

5.1 Introduction

Information on the behaviour of phosphorus in soil is fundamental to understanding plant nutrition and the soil biogeochemical cycle. Phosphorus (P) deficiency is often reported in well-weathered Oxisols and Ultisols because of their strong acidic reactions and abundance of Al^{3+} and Fe^{3+} ions that complex P (Tisdale *et al.*, 1985). In the case of relatively young soils, such as Inceptisol, it has been found that a greater portion of their total P is in an available form, compared to mature soils which are more acid.

Available P deficiency in wheat soils is very common in Australia; farmers need to apply phosphatic fertilisers to maximize wheat yields. Maintenance dressings of P fertiliser to maximise yield vary with crops and soils. For example, wheat needs 15-25 kg fertiliser P ha^{-1} (Martin *et al.*, 1976; <http://www2.dpi.qld.gov.au>, 2006). A soil receiving P for each crop may show lower maximum adsorption capacity than soil receiving no P in the field (Abedin and Saleque, 1998).

Phosphorus usually has a high affinity for soil, resulting in slow downward movement through the soil matrix (Eghball *et al.*, 1990; Sims *et al.*, 1998) or laterally through interflow. In some circumstances, significant amounts of P may move by preferential flow paths (Jensen *et al.*, 1998; Simard *et al.*, 2000) with little adsorption to the soil matrix (Jensen *et al.*, 1998). However, P is likely to be adsorbed by soil Al and Fe in acidic soil (Maguire *et al.*, 2001). The P content of soil and consequential loss of P from soil to water is significant for eutrophication. To determine eutrophication risk there is a need to assess the environmental utility of current tests for P in soil. Sims *et al.* (2000) reported that soil tests clearly do not characterize site hydrology or nutrient-management practices and cannot identify the risk of direct P loss in runoff from fertilisers and organic wastes applied to the soil surface. This suggests that making risk management decisions based solely on conventional agronomic soil tests for P is a flawed approach. To estimate eutrophication risk it may be necessary to substitute current soil test methods with new approaches for assessing the capacity of a soil to retain P rather than lose it by leaching (Edwards and Withers, 1998; Sims *et al.*, 2000).

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

Adsorption and desorption reactions are considered as key aspects of the chemical behavior of P in soil. Adsorption describes the removal of phosphate ions from solution to soil components (Abedin and Saleque, 1998); desorption describes the reverse process of removal of bound soil P to the solution (Kuo *et al.*, 1988). As an equilibrium process the amount of P adsorbed is determined largely by the solution P concentration (Syers and Lu, 1990).

Phosphorus adsorption in soils has been studied extensively (e.g. Barrow, 1978; Barrow, 1983; Beck *et al.*, 1999; Maguire *et al.*, 2001; Siemens *et al.*, 2004). These investigations examined the effect of soil properties, pH, temperature, flooding, and redox potential of soil on P adsorption. As recovery of applied P by crops is typically only 15 to 20 per cent, it is likely that the major portion of the applied P fertiliser is adsorbed to soils at their various adsorption sites. Thus, continuous application of P fertiliser may saturate adsorption sites and make further P addition more available (Abedin and Saleque, 1998). On the other hand growing crops continuously without P applications may expose more sites for P adsorption as a result of desorption. Desorption isotherms characterise the release of adsorbed P but the process is extremely slow, and usually not completed in a period of hours or days. Generally, desorption appears to become very slow after about two days (Tisdale *et al.*, 1985). Bacteria might have the ability to desorb adsorbed P irrespective of the presence of plants in the soil. Evidence for such desorption of P from insoluble P in soil by soil-bacterial-interaction under laboratory conditions has not been reported. Experiments to examine soils for bacterial assisted P desorption in the absence of plants would be valuable in deciding which soil should be selected for a field trial.

Desorption is dependent on the nature of the adsorption complex. For example variscite ($\text{AlPO}_4 \cdot 2\text{H}_2\text{O}$) and strengite ($\text{FePO}_4 \cdot 2\text{H}_2\text{O}$) are more stable than calcium compounds of phosphorus [e.g. $(\text{Ca}_3(\text{PO}_4)_2)$] and are expected to be prominent in acid soils, desorbing P at a slow rate (Haseman *et al.*, 1950; Tan, 1993; Zhang *et al.*, 2001). Phosphorus binding in variscite and strengite is not dependent on the maximum P-buffering capacity of the soils (Kuo *et al.*, 1988). However P desorption is dependent on the P adsorption capacity of soils as estimated by the classical Langmuir equation (Kuo *et al.*, 1988).

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

In the first part of the study described in this chapter, seven soils were assessed for their P adsorption capacity, and the results analysed using different adsorption equations. In the second part of the work described two of these soils were selected and two bacterial isolates described in Chapter 2 were used to estimate the P-desorbing ability of the bacteria.

