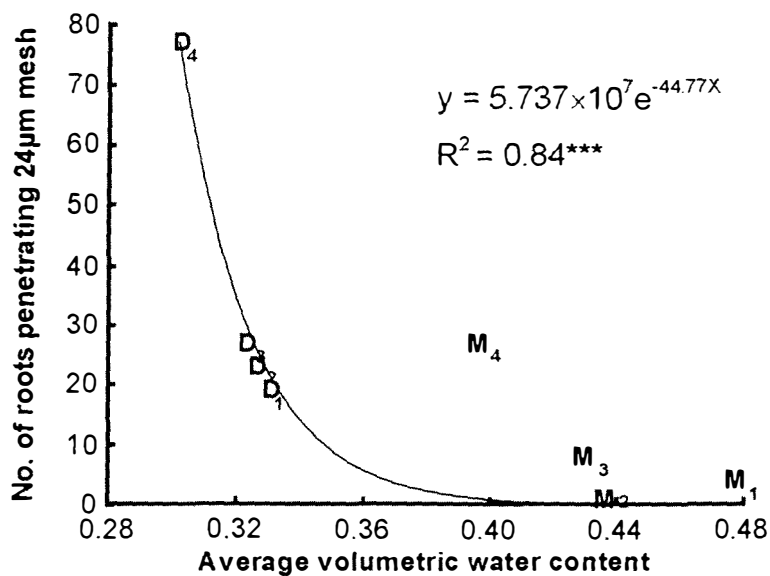
Plants were grown at the end of the rainy season. Figure 7.5 shows that, apart from pot D4, the experimental system controlled the moisture content well for the first 17 d of the experiment while the plants were small and the weather was cloudy. However, during the first day of continuously fine weather (15<sup>th</sup> of October) the moisture contents of all pots dropped markedly. This illustrates how difficult it is to maintain constant water content in a small pot. For the remaining days additional water was added to the tops of the pots daily to maintain the target water contents. However the moisture content was still partially determined by the number of sunshine hours per day so was not maintained uniformly,

particularly in the 10 cm treatment. In Figure 7.6, the number of roots penetrating the mesh is therefore plotted against the average moisture content for the period of the experiment.



**Figure 7.5.** Moisture content for each pot of the moist (M) (10 cm suction) and dry (D) (50 cm suction) treatments during Experiment 7.2. The pots are numbered 1 to 4 – from wettest to driest average moisture content.

There was a significant correlation of 0.73 between the number of roots that penetrated the mesh and the average moisture content of the pot. Regression analysis indicated there was a highly significant exponential decrease in the number of roots that penetrated 24  $\mu\text{m}$  nylon mesh with an increase in average volumetric water content (Figure 7.6). There was one outlier in each treatment (M4 and D4) that had markedly more roots penetrating the mesh (Figure 7.6). This corresponded to the driest pot from each treatment. Outlier D4 had a significant effect on the shape of the relationship in Figure 7.6. The presence of one outlier in each treatment indicates that the experiment would need to be repeated under more controlled moisture conditions if it was important to know the exact nature of the relationship between moisture content and the number of roots penetrating the mesh.

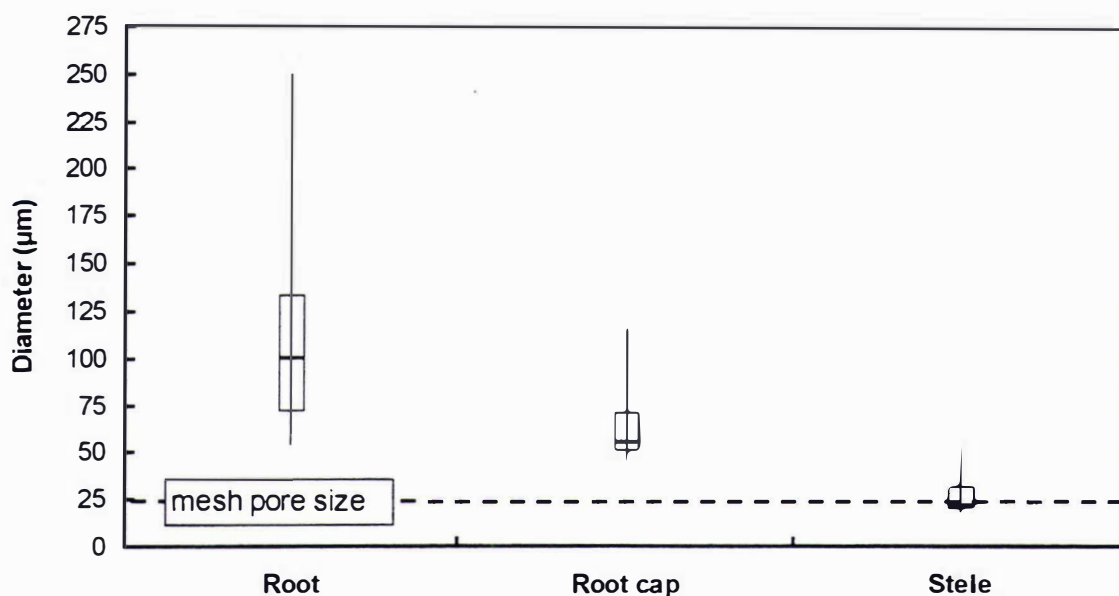


**Figure 7.6.** The relationship between the number of roots that penetrated the 24  $\mu\text{m}$  nylon mesh and the average water content during the 10 d of the different water regimes. The letters M and D represent the moist (10 cm suction) and drier (50 cm suction) treatments, and the pots within each treatment are numbered from wettest to driest. \*\*\* The  $R^2$  is highly significant ( $P < 0.001$ ).

The diameter of the root and also of the root cap was at least two to three times the width of the mesh pores. The staining procedure indicated that the stele width ranged from 19 to 54  $\mu\text{m}$ , with two-thirds of the measurements being less than 24  $\mu\text{m}$  (Figure 7.7), and the bulk of the other values not much greater. Measurement of the two cross sections, one of a thick root and one of a fine root, showed that the stele diameter ranged from 19 to 65  $\mu\text{m}$ , this indicates that the staining technique underestimated the stele width of the coarser roots by approximately 20%.

It appears therefore, that the diameter of the stele maybe an important indicator of the size of pore through which a root may penetrate, but it is not the determining factor. It is likely that mechanical factors have an important bearing on the size of pore through which a root may penetrate: the alignment of the growing root with the pore, the plasticity of the root, and amount of pressure that a root can exert on the mesh pore. The role of the soil water content in determining the pore size through which a root may penetrate is likely due to its effect on soil strength. A root in wet soil is not as well anchored as in dry soil, due to the

low strength of wet soil. Any pressure exerted by the root against the mesh is more likely to move the root, rather than force the root through the pore. A root in wet soil may therefore not be able to exert enough pressure on the root tip to push it through the mesh. In contrast, a root in dry soil is firmly anchored and is less able to bend to the side when being pushed through soil pores by the elongating cells.



**Figure 7.7.** Measurements made on roots that penetrated 24 µm nylon mesh for parameters considered important for roots to penetrate fine pores. The complete range of the data are represented by the line, the upper and lower quartiles by the ends of the box, and the median by the thickened horizontal line. Note that the coarser stele measurements may be underestimated by approximately 20% (see text).

Observations by Scholefield and Hall (1985) indicated that oxygen was important in order for roots to penetrate fine pores. Adequate oxygen is certainly required for root elongation (Eavis 1972, Turner *et al.* 1981, Sato *et al.* 1997). In the current experiment, the average air-filled porosity over the period of the different water regimes was 31–34% for the dry treatment, and 17–25% for the moist treatment. Turner *et al.* (1981) grew rice in solutions aerated with gas mixtures of different O<sub>2</sub> concentrations, and found that rates of root elongation in a range of rice cultivars were reduced below an oxygen concentration of

0.05 L O<sub>2</sub> L<sup>-1</sup> solution. Assuming the soil air is 21% oxygen, an oxygen concentration of 0.05 L O<sub>2</sub> L<sup>-1</sup> soil would require an air-filled porosity of  $(0.05/0.21) \times 100 = 24\%$ . Eavis (1972) found that root elongation rates of peas were reduced at a porosity of  $\leq 30\%$ . Perhaps the ability of a root to penetrate small pores might also be reduced at oxygen concentrations of less than 24–30%. However, the change in soil strength with moisture is likely to be the main factor determining the ability or inability of roots to penetrate pores that are much smaller than their diameter.

### 7.3 Conclusions

Root penetration of the nylon mesh increased the P uptake and growth of upland rice in the TL systems. The number of roots that penetrated the 24 μm nylon mesh was greater in drier soil. It is suggested that this is due to the change in soil strength with moisture content, which resulted in better anchoring of the root in the soil, so that the root could exert more pressure on the root tip to force it through the pore. The greater root penetration in drier soil may also reflect increased oxygen supply.

The ability of a root to penetrate a fine pore is better predicted by the diameter of the stele than the diameter of the root cap. However the factors determining whether a root can penetrate a fine pore are probably physical: the alignment of the root with the pore, and how much pressure the root can exert on the cap to force it through the pore.

Maintenance of volumetric soil moisture contents greater than 0.44 should largely prevent root penetration of 24 μm mesh in future experiments.

## **8. Studies on P uptake mechanisms III. The effect of mycorrhizal infection**

### **8.1 Introduction**

The thin layer experiment described in Chapters 5 and 7 was repeated, this time using 10  $\mu\text{m}$  nylon mesh to prevent roots from growing into the soil. A large infection of mycorrhizal fungi was found in the roots in this thin layer experiment. This chapter therefore examines the effect of VAM infection on P uptake in the TL system, and discusses VAM infection with respect to P uptake in the field, organic anion release, and the collection and storage of soil.

### **8.2 Objective**

The objective of this experiment is the same as the previous thin layer experiments (see Chapter 5) i.e. to identify and quantify mechanisms governing P uptake by rice in aerobic, highly weathered soils. Of particular interest, was the management strategy of banding (localised placement) fertiliser P, and the role of root-released organic anions in enhancing P uptake.

### **8.3 Materials and methods**

The same methods were used as in the second thin layer experiment, except that 10  $\mu\text{m}$  nylon mesh was used instead of 24  $\mu\text{m}$  mesh. Also, a different batch of Cavinti soil was used, the original having run out. The soil was collected from the same site. It was air-dried, sieved to  $< 2$  mm, and stored in a tied plastic bag in a non-air-conditioned room for 10 months. The air-dried water content was 0.18  $\text{g g}^{-1}$  oven-dry soil. Samples were fertilised with 200 and 2000  $\mu\text{g P g}^{-1}$  soil (P200 and P2000 soil) and packed into TL systems to provide banded and fully incorporated treatments as in Section 5.3.2.1 to a bulk density of 1.09  $\text{kg dm}^{-3}$

There were six replicates of each treatment, harvested 41 d after transplanting. Harvesting and subsequent analysis for dry weight, shoot P, and VAM infection are as described in Section 5.3.2.3. Samples were taken for organic acid analysis, but were not analysed, since a large VA mycorrhizal infection was found. Consumption (Hepper and Jakobsen 1983) and production (Graustein *et al.* 1977) of organic compounds by mycorrhizae, as well as leakage of organic anions from damaged fungal hyphae during harvesting and extracting, would have made it impossible to determine the amount of organic anions released by rice into the rhizosphere.

### *Statistics*

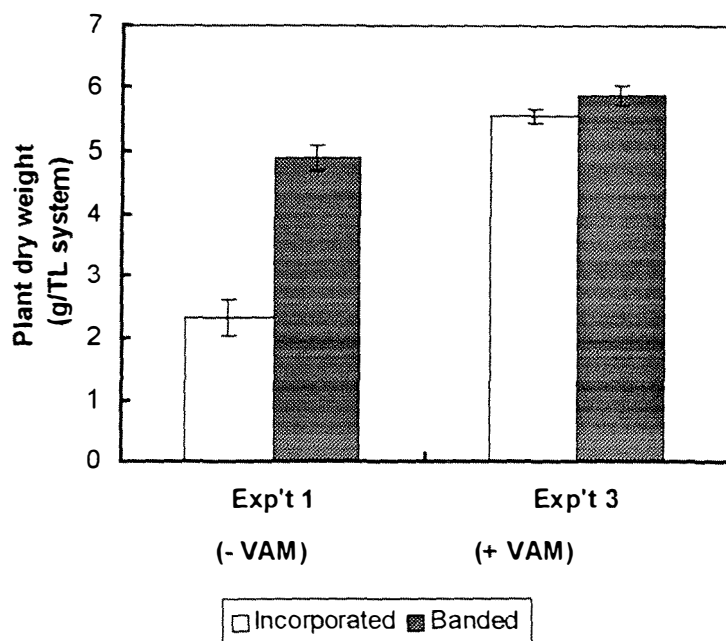
Because the environmental conditions were strictly controlled, it was assumed that statistical comparisons could be made between the first and third thin layer experiments. Differences were determined by ANOVA followed by multiple range tests using the SAS<sup>®</sup> System.

## **8.4 Results**

### **8.4.1 Plant dry weight**

Plant dry weights did not differ significantly between the banded and incorporated treatments, whereas in the first thin layer experiment there was a more than two-fold difference (Figure 8.1). Furthermore, the dry weights of this thin layer experiment were highly significantly ( $P < 0.0001$ ) greater than those of the first – 20% greater for the banded treatment and 140% greater for the incorporated treatment (Figure 8.1).



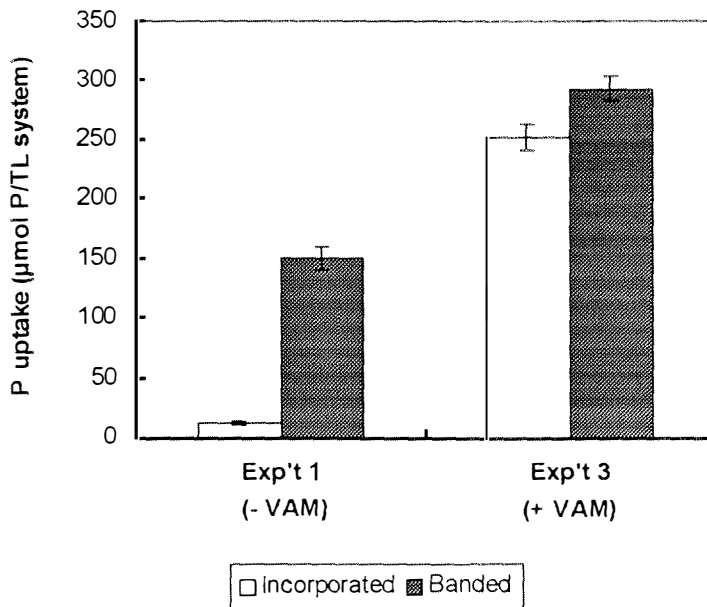


**Figure 8.1.** Total dry weight of upland rice grown in the first TL experiment (without VAM infection) compared with the third TL experiment (55–92% of the total root length infected with VAM). Plants were grown for 6 wk. The data are means  $\pm$  SE.