5.2 Experiment on phosphorus adsorption in some Australian soils

5.2.1 Materials and methods

5.2.1.1 Soil samples

Soil samples (2-3 kg; 0-15 cm depth) were collected from seven sites, six in New South Wales (NSW) and one in Victoria (VIC). These sites were:

1) Camden, NSW, Cobbity (University of Sydney Farm)

This is a duplex soil (Alfisol) (Chan and Barchia, 2007). This soil is from fallow land depleted of almost all nutrients and high in organic matter.

2) Griffith, NSW

This soil is a Mundiwa Clay Loam (Northcote, 1981). This soil is from a rice farm with a cropping pattern of rice-fallow-rice.

3) Narrabri, NSW, Auscott

This soil is a Vertisol (Northcote, 1981) from Auscott cotton field No 5; cropping patterns have been cotton-wheat-fallow. The soil was obtained from fallow land.

4) Rutherglen, VIC

This is a wheat rhizospheric soil as described in Chapter 1 (1.4.1; 2.2.1)), collected from Rutherglen Agricultural Research Station with a cropping pattern of wheat-fallow-wheat. It is classified as a Grey-brown fine sandy loam (Grey Sodosol), (Poutsma and Skene, 1961).

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

5) Wagga Wagga, NSW

This is a soil from a wheat-fallow-wheat cropping area. It was obtained after a wheat harvest and included rhizospheric soil, as described in Chapter 1 (1.4.1; 2.2.1). It is a Red Kandosol (McKenzie *et al.*, 2004).

6) Wee Waa, NSW, Ivanhoe

This is a natural grassland soil collected from 12 km east of Wee Waa. It included rhizospheric soil, as described in Chapter 1 (1.4.1; 2.2.1). It is classified as a red-brown earth/transitional red-brown earth (Inceptisol) (Triantafilis *et al.*, 2002).

7) Yanco, NSW

This soil is from rice field, with a cropping pattern rice-fallow-rice from Yanco, NSW. It is classified as Birganbigil Clay Loam (brown Chromosol) (Doran *et al.*, 2006).

The Narrabri, Wagga Wagga and Wee Waa soils were described in Chapter 2 (2.2.1) and the Camden soil was described in Chapter 4 (4.2.1.1).

5.2.1.2 Analysis of soil samples

The soil samples were air dried, passed through a 2 mm sieve and stored in plastic bags. Soils were analyzed for organic matter, total P and total N at the Wollongbar Analytical Laboratory, NSW Department of Primary Industry, Wollongbar, NSW. The pH, cation exchange capacity (CEC), available P, and texture were determined at the Faculty of Agriculture, Food and Natural Resources, the University of Sydney. The methods used for analysis are described in Chapter 2.

5.2.2 Determination of phosphorus adsorption capacity of seven soil samples

5.2.2.1 Experimental procedure

Two g samples of each soil were weighed into 100 mL centrifuge tubes with three replications. A range of P solutions (0, 10, 20, 30, 40 and 50 mg P L⁻¹) was prepared

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

by dissolving potassium dihydrogen phosphate (KH_2PO_4) in 0.01 M calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) solution in Millipore water and 20 mL aliquots of the solutions were added to the centrifuge tubes to give 0, 100, 200, 300, 400 and 500 mg of added P kg^{-1} of soil. The samples were then shaken for 24 h at room temperature (24°C) at 144 rpm. After 24 h shaking, the tubes were centrifuged at 20,000 g (Sorvall RC 5C; Heraeus; Model-930437) for 12 min, and then filtered through Whatman No. 42 filter paper (Agbenin, 2003). The filtrates were analyzed for available P following the method of Murphy and Riley (1962). The amount of P adsorbed per gram of soil was calculated from the difference in the P added to the soils and the P present in the solution. The P in the control (no P) treatment solution was taken into account. The adsorbed P was calculated as mg kg^{-1} soil.

5.2.2.2 Analysis of adsorption data

Adsorption isotherms describe solute adsorption by solids from an aqueous solution at constant temperature and pressure and show the amount of solute adsorbed as a function of equilibrium concentration in solution. The P adsorption data for the soils used in this study were fitted to the following adsorption equations:

Langmuir adsorption equation:

$$c/(x/m) = 1/kb + c/b \dots\dots\dots [5.1]$$

where c is the equilibrium solution P concentration ($\mu\text{g P mL}^{-1}$), x/m is the mass of P adsorbed per unit mass of soil (mg kg^{-1}), k is a constant related to bonding energy of P to the soil, b is the maximum P adsorption capacity (mg kg^{-1}). A plot of $c/(x/m)$ versus c gives a straight line if the adsorption process fits this model. The values of b and k are obtained from the slope ($1/b$) and the intercept ($1/kb$), respectively.