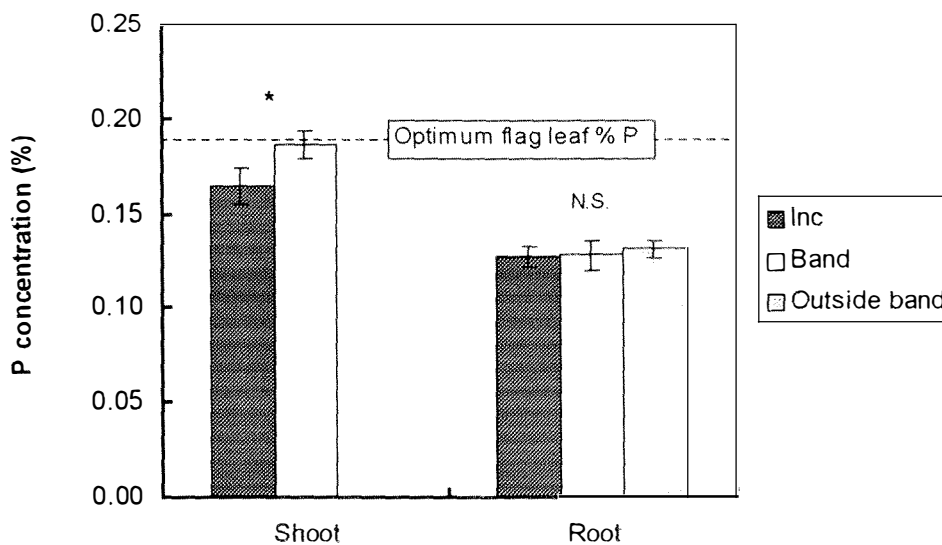
#### 8.4.2 Phosphorus uptake.

The increase ( $P < 0.05$ ) in P uptake in the banded over the incorporated treatment was small (17 %), compared with the six-fold increase in the first thin layer experiment (Figure 8.2). Total plant P uptake was also much larger ( $P < 0.0001$ ) than in the first experiment – two- and greater than ten-times the amounts in the first experiment for the banded and incorporated treatments, respectively (Figure 8.2). The amounts of P taken up from the banded and the incorporated treatments of this thin layer experiment were 6 and 19 times more (respectively) than could be explained by diffusion of readily-available P to the planar rhizosphere (compare Figure 8.2 with Figure 5.8).

The shoot P concentration of the banded treatment (Figure 8.3) was near the optimum for rice at flowering (Jakhro 1985). The shoot P concentration of the incorporated treatment was slightly below the optimum, being significantly ( $P < 0.1$ ) lower than the banded treatment. There was no effect of the different soil P concentrations on the P concentration of the roots.



**Figure 8.2.** A comparison of P uptake from the first TL experiment (without VAM infection) against the third TL experiment (55–92% of the total root length infected with VAM). Upland rice plants were grown for 6 wk. The data are means  $\pm$  SE.

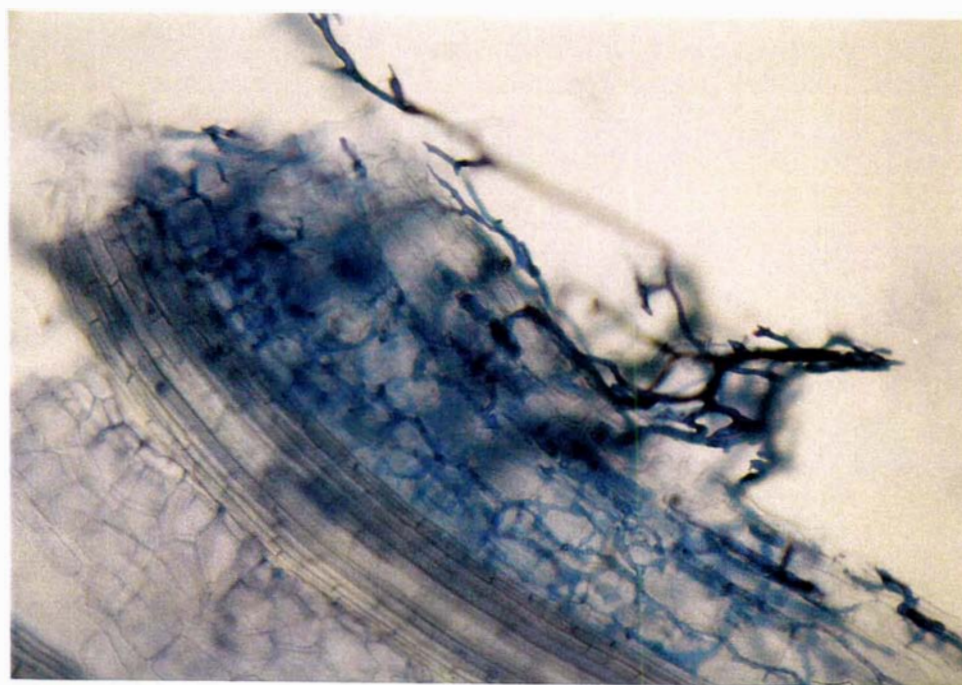


**Figure 8.3.** Root and shoot P concentration of rice grown with P fertiliser incorporated throughout the TL system (Inc) or banded within 10% of the soil volume (Band). For the banded treatment, a separate P concentration is given for roots taken from inside and outside the P-rich band. The optimum flag leaf % P is given for rice at flowering (Jakhro 1985). \* The difference is significant at  $P < 0.1$ . The data are means  $\pm$  SE.

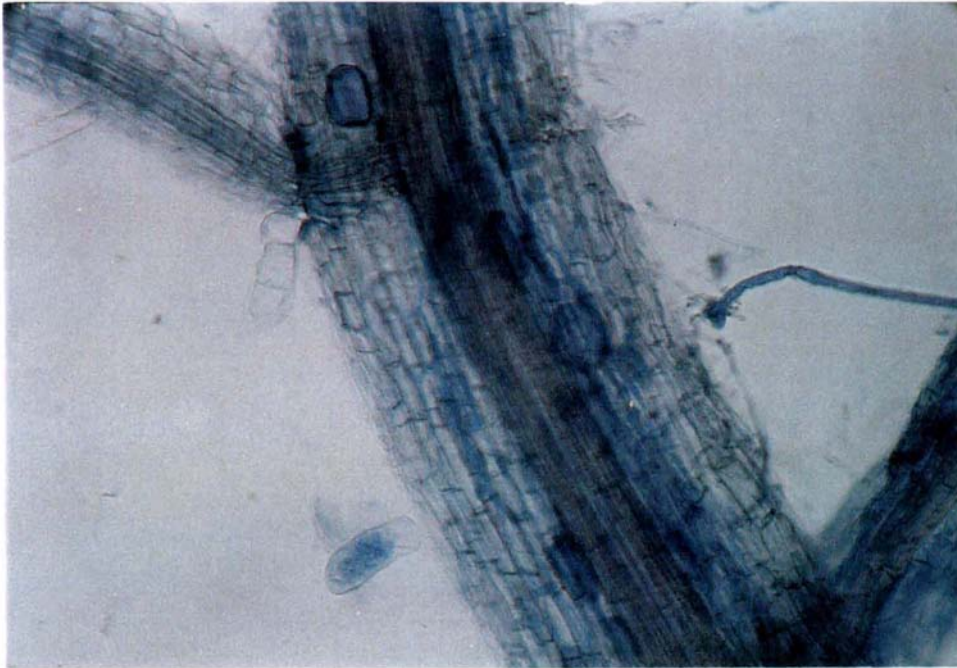
### 8.4.3 Mycorrhizal infection

The much improved growth and P uptake in this experiment could not be attributed to root penetration of the nylon mesh, as the 10  $\mu\text{m}$  mesh had stopped all root penetration. However staining the roots for mycorrhiza revealed that 55–92% of the total root length was infected with VA mycorrhiza (Figures 8.4 – 8.8). The infection was well developed, with abundant intra- and extra-cellular hyphae (Figure 8.4), the formation of vesicles (Figures 8.5 and 8.6), arbuscules (Figure 8.7) and even spores (Figure 8.8).

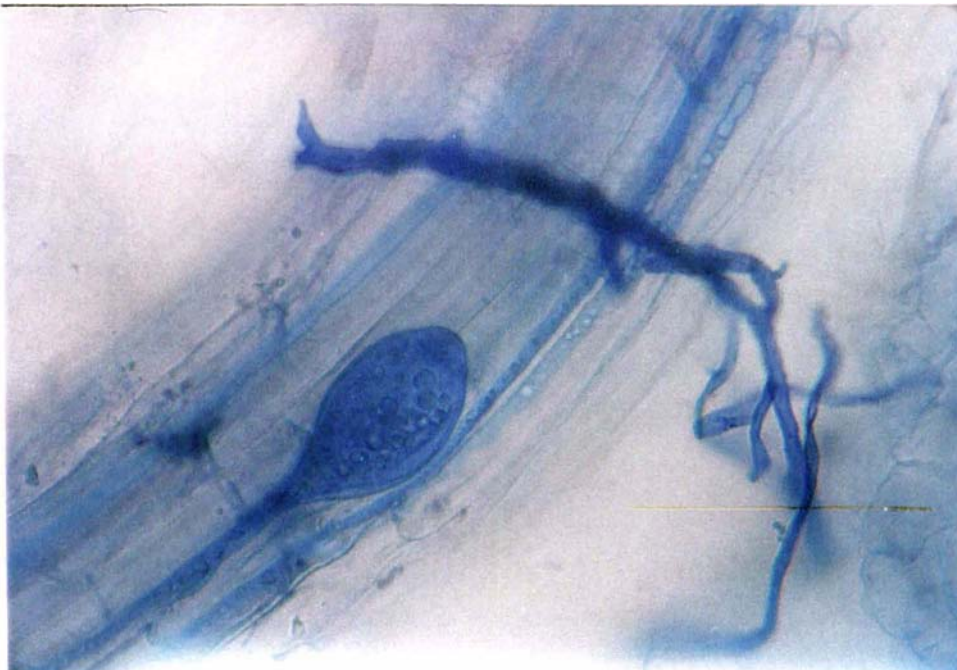
The proportion of root length infected with mycorrhiza was significantly ( $P < 0.01$ ) greater in the P fertilised soil than in the unfertilised soil (Figure 8.9a). Given that the total root length in the P-rich band comprised only approximately 13% of the total root length of the TL systems (calculated using data from the first thin layer experiment, week 5, Table 5.4), the proportion of the total root length that was infected in the banded treatment (66%) was significantly ( $P < 0.05$ ) lower than the incorporated treatment (76%) (Figure 8.9b).



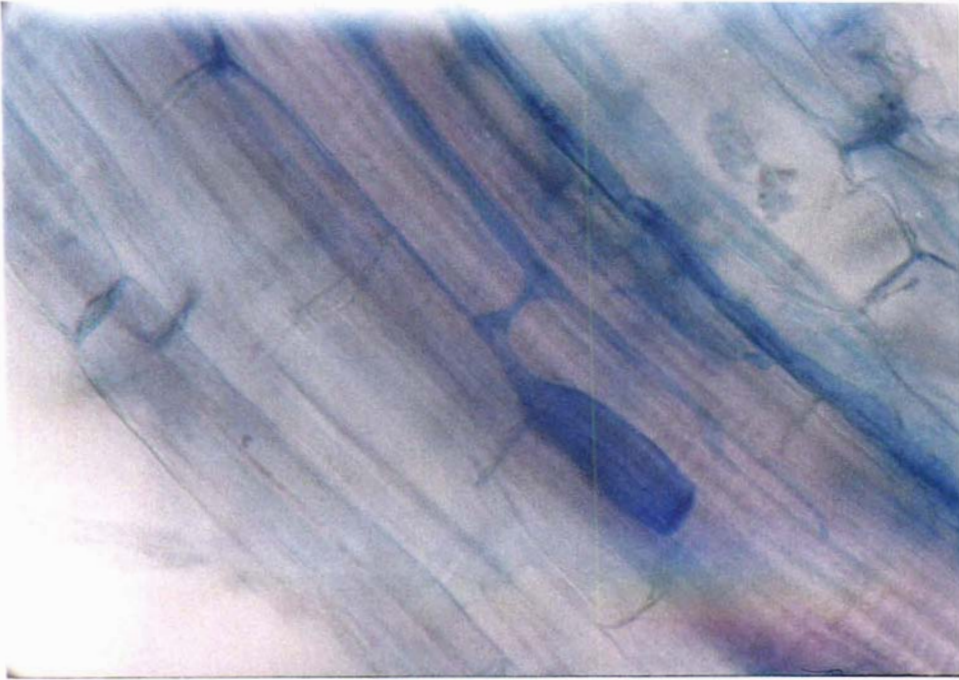
**Figure 8.4.** A rice root heavily infected with VAM. The blue-stained hyphae can be seen both inside and outside the root. Mag. 350 $\times$ .



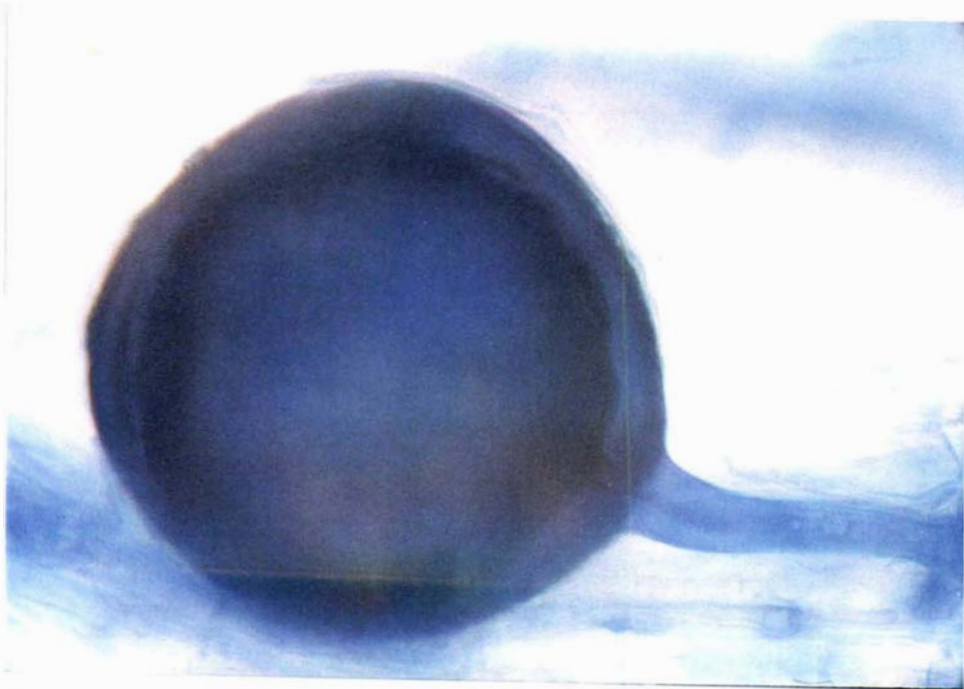
**Figure 8.5.** A rice root, containing a large number of mycorrhizal vesicles (dark blue staining ellipses). Mag. 350 $\times$ .



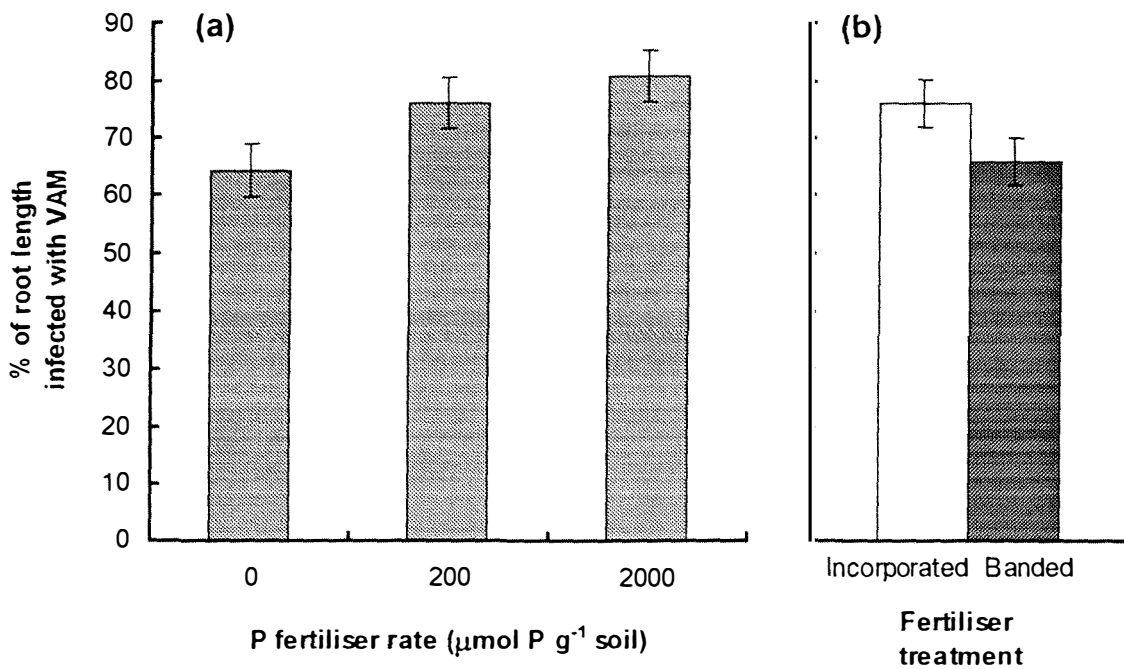
**Figure 8.6.** A mycorrhizal vesicle, mag. 1400 $\times$ .



**Figure 8.7.** An arbuscule (mag. 1400×).



**Figure 8.8.** A mycorrhizal spore, mag. 1400×.



**Figure 8.9.** (a) The effect of P fertiliser rate on the proportion of root length infected with VAM. 0 = the unfertilised soil of the banded treatment, 200 = the incorporated treatment, 2000 = the band of the banded treatment. (b) The proportion of the total root length infected with VAM. The bar is the LSD,  $P < 0.05$ .

## 8.5 Discussion

### 8.5.1 Explaining the observed P uptake

The observed P uptake in the incorporated treatment with VAM (251  $\mu\text{mol/TL}$  system, Figure 8.2) was more than an order of magnitude greater than that calculated for a root-plane absorbing P without solubilisation or VAM (13  $\mu\text{mol/TL}$  system, Figure 5.8). Averaged over all the thin layer soil, an uptake of 251  $\mu\text{mol P}$  is equivalent to a P depletion of 3.7  $\mu\text{mol g}^{-1}$  soil ( $= 251 / (2 \times \text{bulk density} \times \text{volume of TL system})$ ). This depletion is roughly half of the NaOH- $P_i$  fraction (Table 5.1), suggesting that the VAM-infected plants were able to solubilise soil P in some way that the uninfected plants were not. It is not clear how this might have happened. In a preliminary experiment, where plants were grown in thin layers of soil with and without VAM but with the roots allowed to proliferate freely in the soil, the

uninfected plants grew better than the VAM-infected plants in the present experiment (no data collected). Therefore it is unlikely that the VAM hyphae were able to solubilise P that healthily growing rice plants could not.

In the literature, the question of whether VAM hyphae are able to solubilise soil P that is otherwise unavailable to the plant remains a matter of debate. However the majority of evidence indicates that they are not (Tinker and Nye 2000). In experiments with plants grown in  $^{32}\text{P}$ -labelled soil, the specific activity of P in infected and uninfected plants is generally found to be the same, indicating that both plants are accessing P from the same isotopic pools (Tinker and Nye 2000).

If the VAM hyphae cannot solubilise P themselves, then if they are taking up solubilised P this must have been solubilised by the plant. This would mean that the infected plants in the present experiment were growing healthily and solubilising P, but that the plants uninfected in the first thin layer experiment were not, and whatever it is that caused the uninfected plants to be unhealthy was overcome by VAM infection. Perhaps plants need a certain minimum supply of P to have enough adenosine tri-phosphate to actively excrete organic anions. This would explain the observations of Kirk *et al.* (1999a) – that highly stressed rice grown in P100 TL systems showed very little organic anion release, compared with moderately P stressed P1000 plants (see discussion in Section 5.4.5.6).

The comparison between the mycorrhizal plants, and the non-mycorrhizal plants sown directly into the soil, also emphasizes the importance of assessing the magnitude of the increase in P uptake from VAM infection under field conditions.

### **8.5.2 The effect of soil P concentration on the proportion of root length infected with VAM**

The finding that plants with a lower shoot P content (0.16% – incorporated treatment) had a significantly greater mycorrhizal infection than higher P containing plants (0.19% – banded

treatment) (Figure 8.9b) is well documented (Cooper 1984 and references cited therein; documented for rice – Pasolon and Hirata 1993). What is not clear from the literature is the effect of localised placement of P on VAM infection in P-rich zones. Some studies have found that VAM infection decreases in the P-rich zone (Koide and Li 1990, Lu *et al.* 1994), Jasper *et al.* (1979) found that there was no difference, whereas de Miranda *et al.* (1989) found that VAM infection markedly increased in the high P zone. In two of the studies the plants had moderate shoot P concentrations,  $\geq 0.36\%$  for subterranean clover (Jasper *et al.* 1979) and  $\geq 0.30\%$  for maize (Lu *et al.* 1994); also the P concentration of the maize roots growing in the P-rich zones was higher than the roots in the unfertilised zone. These two studies support the hypothesis that the amount of VAM infection shows no change, or decreases, when fertiliser P is added to plants growing in soil supplied with sufficient P (Same *et al.* 1983). In the study of de Miranda (1989) however, the sorghum plants were highly P-stressed, having a shoot P concentration of 0.08%, and a root P concentration of only 0.03%, in the unfertilised treatment. The P concentration increased to 0.22% in the shoot and 0.06% in the zero P roots and 0.08% in the P-fertilised roots when P was applied to one half of the split plot. Studies under P-limited conditions have found that moderate additions of P actually enhance VAM infection (Same *et al.* 1983, Bolan *et al.* 1984, Koide and Li 1990). The observed increase in mycorrhizal infection in the P-rich zone in the present study, and the study of de Miranda (1989), also show an increase in VAM infection in response to increased soil P in P-limited (Figure 8.3) plants. De Miranda *et al.*'s (1989) observations led them to conclude that soil P, as well as plant P, was important in determining the amount of VAM infection in roots. The study of Koide and Li (1990), however, does not fit with this hypothesis, as the sunflower shoot P concentrations were also very low (0.07%, root P concentrations not given). The amount of VAM infection is likely to be related to root cytoplasmic carbohydrate concentration (Bowen 1984), and it might be that sunflowers mobilise carbohydrates differently to rice and sorghum in response to high soil or root cell P concentration.

Therefore, it is likely that VAM infection will increase in roots in P-rich zones from low P status plants, but will decrease or remain unchanged in roots in P-rich zones from plants of



medium to high P status. However, not all of the available data fit this hypothesis, and there is still more research required to elucidate the mechanism as to the effect of plant and soil P status on VAM infection.

The possibility of localisation of mycorrhizal infection in P-rich zones as a P efficient strategy does not appear to be discussed in the literature. Localisation of VAM in P-rich zones would have the same effect on P uptake as the multiplication of roots in P-rich zones (discussed in Section 1.4.3.3). Future fertiliser strategies might involve banding VAM inoculum along with fertiliser as recommended by Howeler *et al.* (1987).

### **8.5.3 The relationship between P deficiency, organic anion release, and VAM infection**

In the present study it was impossible to determine the amount of organic anions produced by upland rice in the rhizosphere soil, for reasons outlined in the methodology (Section 8.3). However, a number of studies suggest that there is a strong link between P deficiency, organic anion release, and VAM infection. Ratnayake *et al.* (1978) and Graham *et al.* (1981) proposed that VAM infection is the result of the release of simple organic compounds due to membranes becoming leaky as a consequence of P deficiency (see Section 1.5.3.2.3a). Both Ratnayake *et al.* (1978) and Graham *et al.* (1981) found that the cytoplasmic concentration of amino acids and reducing sugars declined with P deficiency, yet the concentration of amino acids and reducing sugars exuded increased. This hypothesis is further supported by observations that root exudates, including citric acid (Mosse 1970), have been found to stimulate the spore germination and growth of mycorrhizal hyphae (Hepper 1984).

An alternative hypothesis is that the extent of VAM infection is regulated by the concentration of carbohydrates in the plant root (Jasper *et al.* 1979, Same *et al.* 1983, Bowen 1984, and de Miranda *et al.* 1989). Jasper *et al.* (1979) and Same *et al.* (1983) obtained contrasting results to Ratnayake *et al.* (1978) and Graham *et al.* (1981), and observed an increase in cell carbohydrate concentration with P deficiency. De Miranda *et al.* (1989) found greater VAM

infection in the P-rich zone, as was found in the present study. They argued that if mycorrhizal infection were due to root cell membranes becoming leaky, then they should have observed similar or greater mycorrhizal infection in the unfertilised zone than the fertilised zone. However, it is still possible that root cell membranes may become more permeable, but only in the P-rich zone – although the plants that have been shown to be able to localise organic anion release, rape (Hoffland *et al.* 1989) and lupin (Dinkelaker *et al.* 1989), are both non-mycorrhizal (Vierheilig *et al.* 1995).

For the same reasoning given by de Miranda *et al.* (1989), the findings of this chapter cast more doubt on the hypothesis that mycorrhizal infection is simply due to an increase in membrane permeability as a consequence of P stress. However, the results of Ratnayake *et al.* (1978) and Graham *et al.* (1981), do not support the hypothesis that VAM infection is related to intracellular carbohydrate concentration. It may well be that VAM infection is initiated by an externally released ‘eliciting factor’ (Bowen 1984). Root chemical signals that have been shown to influence spore germination and hyphae development include iso/flavonoids, phenolics and abietic acid (Baker *et al.* 1998). So whilst VAM can be stimulated by organic acids, the two might not be linked as strongly as once thought. However future studies on organic acids present in the rhizosphere should still consider VAM infections, especially since mycorrhiza can produce P-solubilising organic acids, e.g. oxalic acid (Graustein *et al.* 1977) and consume organic acids, e.g. amino acids (Hepper and Jakobsen 1983).

#### **8.5.4 Implications of this experiment on the importance of VAM in enhancing P uptake in rainfed rice**

At the outset of the discussion it must be stated that this experiment does not prove that a VAM infection will increase P uptake in the field – the experimental set-up was not representative of the field, because the mesh prevented the roots from exploring the P-fertilised soil. However this experiment does demonstrate that a heavy VAM infection, which enhanced P uptake, can develop in a time span of 6 wk, given the right conditions. This agrees with the

observations of Iqbal *et al.* (1978) that VAM infection was rapid in rice (see discussion in Section 1.5.3.2.5) and supports the argument that VAM might increase P uptake, even for a fast maturing crop such as rice.

#### **8.5.5 Why were mycorrhizae present in this experiment but not in the first thin layer experiment?**

The viability of mycorrhizal inoculum reduces with disturbance and drying, and the viability of hyphal inoculum markedly reduces if sporulation had commenced at the time of drying (Jasper *et al.* 1993). Hall (1979) found that the amount of plant infection from pelleted inoculum (composed of spores, colonised root pieces and hyphae) declined after drying the inoculum for 2 wk. It is likely that many storage conditions, except very dry storage conditions (Tommerup and Abbott 1981), favour root decomposition and desiccation, which may result in the loss of viability of hyphal fragments found in root pieces. The soil used for the first thin layer experiment, in which no VAM were found, had been stored in a sack outdoors for probably more than 2 years. These humid conditions are likely to have favoured the rapid decomposition of any root fragments containing viable inoculum. The soil for the third thin layer experiment had been collected 10 months before the experiment, from the same site, then air-dried and stored in a tied plastic bag indoors. The decomposition of root fragments would have been much slower in air-dried soil.

The decrease in viability of mycorrhizal inoculum under most soil storage conditions highlights the importance of ensuring that laboratory experiments are representative of the conditions in the field. If technology, such as a new cultivar or fertiliser management strategy, is being developed for an area where the levels of mycorrhizal infection are normally high, then the method of soil storage is an important consideration.

## 8.6 Conclusions

The marked increases in plant dry weight and P uptake observed in the third thin layer experiment, compared with the first, were associated with a VAM infection of rice roots. The VAM inoculum originated from the new batch of Cavinti soil.

This study shows that VAM infections can enhance P uptake under aerobic conditions in time periods as short as 6 wk. Separate studies are necessary to confirm if enhanced P uptake occurs under field conditions where, unlike in this experiment, roots could explore the same volume of soil as the VAM hyphae. The amount of P taken up was much greater than the amount of available P in the soil, being equal to approximately half of the NaOH-Pi, which suggests that solubilisation of P occurred. It is proposed that the solubilisation might have been the result of organic anion release from the more healthy plants. However solubilisation might also have been induced by VAM.

The total amount of VAM infection per unit root length was lower in plants from the banded treatment, which agrees with the existing hypothesis that increasing the shoot P concentration generally decreases the amount of VAM infection, except under very P stressed conditions. This hypothesis is modified by the observation of increased VAM infection in the P-rich band relative to the unfertilised soil, and supports the conclusions of de Miranda *et al.* (1998), that both soil and plant P concentration control VAM infection. Increased VAM infection in P-rich zones in plants with low P concentration is likely to enhance P uptake efficiency and should be investigated further under field conditions.

The effect of VAM on soil P uptake highlights the importance of considering the relevance of VAM to a particular experiment. If VAM are likely to be important then consideration will need to be given to factors that affect the viability of VAM inoculum, such as soil storage and field management prior to planting.

## 9. Studies on P uptake mechanisms IV. Issues associated with growing plants in sterilised soil

### 9.1 Introduction

The rationale behind doing this experiment is the same as discussed in Chapter 5. A number of improvements had been made to the methodology for growing rice in the TL systems, and extracting organic acids, since Chapter 5 (see Materials and methods, Section 9.3). Heavy VA mycorrhizal infection of rice roots in the previous thin layer experiment (Chapter 8) prevented the study of root released organic acids. To overcome the problem caused by VAM infection it was necessary to sterilise Cavinti soil. Different methods for sterilising the soil are discussed below.

Gamma ( $\gamma$ ) irradiation at doses of 2.5–40 kGy, autoclaving at 121°C for 2 h, dry heat (85°C for 2 h) (Jakobsen and Anderson 1982), methyl-bromide fumigation, and aerated steam at 60–80°C for 30 min (Thompson 1990) have all been shown to effectively kill VAM. Heat treatments, particularly autoclaving, can change the chemical characteristics of the soil resulting in toxicities (Bowen and Rovira 1961). Fumigation with methyl bromide can result in high soil concentrations of bromine, which have been found to be toxic to some species, e.g. wheat (Thompson 1990) and carnation (Maw and Kempton 1973). Therefore  $\gamma$  irradiation was chosen as the preferred method.

In this chapter, rice was found to release a considerable amount of  $H^+$  in the P-rich band. Therefore, mathematical modelling was used to predict the effect of  $H^+$  release on the amount of P taken up by upland rice grown in the TL systems.

## 9.2 Objectives

The objectives of this experiment were the same as those of Chapter 5, i.e. to identify and quantify mechanisms governing P uptake by rice in aerobic, highly weathered soils. Of particular interest, was the management strategy of banding (localised placement) fertiliser P, and the role of root-released organic acids in enhancing P uptake.

## 9.3 Materials and methods

The methodology used in this fourth thin layer experiment was the same as for the first thin layer experiment (Section 5.3.2), except for a few improvements that are documented below.

### 9.3.1 Improvements made to the experimental set-up

#### 9.3.1.1 Preliminary experiment to check that irradiation had eliminated VAM

Cavinti soil (2 mm sieved) was sterilised using  $\gamma$  radiation (25 kGy) from a  $^{60}\text{Co}$  source at the Philippine Nuclear Institute, in order to eliminate mycorrhiza-forming fungi. Thin layer systems were set up to check for soil sterility. There were both banded and incorporated treatments, and a non-sterilised control. Only one TL system was prepared of each treatment. Four freshly germinated seeds were planted per system. Roots from the TL systems were harvested 2 and 4 wk after sowing, stained (Koske and Gemma 1989) and then examined under a microscope for VAM.

#### 9.3.1.2 Main experiment

Two soil P concentrations were established by mixing the air-dried, sterilised Cavinti soil with MCP at a rate of 500 and 5000  $\mu\text{g P g}^{-1}$  soil (denoted P500 and P5000, respectively). A higher P rate was used for this experiment as Kirk *et al.* (1999b) found that severely P deficient plants (grown in TL systems fertilised with 100  $\mu\text{g P g}^{-1}$  soil) produced small quantities of organic

acids compared to plants grown at intermediate P concentrations ( $1000 \mu\text{g P g}^{-1}$  soil). Monocalcium phosphate was first thoroughly mixed through a small volume of soil then this soil was mixed with the complete volume. For the incorporated P fertiliser treatment, 38.0 g of air-dried P500 soil was packed uniformly into each thin-layer, giving a bulk density of  $1.03 \text{ kg oven-dry soil dm}^{-3}$ . For the banded treatment, 3.80 g of P5000 soil was packed between the two thin perspex strips, which were glued 1.3 cm apart. Unfertilised soil (P0) was then packed into equal-sized compartments (16.52 g per compartment) either side of the P-fertilised band. Ten micrometre pore-diameter nylon mesh was used to separate plant roots from the soil, since rice roots could penetrate  $24 \mu\text{m}$  nylon mesh (Chapter 7).

There were four planted and four unplanted replicates of each treatment. Plants were harvested 2, 4 and 6 wk after transplanting. Iron was omitted from the nutrient solution from the 2<sup>nd</sup> wk onwards, as it was (wrongly) assumed that the plants were suffering from Fe toxicity.

### **9.3.2 Improvements made to the analytical techniques**

#### **9.3.2.1 Soil measurements**

Soil organic anions were extracted with 25 mM  $\text{H}_2\text{SO}_4$  (see Section 6.1.3.3) at a 1:2 soil:solution (dry-weight basis) ratio for 30 min using a wrist action shaker (one 3 g sample for banded soil, and two 10 g soil samples for the incorporated soil and the unfertilised soil from the banded TL systems). The solutions were centrifuged ( $17,200 g$ ), filtered (Whatman #5) and the filtrate immediately acidified with 0.2 mL (band 0.06 mL) of 2.5 M  $\text{H}_2\text{SO}_4$  (pH 1.3) then frozen. Filtrates were analysed the same day for organic acids by HPLC (Section 4.3.3). Soil solution P was extracted as described previously (Section 5.3.2.4), the pH measured, and the extracts were frozen awaiting P analysis (Murphy and Riley 1962). No P fractionation was performed. The remaining soil was air-dried and the pH measured in 0.01 M  $\text{CaCl}_2$  a 1:2.5 soil:solution ratio.

### 9.3.2.2 Plant measurements

Subsamples (250 mg) of dried shoot or root were digested with concentrated  $\text{H}_2\text{SO}_4$  and 30%  $\text{H}_2\text{O}_2$  (Jones and Case 1990) and the digest analysed for P (Murphy and Riley 1962), and Fe, Al and Mn by ICP–AES (for the week 2 harvest, only Fe was determined, using AAS). At the transplanting stage, representative seedlings were also dried, weighed and their P and Fe contents determined as above.

### 9.3.2.3 Modelling P uptake

Phosphorus uptake by upland rice in the absence of solubilisation was modelled as described in Section 5.3.2.5, except for two differences outlined as follows. Firstly, the P buffer power, over the range of P taken up by plants grown in the fourth thin layer experiment, was better described by a Freundlich isotherm (Figure 4.2) than by a linear relationship (as used in Chapter 5). This is because the desorption isotherm became much more curvi-linear at high soil P concentrations. The model used in this chapter, therefore, calculated  $b_p$  from the slope of the Freundlich relationship in Figure 4.2 using Equation 4.3.

Secondly, the assumption  $[\text{P}_L]_0 \ll K_m$  (Equation 5.3a) did not hold for the P5000 soil in this experiment. Therefore it was necessary to use Equation 5.3 in the model to calculate P uptake by the roots, which requires values for  $F_{\max}$  and  $K_m$ . It was not possible to measure  $F_{\max}$  or  $K_m$  under the experimental conditions, therefore values for these parameters were estimated using the model. The estimated values are discussed in relation to those found in the literature (Section 9.4.2.1).

The initial concentrations of P in solution ( $[\text{P}_L]$ ) are taken from the P desorption isotherms (Figure 4.2). The moisture content ( $\theta$ ) is measured in the current experiment. The remaining values are the same as those used in Table 5.3. The output from the model is shown in Figure 9.8.



### 9.3.2.4 Phosphate desorption isotherms for P5000 Cavinti soil at different pH

Phosphorus desorption isotherms at three pHs were made as follows. Three 25 L volumes of 0.01 M CaCl<sub>2</sub> solution, containing 50 mg L<sup>-1</sup> HgCl<sub>2</sub>, were prepared at initial pHs of 3.58, 4.18 and 4.58. The pH was adjusted with HCl. These solutions were added to containers of six different volumes (50, 100, 250, 500, 1000 and 2000 mL) containing 2 g of sterilised P5000 air-dried Cavinti soil. Varying small additions of HCl were made to each container to give final solution pHs near the target initial value. The solutions were shaken, extracted, and analysed for final pH and P as described in Section 4.3.3. The mean ± max/min final pHs of the solutions within each isotherm were 4.62 ± 0.05, 4.4 ± 0.1 and 3.8 ± 0.2.

### 9.3.2.5 Model 3: P uptake with solubilisation by acidification

The following two equations describe the diffusion and interaction of P and H<sup>+</sup> in the soil (Kirk *et al.* 1999b, Equations 12 and 13)

$$b_{P^*} \frac{\partial}{\partial t} ([P_L^*] - \lambda_H [H_L]) = \frac{\partial}{\partial x} \left( D_{LP^*} \theta f \frac{\partial [P_L^*]}{\partial x} \right) \quad (9.1)$$

$$b_H \frac{\partial [H_L]}{\partial t} = \frac{\partial}{\partial x} \left( D_{LH} \theta f \frac{\partial [H_L]}{\partial x} \right) \quad (9.2)$$

Implicit in these equations is that the diffusion of H<sup>+</sup> is not affected by the diffusion of P. There are two possible mechanisms that might affect the diffusion of H<sup>+</sup>: (a) H<sub>2</sub>PO<sub>4</sub><sup>-</sup> may act as a carrier for H<sup>+</sup>, or (b) P desorption from the soil solid might influence the reaction of H<sup>+</sup> with the soil solid. Possibility (a) is unlikely because at the pH of the TL system (pH=4.6), almost all the P in solution will be as H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, so there won't be significant transfer of H<sup>+</sup> by counter diffusion of HPO<sub>4</sub><sup>2-</sup> towards the low pH zone or H<sub>3</sub>PO<sub>4</sub> away from it. Possibility (b) is also unlikely, because much more H<sup>+</sup> reacts with the soil than the amount of P removed (67 μmol H<sup>+</sup> g<sup>-1</sup> soil versus approximately 5 μmol P g<sup>-1</sup> soil, when the soil pH was decreased by approximately 0.8 unit (Figure 9.9).

Also, it is assumed that  $H^+$  ions are carried through the soil solely by the movement of the acid-base pair  $H_3O^+ - H_2O$ ; the contribution made by other acid-base pairs, such as  $H_2CO_3 - HCO_3^-$ , is small over the pH range in the present experiment. The following boundary conditions apply, analogous to those for Equation (5.1):

$$D_{LP} \cdot \theta f \frac{d[P_L^*]}{dx} = -\alpha [P_L^*], \quad D_{LH} \theta f \frac{d[H_L]}{dx} = F_H \quad x = l_h \quad t \geq 0 \quad (9.3)$$

$$D_{LP} \cdot \theta f \frac{d[P_L^*]}{dx} = 0, \quad D_{LH} \theta f \frac{d[H_L]}{dx} = 0 \quad x = L \quad t \geq 0. \quad (9.4)$$

The equations were solved using numerical methods as in Chapter 5.

### 9.3.2.6 Statistics

Significant differences were determined by analysis of variance using the SAS<sup>®</sup> System. Data were log transformed where the variance was proportional to the mean, e.g. when comparing the amount of  $H^+$  released from roots in the P5000 and P0 soil.

## 9.4 Results and discussion

### 9.4.1.1 Effect of $\gamma$ irradiation

The preliminary experiment showed that there was no VAM infection in the sterilised soil 4 wk after transplanting into the TL system. Furthermore, there was only 2.4% infection in the non-sterilised Cavinti soil indicating that the viability of the inoculum had declined rapidly during the 3 months of storage.

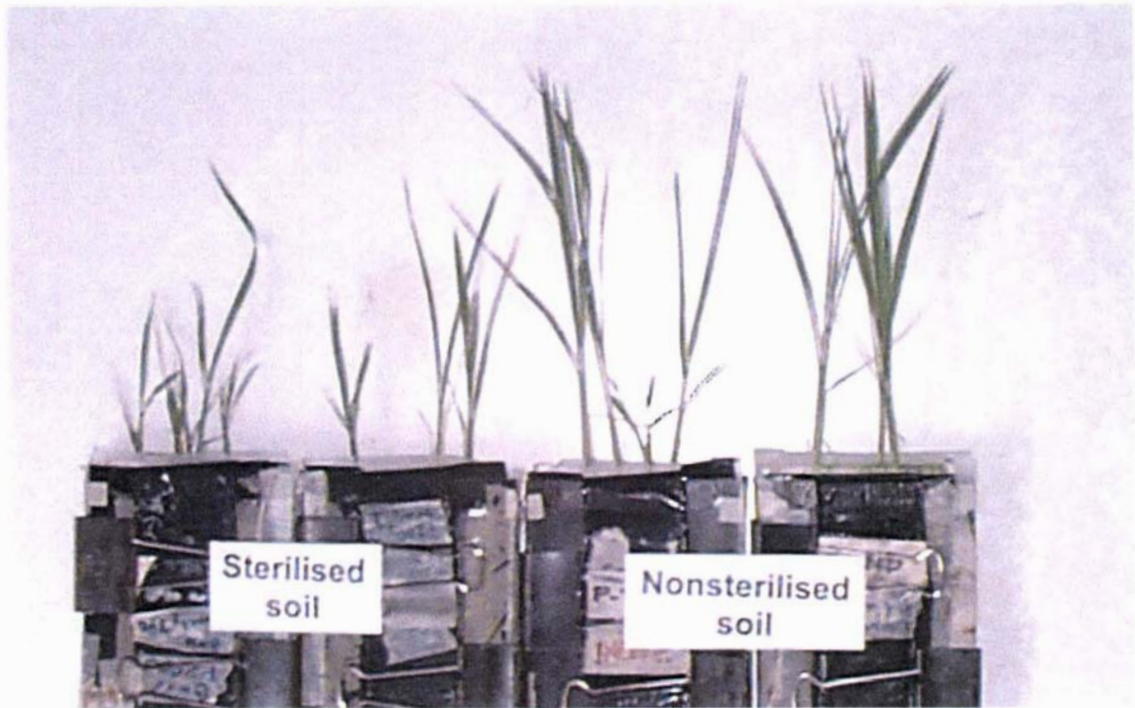
The average height of plants in the sterilised treatment was only approximately half the height of plants in the non-sterilised soil (Figure 9.1), although there was quite large variation in the data, which meant that the difference was not statistically significant. The leaves of rice grown in the sterilised soil had brown spots, some were dead and others had brown lines down the

centre (Figure 9.2). The tips of the leaves were pale, almost white. These symptoms are consistent with Mn toxicity (Section 1.3.1.3). No brown lines or other toxicity symptoms were observed in the plants in non-sterilised soil, although one plant grew poorly (Figure 9.1).

Towards the end of the experiment, the less affected plants grown in sterilised soil had begun to recover. It was therefore assumed that, as in the case of high P, that older plants transplanted at 2 wk would be more tolerant of high soil  $Mn^{2+}$  concentrations than freshly germinated seeds.

#### 9.4.1.2 Plant dry weight

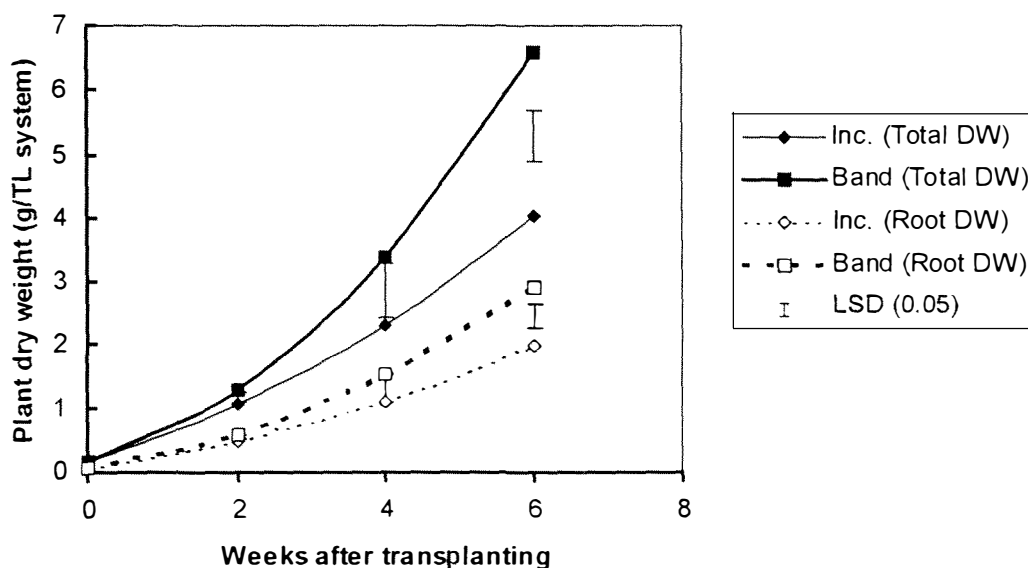
Plant dry weight showed a parabolic increase during the course of the experiment (Figure 9.3). A significant difference between the two treatments was evident after only 2 wk of growth. By the end of the experiment, plants in the banded treatments were more than one-and-a-half times the weight of plants from the incorporated treatments. Both shoot and root dry weight followed the same trend as the total dry weight. Plant growth in the incorporated P500 treatment was poor (< 4 g DM in 6 wk, Figure 9.3), as Kirk *et al.* (1999b) obtained plant dry weights of > 6 g DM in 6 wk in incorporated P100 TL systems. This suggests that some factor other than P deficiency was limiting growth (see Section 9.4.3).



**Figure 9.1.** Upland rice grown in sterilised ( $\gamma$  irradiation, 25 kGy) and non sterilised Cavinti soil 4 wk after germination.



**Figure 9.2.** Close up of the leaves of upland rice grown in sterilised ( $\gamma$  irradiation, 25 kGy) Cavinti soil. The brown streaks are evidence of Mn toxicity.



**Figure 9.3.** Dry weight of upland rice grown in sterilised soil in the fourth thin layer experiment. Phosphate fertiliser was either incorporated throughout the entire TL system (Inc.), banded in 10% of the TL system (Band).

#### 9.4.1.3 Soil pH

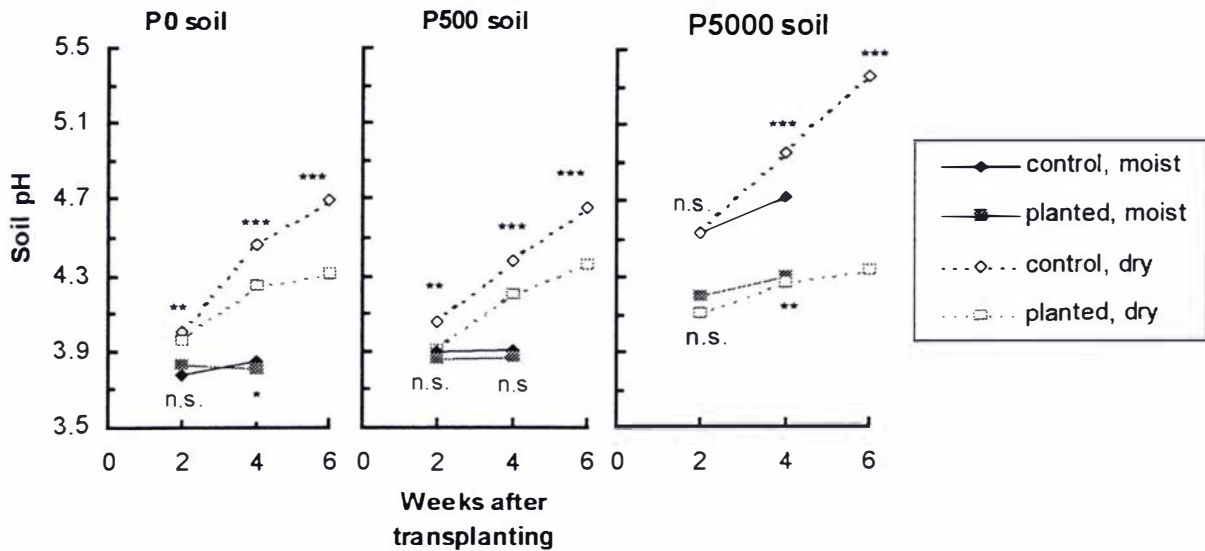
##### a) pH measured on air-dried soil samples

The  $\text{pH}_{\text{CaCl}_2}$  of all air-dried soils increased over time (Figure 9.4). The pH of the control soil increased significantly more than the planted soil at all soil P concentrations, and the difference in pH between the control and planted soil increased with time. The difference in pH between planted and unplanted soil was approximately three times greater in the banded soil compared to the P500 or P0 soils. The pH buffer curve for the P5000 soil (Figure 9.5) shows that the acid release in the planted soil is equivalent to 30, 46 and 77  $\mu\text{mol H}^+ \text{g}^{-1}$  air-dried soil for 2, 4 and 6 wk after harvest, respectively.

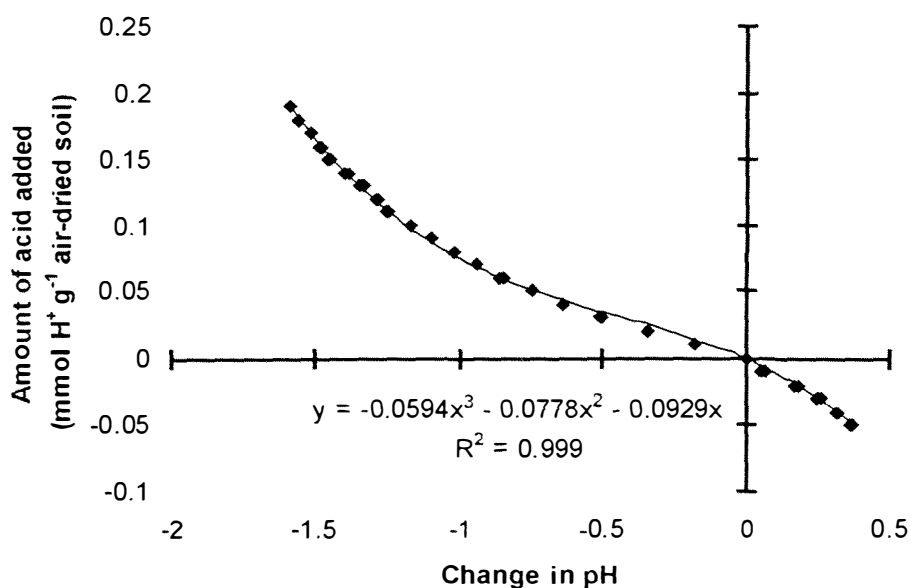
##### b) pH measured on moist soil samples

The  $\text{pH}_{\text{CaCl}_2}$  of the moist soils showed no increase in pH over time in the P0 and P500 soils, and no difference in pH between planted and unplanted soil (data for weeks 2 and 4 only) in these treatments (Figure 9.4). In the P5000 soil the rate of increase in pH over time was less

than half of that observed when the soil was air-dried, but the soil in the planted treatment was still highly significantly ( $P < 0.01$ ) more acidic than the unplanted soil 4 wk after transplanting. The amount of  $H^+$  release 4 wk after transplanting, calculated using the buffer curves for the P5000 soil (Figure 9.5) and unfertilised Cavinti soil (Kirk *et al.* 1999b) showed that there was highly significantly ( $P < 0.001$ ) more  $H^+$  released per gram of root in the banded soil compared to the unfertilised soil outside the band (1.39 compared to 0.08  $\text{mmol } H^+ \text{ g}^{-1}$  root DM, respectively).



**Figure 9.4.** Soil pH of moist soil and air-dried soil, 1:2.5 soil:CaCl<sub>2</sub> ratio, from the TL systems: P0 = unfertilised soil outside the band, P500 = the incorporated treatment, and P5000 = the high P band. Significant differences between the planted and control treatments are indicated as follows: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , n.s.=not significant. Significant differences for air-dried soils are indicated above the data points, differences for moist soils are indicated below the data points.



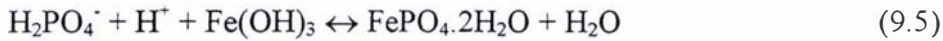
**Figure 9.5.** pH buffer curve for P5000 Cavinti soil. Note: the native pH of P5000 Cavinti soil is 4.8.

#### *Explaining the pH changes*

The agreement in pH between that measured in air-dried soil and that measured in moist soil is poor. It is assumed that the pH measured in the moist soil is more representative of the true pH in the TL systems, and that the pH of the air-dried soils must be subject to some artifact upon air-drying.

Kirk *et al.* (unpublished) found that after adding MCP to Cavinti soil, the soil pH initially decreases, then increases again until reaching a stable value. The higher the rate of MCP addition, the longer the soil pH takes to stabilise. The incubation time of 4 wk prior to packing the TL systems was based on the time the P1000 soil took to stabilise; Figure 9.4 shows that the pH of the moist P5000 soil was still increasing after 8 wk (4 wk incubation plus 4 wk in the TL systems). The initial soil pH decrease is due to the high concentration of MCP, since a saturated solution of MCP has a pH of 1.0 (Talibudeen 1981). The subsequent pH increase is

probably a consequence of both ligand exchange of phosphate for  $\text{OH}^-$  (Section 4.4.2) and Fe- and Al-phosphate formation (e.g. strengite ( $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$ ), Equation 9.5).



The decrease in soil pH in the planted (relative to unplanted) soil in the P-rich band, implies that there was  $\text{H}^+$  release from the roots. Hydrogen ion release is largely governed by the cation:anion balance of the plant. It is likely that the large release of  $\text{H}^+$  in the band was a localised response by roots maintaining cellular electroneutrality, balancing a large uptake of  $\text{Ca}^{2+}$  from the Ca-rich MCP band, as rice takes up approximately twice the number of moles of positive charge in the form of  $\text{Ca}^{2+}$ , than negative charge as  $\text{H}_2\text{PO}_4^-$  (Grist 1975).

#### 9.4.1.4 Phosphorus in solution

The solution P concentrations at the start of the experiment (Table 9.1) were very low in the P500 soil, and extremely low in the unfertilised soil. A ten-fold increase in the soil P concentration, from 500 to 5000  $\mu\text{g P g}^{-1}$  soil, resulted in a 600% increase in P in solution.

#### 9.4.1.5 Plant P uptake

Upland rice showed a linear increase in P uptake throughout the duration of the experiment (Figure 9.6), except for the week 4 incorporated treatment, where two replicates grew poorly, hence the large standard error for this treatment (see footnote to Figure 9.8). By the 6<sup>th</sup> wk plants from the banded treatment had taken up significantly more P ( $> 3\times$ ) than plants from the incorporated treatment. The rate of P uptake was insufficient to match the P required for growth: the P concentration of the plants from the incorporated treatment dropped to the critical concentration of 0.1% (Tanaka and Yoshida 1970) after only 2 wk of growth, and the plants from the banded P treatment reached this critical concentration after 6 wk growth (Figure 9.7).

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<sup>†</sup> In acidic soils, most P would be taken up as  $\text{H}_2\text{PO}_4^-$ , since the  $\text{p}K$  for the reaction  $\text{H}_2\text{PO}_4^- \leftrightarrow \text{HPO}_4^{2-} + \text{H}^+$  is 7.20 (Dean 1985).



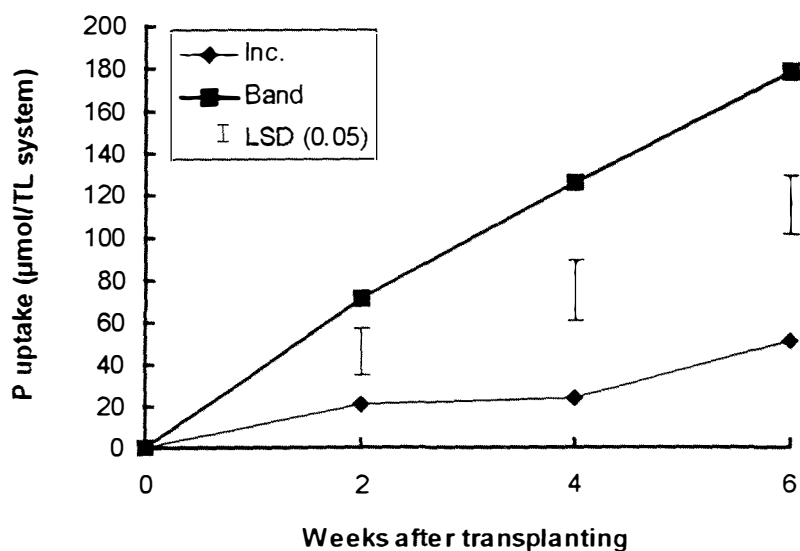


Figure 9.6. P uptake per TL system when P fertiliser is incorporated throughout the soil (Inc.) or banded within 10% of the soil volume (Band).

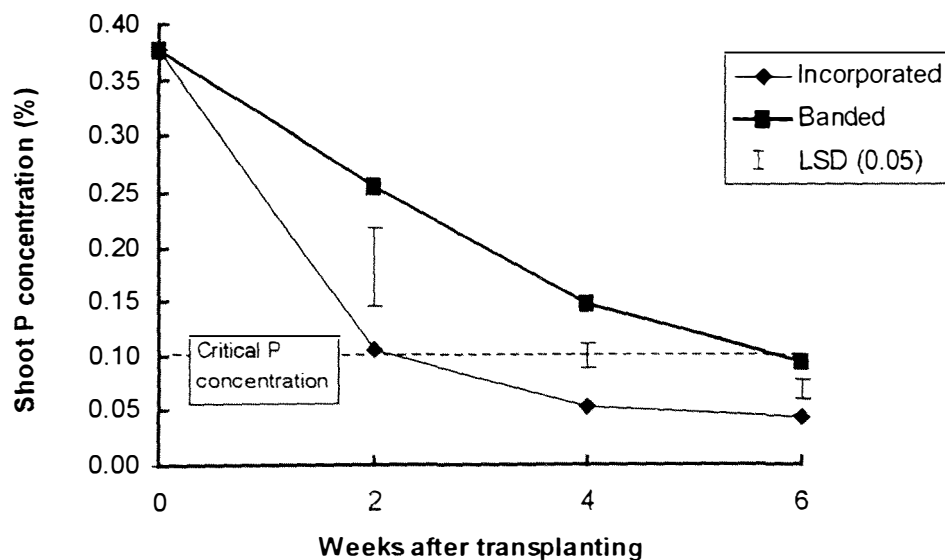


Figure 9.7. Shoot P concentration for upland rice grown in incorporated or banded TL systems. The critical shoot P concentration below which P deficiency is likely (Tanaka and Yoshida 1970) is shown as a dashed line.

## 9.4.2 Modelling P uptake

### 9.4.2.1 Modelling P uptake in the absence of solubilisation (Model 1)

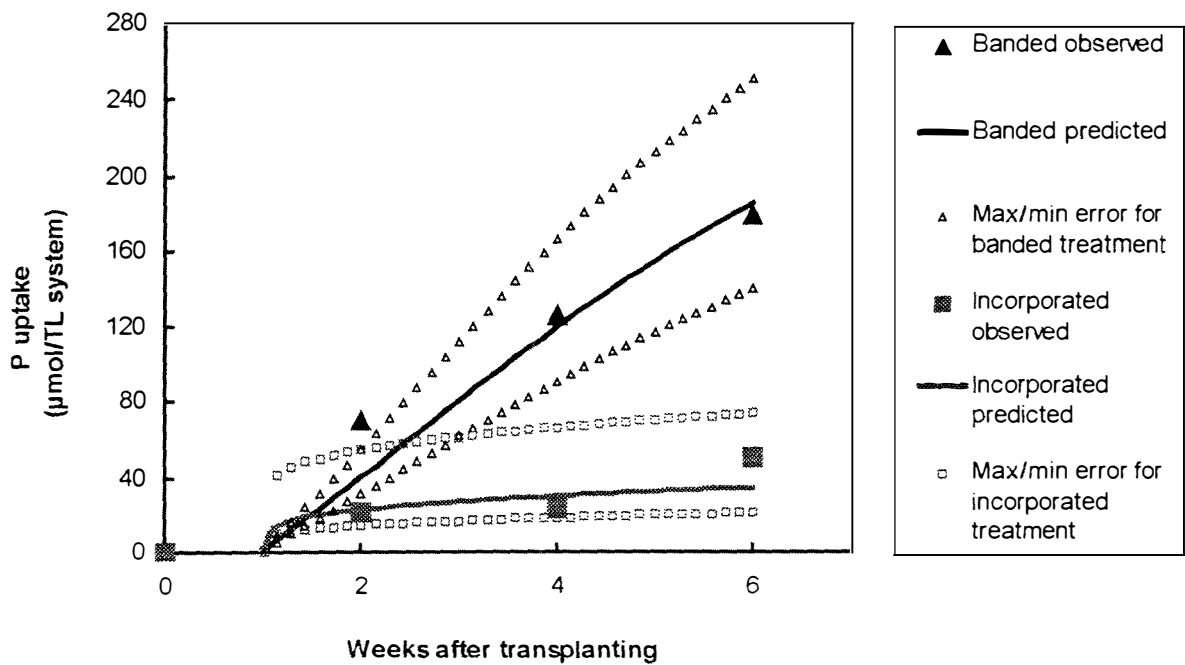
**Table 9.1.** Parameters used to model P uptake in the 4<sup>th</sup> thin layer experiment. Minimum and maximum values are given in parenthesis.

Parameter	Units	P500 soil		P5000 soil	
		Value	(Min – Max)	Value	(Min – Max)
[P <sub>L</sub> ] initial	mol dm <sup>-3</sup>	0.55×10 <sup>-6</sup>	(0.38×10 <sup>-6</sup> – 0.75×10 <sup>-6</sup> )	330×10 <sup>-6</sup>	(300×10 <sup>-6</sup> – 360×10 <sup>-6</sup> )
[P] initial	mol dm <sup>-3</sup> soil	0.017	–	0.132	–
<i>b</i> <sub>P</sub> (linear)	–	4500	(3500–5500)	–	–
<i>b</i> <sub>P</sub> (Freundlich) #, where <i>a</i> =	–	–	–	–6.57×10 <sup>-6</sup> †	(-1.94×10 <sup>-4</sup> – -6.57×10 <sup>-5</sup> )
and <i>b</i> =	–	–	–	-0.611†	(-0.492– -0.611)
<i>θ</i>	–	0.49	–	0.49	–
<i>A</i>	dm <sup>2</sup>	2.08	–	0.208	–
<i>F</i> <sub>max</sub>	mol dm <sup>-2</sup> s <sup>-1</sup>	2.5×10 <sup>-10</sup>	–	2.5×10 <sup>-10</sup>	(2×10 <sup>-10</sup> –3×10 <sup>-10</sup> )
<i>K</i> <sub>m</sub>	mol	6×10 <sup>-6</sup>	–	6×10 <sup>-6</sup>	–
<i>l</i> <sub>h</sub>	dm	0.0036	(0.0036–0.0053)	0.0036	(0.0036–0.0053)
<i>f</i>	–	0.4	(0.4–0.44)	0.4	(0.4–0.44)

†These values differ from those given in Table 4.2 because the units in the model are mol kg<sup>-1</sup> soil, whereas the units in Table 4.2 are μmol kg<sup>-1</sup> soil.

#The buffer power is calculated from the slope of a Freundlich isotherm using the equation  $b_p = ab[PL]^{b-1}$

Diffusion of readily-available P was sufficient to explain the observed P uptake from both treatments (Figure 9.8). The observed P uptake is underestimated at the beginning of the experiment in the P5000 treatment, presumably because there is some P uptake during the first week after planting, whereas the model assumes no P uptake during this period.



**Figure 9.8.** Observed and predicted P uptake by upland rice grown in the fourth thin layer experiment. The standard error in the observed uptake is approximately equal to the size of the symbols<sup>†</sup>. The maximum and minimum predicted errors (from Table 9.1) are plotted as open symbols.

#### *The importance of $F_{max}$*

The predicted uptake in the P5000 soil was sensitive to  $F_{max}$ . Increasing  $F_{max}$  increased the predicted uptake, indicating that uptake in the P5000 soil was limited by root properties rather than by the diffusion of P through the soil. In contrast, for all the other experiments in this thesis, where soil P concentrations were smaller, the value of the root absorbing power had no effect on the predicted uptake, indicating that diffusion through the soil was rate-limiting.

The values of  $F_{max}$  and  $K_m$  that best explained uptake in the P5000 treatment were  $250 \text{ pmol dm}^{-2} \text{ s}^{-1}$  and  $6 \text{ }\mu\text{M}$ , respectively. Teo *et al.* (1992) measured P absorption by 8-wk-old rice plants using steady-state P depletion isotherms in full strength Yoshida nutrient

<sup>†</sup> except for the incorporated treatment, week 4, where the SE of the mean is  $1.7\times$  the size of the symbol

solution, and obtained  $F_{\max} = 124 \text{ pmol dm}^{-2} \text{ s}^{-1}$  and  $K_m = 3.4 \text{ }\mu\text{M}$ . However, Tinker and Nye (2000) stated that perturbation isotherms are a more appropriate method to measure Michaelis-Menten parameters than steady-state depletion isotherms. Jungk *et al.* (1990) determined Michaelis-Menten parameters on maize grown at a range of P concentrations (from 100 down to 0.1  $\mu\text{M}$ ) using perturbation isotherms, and found that  $F_{\max}^\dagger$  increased from 65 to 340  $\text{pmol dm}^{-2} \text{ s}^{-1}$  and  $K_m$  from 3.4 to 6.1  $\mu\text{M}$ , as the solution P concentration decreased. Therefore the values used here are reasonable. The influx rates across individual root surfaces will have been lower than calculated for the root plane because the true absorbing root surface is somewhat larger than the surface of the root plane. Near the beginning of the experiment, when  $F_{\max}$  is most likely to have been limiting, the data in Table 5.4 show that the true root surface area was more than twice the root-plane area, assuming an average root radius of 100  $\mu\text{m}$ .

The fact that root uptake properties limited uptake rates in the high P concentration treatments raises the question “What is the importance of plants having a high  $F_{\max}$  when fertiliser is banded in the field?” In the limit, as  $F_{\max}$  tends to infinity, the volume of soil that needs to be fertilised tends to zero; and since the proportion of added P that is immobilised decreases as the volume of soil fertilised decreases, fertiliser efficiency should increase greatly with increases in  $F_{\max}$ . In practice, processes at the cellular level limit  $F_{\max}$  – the root’s ability to absorb and assimilate P. Genetic differences in  $F_{\max}$  values have been reported. Baligar and Barber (1979) found considerable variation in  $F_{\max}$  between two lines of maize. However the lines with a higher  $F_{\max}$  also had a much higher  $C_{\min}$  (the minimum concentration in solution at which nutrient uptake can occur). This suggests that whilst breeding for plants with a higher  $F_{\max}$  may improve P uptake from the P-rich band, this improvement may come at the expense of performance at low solution P concentrations. Ideally,  $F_{\max}$  should be increased without raising  $C_{\min}$ . Other possible mechanisms to enhance P uptake from the band may include mycorrhizal infection (Figure 8.2), or increased root proliferation in the P-rich zone (Table 5.4).

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<sup>†</sup>  $F_{\max}$  was calculated by dividing  $I_{\max}$  (the maximum uptake per unit root length) by the root circumference, assuming an average root radius of 175  $\mu\text{m}$  (Varney *et al.* 1991).

#### 9.4.2.2 Modelling P uptake to estimate the importance of H<sup>+</sup> release as a means of solubilising P (Model 3)

Figure 9.4 shows that that rice roots released a significant amount of H<sup>+</sup> ions in the P-rich band. In soils containing apatite or fertilised with RPR, acid release has been shown to significantly enhance the rate of P dissolution in clover (Trolove *et al.* 1996). The amount of acid-soluble P in Cavinti soil is small (Hedley *et al.* 1994), however, acid-soluble calcium phosphates, e.g. dicalcium phosphate, may have formed in the highly fertilised band (Sample *et al.* 1980). It was therefore of interest to estimate the amount of P released from the band by H<sup>+</sup> release. Model 3 (Section 9.3.2.4) was used to predict the amount of P taken up by upland rice from the banded TL systems allowing for the solubilising effect of H<sup>+</sup>. Note that this model uses a linear relationship for  $b_p$ , and hence the shape of the curve does not match the shape of the observed data well (compare Figure 9.10 with Figure 9.8), however the model is still valid to estimate the extra P released due to the solubilising effect of H<sup>+</sup>. The parameter values used are given in Table 9.2, and were obtained as described below.

The P buffer power in the presence of H<sup>+</sup> ( $b_{p^*}$ ) was taken as 65, which is a good linear fit for the data in Figure 9.9 over the range of P desorbed in the thin layers. This value agrees well with the average of the P5000 Freundlich isotherm (69) over the same range of P desorbed, indicating that there was little change in  $b_p$  upon addition of H<sup>+</sup>. The quantity  $(-\Delta[P]/\Delta[H])_{[P_L]}$  can be estimated from the data in Figure 9.9 as follows. The pH 3.8 isotherm shows that approximately 5.5 mmol P kg<sup>-1</sup>† was removed from the soil to leave the P concentration at its initial value of 330 μM (Table 9.1), and the final pH was approximately 3.65 (data not shown). From the soil pH buffer curve (Figure 9.5), the quantity of H<sup>+</sup> that reacted with the soil in changing the pH from 4.58 to 3.65 was 0.067 mmol H<sup>+</sup> g<sup>-1</sup> soil. Therefore,

$(-\Delta[P]/\Delta[H])_{[P_L]} \approx 0.082 \text{ mol P mol}^{-1} \text{ H}^+$ , and with  $b_p = 65$ , the P×H<sup>+</sup> interaction coefficient ( $\lambda_H$ ) =  $0.082 \times 350 / 65 = 0.44$ .

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† Note that it was necessary to extrapolate the pH 4.62 isotherm by 10 μM to estimate this value

And since 
$$b_H = \frac{\Delta[H]}{\Delta[H_L]}$$

$$b_H = \frac{-1.03 \times 0.067}{10^{-4.58} - 10^{-3.65}} = 350.$$

The flux of  $H^+$  ions moving out from the root plane is calculated from the net change in acidity in the band soil. Since the data for the air-dried soil appears to be subject to drying artifacts (see Section 9.4.1.3) and the week 6 moist data is missing, the change in pH in the P5000 soil is conservatively extrapolated from the moist pH curves (Figure 9.4) as being approximately 0.5 pH unit. Using the buffer curve (Figure 9.5) this equates to  $0.034 \text{ mmol g}^{-1}$  soil. Thus, considering the thin layer thickness ( $0.03 \text{ dm}$ ) and the bulk density ( $1.03 \text{ kg dm}^{-3}$ ), and by assuming a constant flux over the 5 wk following the transplanting shock,

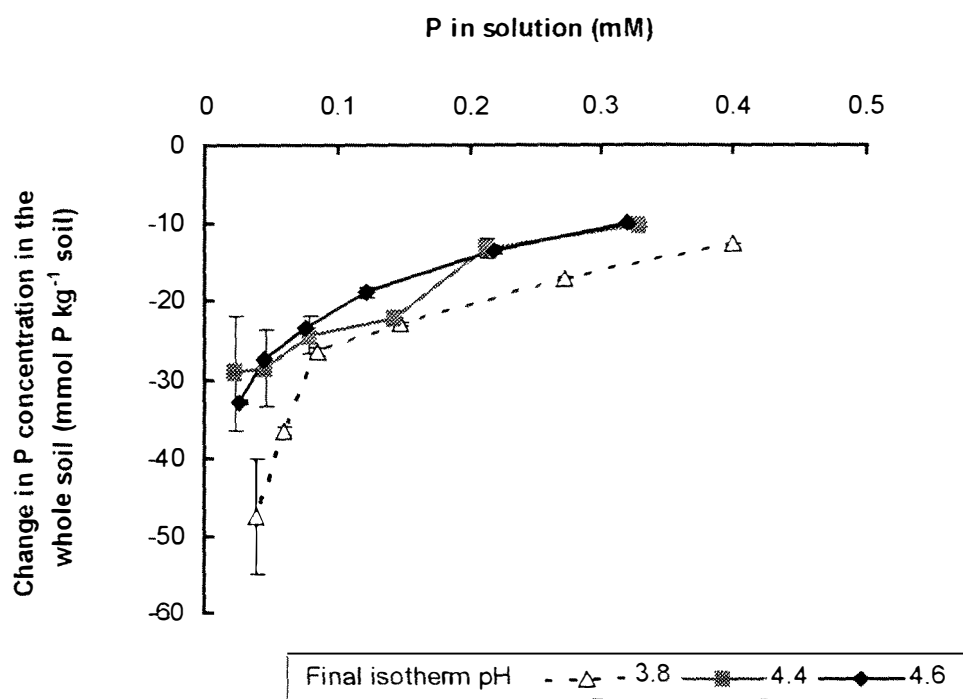
$$F_H = 0.034 \times 0.03 \times 1.03 / (5 \times 7 \times 24 \times 60 \times 60) = 4 \times 10^{-10} \text{ mol dm}^{-2} \text{ s}^{-1}.$$

**Table 9.2** Values of additional parameters required by Model 3 to predict P uptake from the banded treatment as a result of  $H^+$  release from the roots covering the P-fertilised band. Other parameters were the same as those given in Table 9.1.

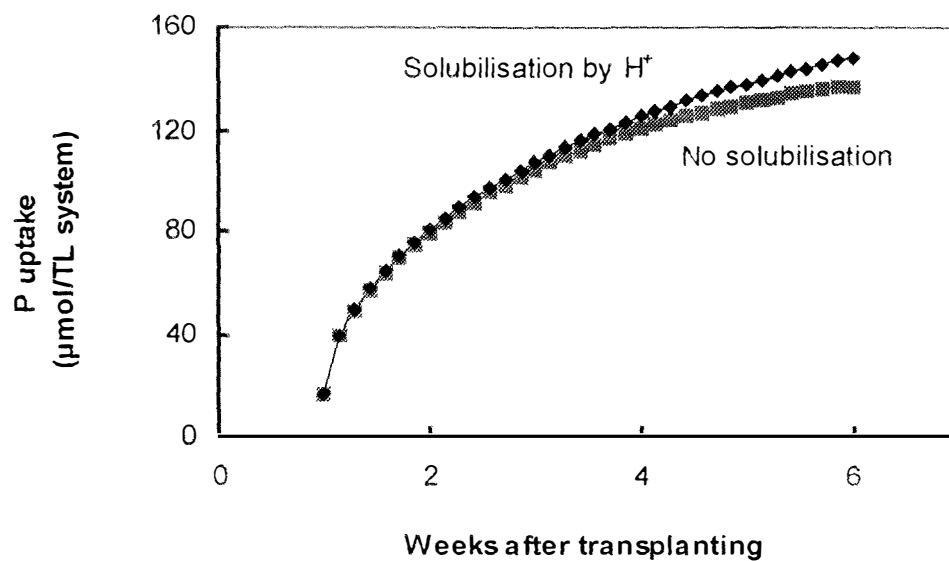
Parameter <sup>†</sup>	Units	Value
$b_H$	–	350
$b_{P^*}$	–	65
$[P_L^*]$	$\text{mol dm}^{-3}$	$3.3 \times 10^{-4}$
$\lambda_H$	–	0.44
$F_H$	$\text{mol dm}^{-2} \text{ s}^{-1}$	$4 \times 10^{-10}$
$p_{CO_2}$	atm	$5 \times 10^{-4}$
$\text{pH}_i$	–	4.5
$k_H$	$\text{s}^{-1}$	0
$D_{LP^*}$	$\text{dm}^2 \text{ s}^{-1}$	$8.9 \times 10^{-8}$
$D_{LH}$	$\text{dm}^2 \text{ s}^{-1}$	$9.55 \times 10^{-7}$

<sup>†</sup> these symbols are defined at the front of this thesis

Model 3 predicts little increase in the amount of P taken up due to solubilisation by  $H^+$  in the first 4 wk after transplanting, and only an 8% increase in P taken up by rice after 6 wk growth (Figure 9.10), assuming a decrease in soil pH of 0.5 relative to the control. This small increase in predicted P uptake agrees with the almost negligible difference in the amount of P desorbed between the pH 4.6 and 4.4 isotherms (Figure 9.9). This suggests that  $H^+$  release has only a small solubilising effect on P from MCP-fertilised bands in acidic soils. However Figure 9.10 shows that the proportion of P supplied by  $H^+$  solubilisation increased with time. In the field, where roots are continually exploring new soil volumes, rather than being confined to the same planar area, the pH decrease around the root is likely to be smaller. The size of the pH drop in field grown rice also depends on the form of N present, being greater in soils where  $NH_4^+$  is the main source of N (Section 1.3.3.3), e.g. in low oxygen or low pH soils. In soils where  $NO_3^-$  is the dominant N source, any solubilising effect is likely to be confined to a small area of soil around the root tip and elongation zone (see Figure 9.11).

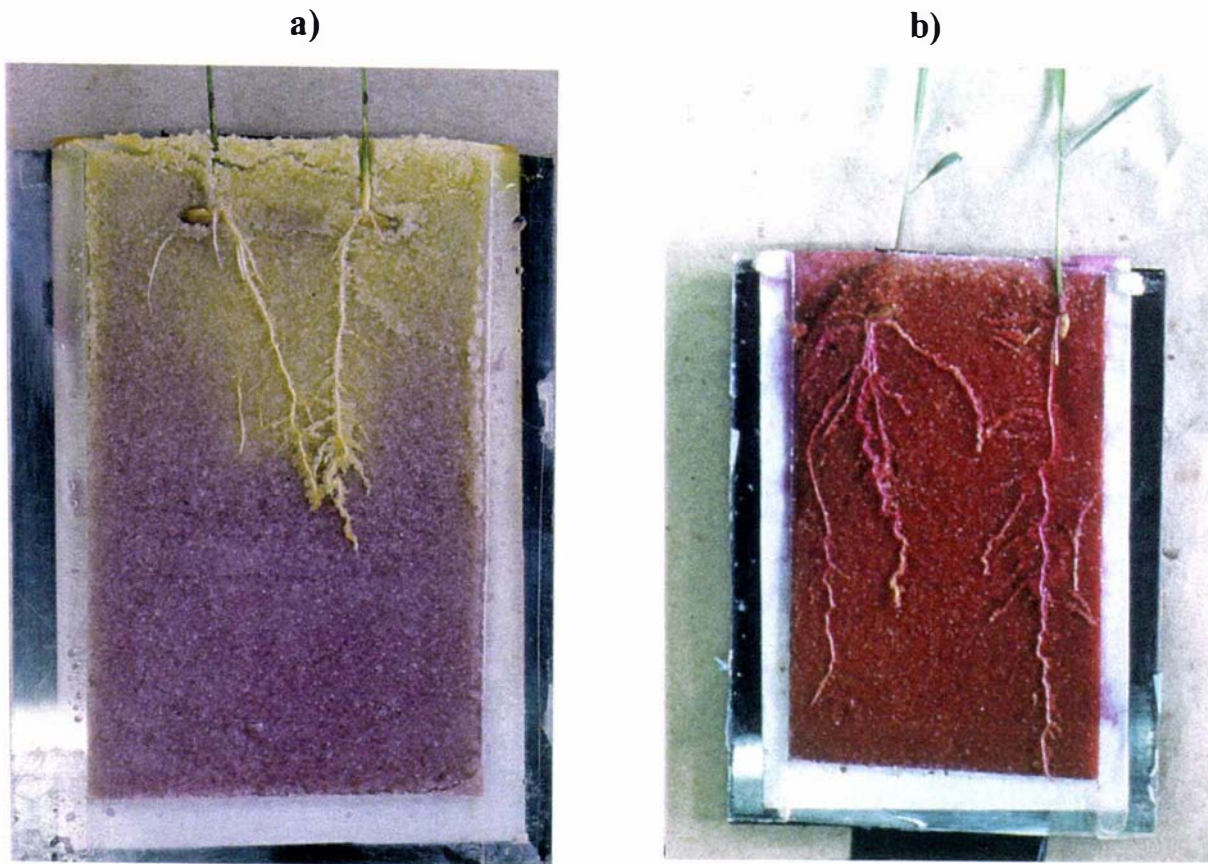


**Figure 9.9.** P desorption isotherms for the P5000 Cavinti soil in the presence of added  $H^+$ . The pH (0.01 M  $CaCl_2$  1:2.5 soil:solution ratio) of P5000 Cavinti soil is 4.58. The data are means  $\pm$  SE,  $n=2$ .



**Figure 9.10.** The predicted uptake of P by upland rice grown in TL systems with and without accounting for solubilisation by  $\text{H}^+$ .





**Figure 9.11.** Acid release patterns from 2-wk-old upland rice grown on sand with N supplied as either (a)  $\text{NH}_4\text{NO}_3$  or (b)  $\text{Ca}(\text{NO}_3)_2$ . The sand is stained with bromocresol purple, which is yellow below pH 4. All nutrients were supplied by P-free Yoshida nutrient solution (Yoshida *et al.* 1976), which was adjusted to pH 5.0. Acid release declined greatly when  $\text{NO}_3^-$  was the sole N source, although acid release was still observed from the root tips.

### 9.4.3 Uptake of Fe, Mn, and Al

Brown blotches appeared on the leaves within a few days after transplanting. Plants in the banded treatment recovered after about 1 wk but plants in the incorporated treatment took more than 2 wk to recover. Plant shoot analysis 4 wk after transplanting showed high concentrations of Mn (Table 9.3), 20% above the critical concentration for toxicity of

2500  $\mu\text{g Mn g}^{-1}$  DW (Tanaka and Yoshida 1970). Leaf Mn concentrations were lower after 6 wk, particularly in the banded treatment.

In contrast, shoot tissue analysis indicated that the rice plants were deficient in Fe and Al after 4 wk of growth (Table 9.3). However the leaves showed no visible signs of Fe deficiency. The concentrations of these elements measured in the root were very high. This was almost certainly the result of soil contamination or perhaps caking on the outside of the root. Therefore, unless very thorough washing is done there is little value in analysing roots for these elements.

**Table 9.3.** Shoot Fe, Al, and Mn concentrations for upland rice grown in TL systems where P fertiliser was either incorporated throughout the soil (Inc.) or banded in 10% of the soil volume.

Week	Fe ( $\mu\text{g Fe g}^{-1}$ DW)		Al ( $\mu\text{g Al g}^{-1}$ DW)		Mn ( $\mu\text{g Mn g}^{-1}$ DW)	
	Inc.	Banded	Inc.	Banded	Inc.	Banded
2	111	125	–	–	–	–
4	66	54	13	13	3209	3165
6	75	60	74	33	3004	2483
<b>Critical conc. #</b>	<b>Deficient</b> <70	<b>Toxic</b> >300	<b>Deficient</b> <30	<b>Toxic</b> >300	<b>Deficient</b> <20	<b>Toxic</b> >2500

# Tanaka and Yoshida (1970).

#### 9.4.3.1 Manganese toxicity

The reason why Mn toxicity developed in this experiment was presumably because sterilisation of the soil prevents microbial oxidation of  $\text{Mn}^{2+}$  under aerobic conditions, however the chemical reduction that produces  $\text{Mn}^{2+}$  continues (Section 1.3.1.3). Since there was a period of 2 months between soil sterilisation and transplanting (when the soil would have been inadvertently inoculated with bacteria from the rice roots) this would have resulted in a build-up of toxic  $\text{Mn}^{2+}$  in the soil. If it is necessary to sterilise a  $\text{Mn}^{2+}$ -rich soil to eliminate VAM-

forming fungi, then the soil should be reinoculated with bacteria, and incubated moist until the soil solution concentrations of  $Mn^{2+}$  have dropped below the toxic threshold.

Toxic tissue Mn concentrations have been shown to reduce iron uptake in pineapples (Sideris and Young 1949, cited by Heenan and Campbell 1983) so this may be the reason for the low Fe concentrations in this experiment, although high tissue Mn concentrations have been shown not to affect Fe uptake in barley, tomatoes and soybeans (Heenan and Campbell 1983). There does not seem to be published data for rice. The drop in shoot Mn concentration of rice in the week 6 banded treatment is probably because the rhizosphere microbial population was quicker to re-establish in the high-P band. Alternatively, Robson and Loneragan (1970) found that forage legumes absorbed less Mn in the presence of high Ca, so perhaps the banding treatment enhanced the availability and uptake of Ca, which in turn may have reduced Mn uptake.

#### **9.4.4 Organic anion release**

No significant quantities of organic anions were detected in the rhizosphere soil. It is possible that  $Mn^{2+}$  toxicity prevented normal function and thus no significant amounts of organic anions were released. One of the symptoms of  $Mn^{2+}$  toxicity is a high concentration of organic acids (especially aconitic acid) in shoot and root tissue (Scott *et al.* 1985, Burke *et al.* 1990, Macfie *et al.* 1994). Burke *et al.* (1990) suggested that high intracellular concentrations of  $Mn^{2+}$  reduce the activity of enzymes involved in the normal metabolism of carboxylic acids. Hue (1988) found that the addition of sewage sludge rich in organic acids to a manganiferous Oxisol caused Mn toxicity in lettuce, suggesting that organic acid release would be harmful to the plant in some  $Mn^{2+}$ -rich soils (although the addition of citrate did not increase the amount of  $CaCl_2$ -extractable Mn in Cavinti soil (Figure 4.7)). Plants adapted to acid soils, such as upland rice, might therefore have mechanisms that prevent organic acid release when soil Mn concentrations are high.

In order to investigate the role of organic acids in enhancing P uptake, future experiments should be conducted in soils with no other limitations to growth (e.g.  $Mn^{2+}$  toxicity) apart from

P deficiency. If it can be shown that organic acids are important under such conditions, then experiments could be conducted to determine the role of organic acids under various other field conditions, such as a  $\text{Mn}^{2+}$  toxic soil.

## 9.5 Conclusions

Sterilising a Mn-rich soil can cause Mn toxicity in upland rice. It is recommended that future experiments using soil sterilised against VAM should be re-inoculated with bacteria and incubated moist for some time until soil solution concentrations of Mn have dropped.

The growth and P uptake of upland rice was poorer than expected in the fourth thin layer experiment because plants were suffering from Mn toxicity. Mathematical modelling indicated that diffusion of readily-available P could supply adequate P to explain the observed P uptake, in fact, at the high P concentrations in the P5000 band, the rate of P supply was initially greater than the rate at which roots could absorb P. There was no evidence that solubilisation of P occurred in this experiment. It therefore appears that Mn toxicity might interfere with P uptake efficiency mechanisms in rice.

Upland rice was found to release significantly more  $\text{H}^+$  in P-rich bands, compared to the surrounding unfertilised soil. This P-efficient mechanism was probably a response to a large  $\text{Ca}^{2+}$  uptake from the MCP-fertilised band. Mathematical modelling indicated that  $\text{H}^+$  release has only a small solubilising effect on P from MCP-fertilised bands in acidic soils, but that  $\text{H}^+$  release would be more important in low pH soil, e.g. soil next to the root surface.

## 10. Summary, conclusions and recommendations for future work

### 10.1 Summary and conclusions

The introductory chapter identified the need to increase world rice production. Scobie *et al.* (1993) estimated that 30% of the projected 70% increase in global rice demand by the year 2030 must come from rainfed rice. Here rice is grown on acidic, nutrient-deficient soils as a staple food crop by farmers who have little or no money. Hence there is a need to develop agronomic practices and cultivars that provide good yields under less-favourable rainfed conditions but also have responsiveness to improved inputs and management.

One of the major problems of rainfed and upland agro-ecosystems is soil acidity. In very acid soils rice growth is limited by Al toxicity. In Chapter 2, a root length bioassay was used in combination with two different soil extraction and measurement techniques to identify soils that were Al toxic to rice roots. A soil test that involved the extraction of soil solution by centrifugation was a more accurate indicator of Al toxicity to rice roots than Al extracted in 0.02 M CaCl<sub>2</sub>. The Wharekohe silt loam subsoil used in Chapter 2 had to be acidified to pH < 4.3, before a reduction in root growth was observed. Rice root extension in the Cavinti Soil (the experimental soil used in the Philippines) showed no signs of Al toxicity. It was concluded that Al toxicity would not be a problem in future studies using the Cavinti soil to study root growth and P uptake in the rice rhizosphere.

The soils in which upland and rainfed rice are grown are often deficient in a wide range of nutrients, and may also suffer from metal toxicities. Two of the most common deficient nutrients are N and P. A low cost strategy for supplying these nutrients is the use of leguminous green manure and reactive phosphate rock. Published research indicates that incorporation of green manures with RPR may enhance or inhibit P release from RPR, dependent on green manure properties and experimental conditions. The rhizosphere

chemistry of green manure + RPR fertilised soils has been little studied. Chapter 3 showed that the decomposition and N mineralisation of green manure slowed the dissolution of RPR by increasing the bulk soil pH. However, subsequent plant uptake of  $\text{NH}_4^+$  caused rice roots to release  $\text{H}^+$ , which caused the rhizosphere pH to drop and partially compensated for the pH increase due to the mineralisation of green manure.

In Ultisols and Oxisols (the predominant soil groups in which rainfed and upland rice are grown), only 5-20 % of the applied soluble P fertiliser is taken up by the subsequent rice crop. There is therefore significant scope to improve crop uptake of fertiliser P. A management strategy that has been shown to increase the efficiency of P uptake is banding or localised placement of P. Plant strategies that can increase the efficiency of P uptake include: association with mycorrhiza, the release of solubilising agents ( $\text{H}^+$ , chelating agents, enzymes), and root geometry effects (developing long, fine, hairy roots, and multiplying roots in P-rich zones). The remainder of the research in this thesis was targeted at demonstrating the effects of P placement on improving the efficiency of fertiliser use by rice, and elucidating the rhizosphere processes that make upland rice efficient at utilising soil and fertiliser P.

In Chapter 4, laboratory extractions showed that both banding of P, and root exudation of citrate, could theoretically increase the availability of P fertiliser to plants. Furthermore, there was a positive interaction between increasing the soil P concentration (which occurs in banding) and the effect of citrate, on the amount of P dissolved into solution. This indicated that the practice of banding P fertiliser could potentially increase the efficiency of P mobilisation by root-released citrate.

In Chapter 5, rice was grown with its roots 'sandwiched' between thin layers of soil to study these mechanisms. Thin layers of soil were used so that all the soil could be considered root-influenced, and root-induced changes in the soil studied accordingly. The thin layers of soil were fertilised either by incorporating the fertiliser throughout the entire soil volume or by banding the same amount of fertiliser in one-tenth of the soil volume. Banding led to a one-hundred-fold increase in the concentration of P in solution, compared to the incorporated treatment. Plants in the banded treatment took up more than ten times

the amount of P and produced more than twice the dry weight of plants in the incorporated treatment. It was concluded that banding of P fertiliser enhances the availability P to rice grown in thin layers, compared to incorporation of P fertiliser throughout the soil. Rice has a strong ability to multiply roots in P-rich zones, which should further enhance P uptake when fertiliser is banded in the field.

A computer model showed that only one-third of the observed increase in P uptake in the banded treatment could be explained by diffusion of readily-available P to the roots. No adequate explanation was found to explain the observed increase in P uptake in the banded treatment, and it was proposed that the increase might have come from root release of organic acids.

Attempts to extract and measure organic acid concentrations in rhizosphere soil were unsuccessful. Methodological problems associated with this were addressed in Chapter 6. Sulphuric acid was found to be a better extractant of organic anions from the highly-weathered soil used than HCl,  $\text{H}_3\text{PO}_4$ , or alkaline  $\text{KH}_2\text{PO}_4$ . The optimal procedure for extracting organic acids from such soils appears to be a 30 min shake with a 1:2 soil:solution ratio of 50 mM  $\text{H}_2\text{SO}_4$ , followed by centrifugation at 17,000 g and filtration (0.2  $\mu\text{m}$  pore size). Samples should be immediately acidified to pH 1.7 and analysed within 24 h.

With these improvements to the organic acid extraction procedure the thin layer experiment described in Chapter 5 was repeated. A number of further problems with the experimental method were encountered (Chapters 7-9) providing the opportunity to learn more about factors affecting root growth and P uptake. In Chapter 7, rice roots were found to be able to penetrate 24  $\mu\text{m}$  nylon mesh (which had been used to create a planar rhizosphere) under certain conditions, even though the diameter of the smallest rice roots was 70  $\mu\text{m}$ . A further experiment showed that the number of roots that can penetrate a rigid pore increases as the soil dries. It was hypothesised that roots are more firmly anchored in dry soil, and can therefore exert more pressure on the root tip to force it through the small pore. The size of the root that could penetrate the nylon mesh was more closely related to the diameter of the stele, than the diameter of the root or root cap.

A large infection of VA mycorrhiza (Chapter 8) resulted in a significant increase in plant growth and P uptake, particularly in the incorporated treatment. This result highlights the fact that VAM infection can cause a significant increase in P uptake within a few weeks under the right conditions. Sterilisation of the soil ( $\gamma$  irradiation, 25 kGy) to remove the VAM infection induced Mn toxicity in rice (Chapter 9). Plant growth was poor in the Mn-toxic thin layers, and the observed P uptake could be explained by diffusion of readily-available and  $H^+$ -soluble P. Rice was observed to concentrate  $H^+$  release in MCP-fertilised zones. This rhizosphere acidification could enhance the availability of acid-soluble P. In reality, the amounts of acid-soluble P in highly-weathered acidic soils are generally low and mathematical modelling indicated that the amount of P solubilised by  $H^+$  release from the Ultisol studied was small. No release of organic acids was observed in the rhizosphere of Mn-toxic upland rice.

The major conclusions from the research in Chapters 5 and 8 was that P efficient uptake by upland rice appears to be achieved by solubilisation of soil P from the rhizosphere – probably by organic acid release and improved uptake through VAM. Indirect evidence of P solubilisation and efficient uptake was provided through use of simulation modelling of P uptake. The complex problem of efficiently extracting organic acids from *in vivo* rhizosphere soil prevented the quantity and form of organic acid from being identified. Therefore, a number of improvements to the experimental technique are suggested below.

## 10.2 Future work.

Due to the many interactions among roots, soil, and soil micro-organisms involved in P uptake, future studies endeavouring to quantify the role of root-released organic acids from soil should strive to develop better *in vivo* rhizosphere study techniques. Concentrations of organic acid released are small and their half-life in the soil solution is short. One option would be to develop sink materials that would absorb organic anions more strongly than hydrous oxide surfaces, e.g. specific resins. Such materials could be placed in rhizosphere soil and withdrawn for analysis. A second option would be to place Rhizon soil moisture



samplers (RSMSs) in the rhizosphere. These could be sampled regularly throughout the course of the experiment. Rhizosphere soil could be generated using the method described in Chapter 3. The use of a larger pot would be able to supply more water than the thin layers, which would mean that the pot water content would not need to be so high, lessening the likelihood of localised reduction enhancing P availability. Resin strips or RSMSs could be placed in the top cell just above the root mat. The lower cell could be sliced and rhizosphere P depletion measured as described in Hedley *et al.* (1994) and modelled (Kirk 1999); measured soil solution organic acid data (or calculated values, if resin strips were used) could then be added into the model.

Another novel approach to investigating the role of organic acids in enhancing P uptake would be to follow that of de la Fuente *et al.* (1997), who inserted bacterial genes for citric acid production into tobacco and papaya plants. The resulting plants excreted four times more citric acid than the non-genetically engineered clones, and showed increased tolerance to Al. However no data were reported for P uptake. Such an experimental approach would be expensive, and require a wide range of expertise, but would certainly provide valuable information on the potential of enhanced citric acid release to improve P uptake by upland and rainfed rice under field conditions.

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## Appendix A

**Table A.1.** The stability constants for the possible metal-ligand complexes that may form in solution for Experiment 4.3.3. Key to numbers in the heading rows: the first number denotes the number of metal ions, the second number denotes the number of ligands, the third number (if given) denotes the number of H<sup>+</sup> ions, or OH<sup>-</sup> ions if the number is negative. Source: GEOCHEM-PC 2.0 geochem\geodat-b.lig (Parker *et al.* 1995).

<b>Metal-ligand</b>	<b>1 1 -1</b>	<b>1 1 0</b>	<b>1 1 1</b>	<b>1 1 2</b>	<b>1 2 0</b>	<b>1 3 0</b>	<b>2 2 -2</b>
Ca-Cl		-1.00					
Ca-PO <sub>4</sub>		6.50	15.10	21.00			
Ca-citrate		4.80	9.30	12.60			
Fe-Cl		1.50			2.10	0.80	
Fe-PO <sub>4</sub>			23.30	25.00			
Fe-citrate	10.12	13.15	14.44		20.14		24.53
Mn-Cl		0.60			0.04		
Mn-citrate		5.00	9.20				-4.80
Al-PO <sub>4</sub>			19.80	22.70			
Al-citrate	6.40	9.90	12.90				
	<b>1 1</b>	<b>1 2</b>	<b>1 3</b>	<b>1 4</b>	<b>2 1</b>	<b>2 2</b>	<b>2 3</b>
Ca-OH	-12.70	-28.00					
Fe-OH	-2.20	-5.70	-13.10	-21.60		-2.90	
Mn-OH	-11.00	-22.20	-34.00	-48.30	-10.60		-23.90
Al-OH	-5.00	-10.10	-16.80	-22.70			
	<b>1 1</b>	<b>2 1</b>	<b>3 1</b>				
H-Cl	-8.40						
H-PO <sub>4</sub>	12.35	19.55	21.70				
H-citrate	6.34	11.12	14.21				

## Appendix B

**Table A.2.** Composition of the modified Yoshida *et al.* (1976) nutrient solution used for the thin layer experiments.

Element	Form supplied	Concentration of element in nutrient solution ( $\mu\text{M}$ )
N	$\text{NH}_4\text{NO}_3$	2860
P	$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	32 <sup>†</sup>
K	$\text{K}_2\text{SO}_4$	1030
Ca	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	1000
Mg	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1650
Mn	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	9.1
Mo	$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	0.52
B	$\text{H}_3\text{BO}_3$	19
Zn	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.15
Cu	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.16
Fe	Fe-EDTA	89

<sup>†</sup>Phosphorus was only included in the solution prior to transplanting

## Appendix C

### Calculation of the phosphate-citrate interaction coefficient, $\lambda_C$

Changes in the concentrations of P and citrate (C) in the whole soil,  $\delta[P]$  and  $\delta[C]$ , should be related to the corresponding changes in the concentrations in solution,  $\delta[P_L^*]$  and  $\delta[C_L]$ . Because  $\delta[P_L^*]$  is a function of both  $\delta[P]$  and  $\delta[C]$ ,

$$\delta[P_L^*] = \left( \frac{\partial[P_L^*]}{\partial[P]} \right)_C \delta[P] + \left( \frac{\partial[P_L^*]}{\partial[C]} \right)_P \delta[C] \quad (\text{A.1})$$

where the subscripts outside the brackets indicate that the partial derivatives are taken at constant [P] or [C]. At constant  $[P_L^*]$  (e.g. comparing the curves in Fig. 4.11a,b at a particular  $[P_L^*]$  value),  $\delta[P_L^*] = 0$  and therefore

$$\left( \frac{\partial[P]}{\partial[C]} \right)_{[P_L^*]} = - \left( \frac{\partial[P]}{\partial[P_L^*]} \right)_C \left( \frac{\partial[P_L^*]}{\partial[C]} \right)_P \quad (\text{A.2})$$

Using the chain rule to expand the last term at constant P,

$$\left( \frac{\partial[P]}{\partial[C]} \right)_{[P_L^*]} = - \left( \frac{\partial[P]}{\partial[P_L^*]} \right)_C \left( \frac{\partial[P_L^*]}{\partial[C_L]} \right)_P \left( \frac{\partial[C_L]}{\partial[C]} \right)_P, \quad (\text{A.3})$$

or

$$\left( \frac{\partial[P]}{\partial[C]} \right)_{[P_L^*]} = -\lambda_C \frac{b_{P^*}}{b_C} \quad (\text{A.4})$$

where  $\lambda_C$  is the P-C interaction coefficient,  $\left( \frac{\partial[P_L^*]}{\partial[C_L]} \right)_P$ ;

$b_{P^*}$  the  $P^*$  buffer power at constant [C],  $\left( \frac{\partial[P]}{\partial[P_L^*]} \right)_C$ ;

and  $b_C$  the C buffer power and at constant [P],  $\left( \frac{\partial[C]}{\partial[C_L]} \right)_P$ .

In words,  $\lambda_c$  is defined as the quantity of P that must be removed from the soil for a given uniform addition of C to leave the concentration of P in solution unchanged. The parameter  $\lambda_c$  is necessary for Model 2, and was calculated for P5000 Cavinti soil, and estimated for P2000 Cavinti soil, according to the method described in Kirk *et al.* (1999), as detailed below.

The value of  $\lambda_c$  for the P5000 soil should be calculated for a representative solution P concentration, i.e. half of the initial solution P concentration (0.165 mM), for a moderate amount of P desorbed (<50 mmol P kg<sup>-1</sup> soil. Figure A.1 shows that the quantity of P that must be removed from the soil to leave the solution P concentration at 0.165 mM as [C] increases from approximately 0.04 mM to 0.18 mM is (48-21) mmol kg<sup>-1</sup> × 1.03 kg L<sup>-1</sup> = 28 mmol P L<sup>-1</sup> soil.

$$\left( \frac{-\Delta[P]}{\Delta[C]} \right)_{[P_L^*]} = \lambda_c \frac{b_{P^*}}{b_C} \quad (\text{A.5})$$

$$\text{i.e. } \lambda_c = \left( \frac{-\Delta[P]}{\Delta[C]} \right)_{[P_L^*]} \left( \frac{b_C}{b_{P^*}} \right) = \left( \frac{-\Delta[P]}{\Delta[C_L]} \right)_{[P_L^*]} \div b_{P^*} \quad (\text{A.6})$$

From Figure A.1,  $\Delta[C_L]$  over the range of  $\Delta[P]$  is 0.18 - 0.04 = 0.14

The average P buffer power ( $b_P$ ) over the range of P desorbed can be best estimated as the average slopes of the 0.1 and 0.25 mM citrate, P desorption isotherms.

$$\text{Slope of 0.1 mM citrate P desorption isotherm} = \frac{(47 - 20) \times 1.03}{0.10 - 0.17} = -398$$

$$\text{Slope of 0.25 mM citrate P desorption isotherm} = \frac{(60 - 14) \times 1.03}{0.12 - 0.30} = -264$$

$$\text{Average P buffer power} = \frac{(-398 - 264)}{2} \approx -330$$

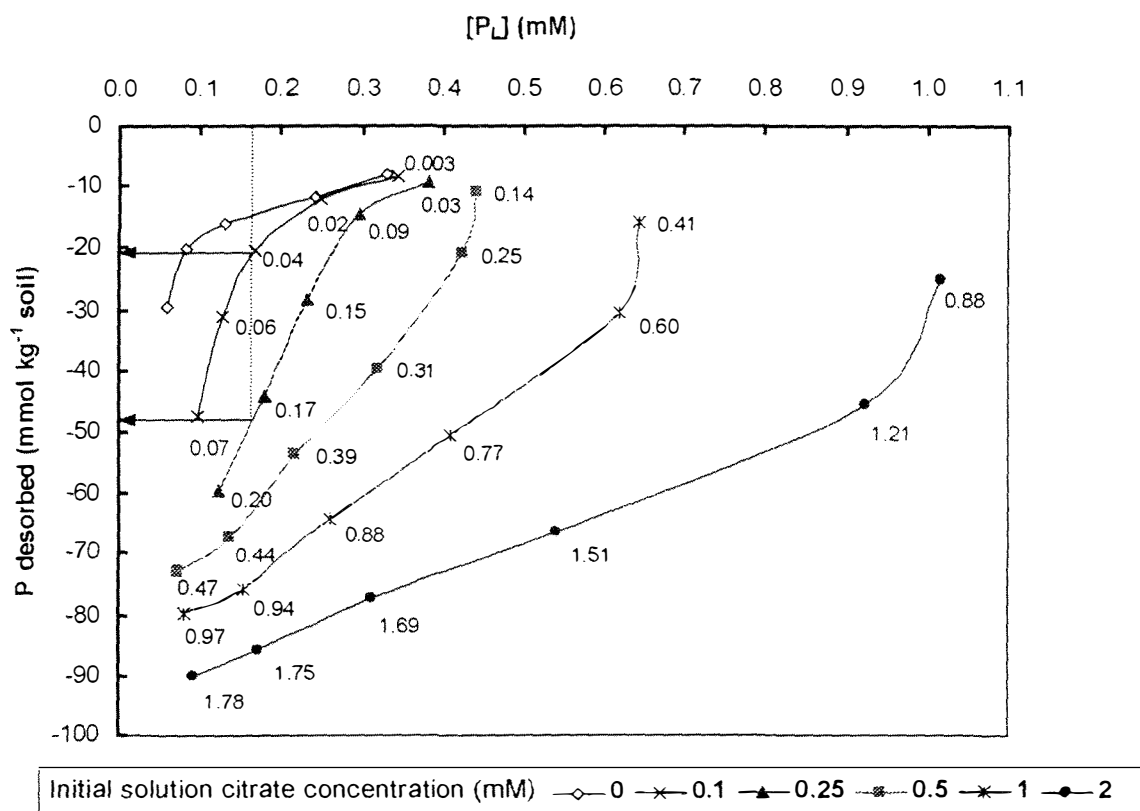
$$\text{Therefore } \lambda_c = \frac{28}{0.14} \div 330 = 0.61$$



This is markedly higher than the value of 0.025 calculated by Kirk *et al.* (1999) for P1000 soil. The higher value of  $\lambda_C$  indicates that more P is solubilised per unit of citrate addition at high soil P rates than lower soil P rates, as discussed in Section 4.4.3.8. To estimate a value for  $\lambda_C$  for the P2000 soil (Section 5.4.5.6), a value was chosen that is one quarter of the way between 0.025 and 0.65 (since P2000 soil is one quarter of the way between P1000 and P5000 soil), i.e. 0.17.

The  $b_{P^*}$  in the presence of citrate was estimated by assuming a value slightly lower than that of 200 measured for P1000 Cavinti soil by Kirk *et al.* (1999b), since  $b_P$  decreases as the soil P concentration increases (Table 4.2). Therefore a  $b_{P^*}$  of 150 was used.

The value of  $[P_L^*]$  used was taken to be one quarter of the way between the P1000 Cavinti soil (8  $\mu\text{M}$ , Kirk *et al.* 1999b) and P5000 Cavinti soil (330  $\mu\text{M}$ , Figure A1), i.e. 90  $\mu\text{M}$ .



**Figure A.1.** P desorption isotherms for P5000 Cavinti soil. The numbers beside each data point are the final solution citrate concentrations (mM) after 16 h of shaking. The points are means,  $n=2$ . The value of  $\lambda_C$  (see text) is calculated for a solution P concentration of 0.165 mM, shown by the dotted line.

## Appendix D

### Estimation of the amount of citrate contained in bacteria in a rhizosphere extract

Rouatt *et al.* (1960) found that the rhizosphere soil of 5-wk-old wheat plants contains  $1.2 \times 10^9$  bacteria  $\text{g}^{-1}$  soil. Assuming each cell has a volume of  $1 \mu\text{m}^3$  (Parkinson *et al.* 1971), then the total cellular volume extracted from 1 g of soil is  $1.2 \times 10^9 \mu\text{m}^3$  ( $\equiv 1.2 \mu\text{L}$ ).

Note: fungal hyphae were not included in the calculation as only undamaged fungal hyphae would contribute to an increase in citrate concentration after freezing – presumably all undamaged hyphae would be removed by the Whatman #5 filter paper.

Sixty grams of rhizosphere soil was extracted, therefore 72  $\mu\text{L}$  of micro-organisms could have been extracted in the 120 mL sample, or 0.6 mL of micro-organisms per litre of rhizosphere solution. This assumes that all of the bacteria resuspended after centrifugation at 17,000 g, and passed through a Whatman #5 filter paper.

It was assumed that the maximum concentration in the bacterial cytoplasm would be approximately 30 mM, which was the average intracellular citrate concentration of *Penicillium simplissimum* - a citrate-producing fungus (Gallmetzer *et al.* 1998, Figure 1). Therefore, the concentration of citrate in the rhizosphere extracts originating from microbial cell lysis would be:

$$(6 \times 10^{-4} \text{ L cells L}^{-1} \text{ extract}) \times (30 \times 10^{-3} \text{ mol citrate cell}^{-1} \text{ L}^{-1}) = 18 \mu\text{M citrate.}$$

The median peak heights in Figure 6.4c correspond to citrate concentrations of 74  $\mu\text{M}$  for the non-acidified treatment, and 41  $\mu\text{M}$  for the frozen treatment. Thus, assuming no change in cellular citrate concentration during freezing, or just prior to cell death in the unfrozen treatments, cell lysis explains only 24 – 44% of the observed ‘citrate’ peak, for the unfrozen non-acidified, and frozen treatments, respectively.