Freundlich adsorption equation:

$$\frac{x}{m} = ac^b \dots\dots\dots [5.2]$$

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

By rearranging;

$$\log \frac{x}{m} = \log a + b \log c \dots\dots\dots [5.3]$$

where, x/m is the mass of P adsorbed per unit mass of soils (mg kg^{-1}), c is the equilibrium solution P concentration ($\mu\text{g mL}^{-1}$), a and b are constants. A plot of $\log (x/m)$ versus $\log c$ gives a straight line if the adsorption process fit the model. The values of a and b are obtained from the intercept ($\log a$) and the slope (b), respectively.

Temkin adsorption equation:

$$\frac{x}{m} = a + b \ln c \dots\dots\dots [5.4]$$

where, x/m is the mass of P adsorbed per unit mass of soil (mg kg^{-1}), c is equilibrium solution P concentration ($\mu\text{g mL}^{-1}$), a and b are constants. A plot of x/m against $\ln c$ gives a straight line if the model fits the adsorption process. The values of a and b are obtained from the intercept (a) and the slope (b), respectively. The b value of the Temkin equation is considered as the P-buffering capacity (retention capacity of adsorbed P) of soil (mg kg^{-1}).

Statistical analyses were carried out using statistical software GenStat (Payne *et al.*, 2003).

5.2.3 Results

5.2.3.1 Physico-chemical properties of soils

The physico-chemical properties of the seven soils collected in NSW and Victoria are shown in the Table 5.1.

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

Table 5.1 Physico-chemical properties of seven soils collected from NSW and VIC.

Soils were from NSW except the Rutherglen soil from Victoria. Total P, total N and OM were determined at Wollongbar Analytical Laboratory; other parameters were determined in Sydney (2.2.1).

Physico-chemical properties	Soils						
	1	2	3	4	5	6	7
	Camden	Griffith	Narrabri	Rutherglen	Wagga	Wee Waa	Yanco
total P (mg kg ⁻¹)	150.0	240.0	280.0	370.0	150.0	300.0	380.0
total N (mg kg ⁻¹)	1900.0	1600.0	1300.0	2600.0	890.0	1500.0	1200.0
% OM	5.1	4.6	2.5	7.6	3.0	4.6	3.2
available P (mg kg ⁻¹)	3.9	11.3	11.1	30.3	17.0	3.8	18.1
pH	5.4	4.1	7.4	5.4	4.8	5.7	6.4
CEC (cmol ^c kg ⁻¹)	5.6	6.5	32.9	4.4	4.5	3.3	12.6
sand (%)	59.5	56.0	33.1	55.5	63.0	83.0	63.3
silt (%)	26.2	9.8	17.3	34.6	17.3	7.3	7.4
clay (%)	14.3	34.2	49.5	9.9	19.7	9.7	29.4
soil texture	Sandy loam	Sandy clay loam	Clay	Sandy loam	Sandy loam	Loamy sand	Sandy clay loam

5.2.3.2 Phosphorus remaining in solution after treatment of soil samples with potassium dihydrogen phosphate

The results of assays of P in the filtrate from soil samples treated with varying quantities of P (5.2.2.1) are shown in Table 5.2.

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

Table 5.2 The amount of P remaining in the equilibrium solution of soils treated with varying amounts of P.

Soil samples were treated with up to 500 mg P kg⁻¹ soil for 24 h at room temperature by mixing the soil samples with KH₂PO₄ in 0.01 M CaCl₂ solution. The equilibrium solution was separated from the soil by centrifugation and filtration and assayed for P (5.2.2.1).

For the zero added P procedure, soil samples were treated with 0.01 M CaCl₂ solution.

Added P (mg kg ⁻¹ soil)	P remaining in the Equilibrium Solution (µg mL ⁻¹)						
	1	2	3	4	5	6	7
	Camden	Griffith	Narrabri	Rutherglen	Wagga	Wee Waa	Yanco
0	0.00 fB*	0.00 cB	0.08 fB	0.41 fB	0.30 fB	1.16 fA	0.08 eB
100	1.99 eB	0.08 cD	1.09 eC	5.32 eA	5.17 eA	5.70 eA	0.45 eD
200	7.42 dC	0.26 cF	4.39 dD	12.78 dA	11.51 dB	11.96 dB	3.34 dE
300	13.76 cC	0.79 cF	8.77 cD	20.95 cA	18.29 cB	20.84 cA	6.26 cE
400	22.00 bD	1.99 bG	14.54 bE	30.55 bA	27.25 bC	29.91 bB	11.66 bF
500	30.40 aC	3.34 aF	19.42 aD	38.64 aA	36.24 aB	36.92 aB	17.62 aE

Soil and added P rate interaction was significant at F probability level of <0.001 with LSD (0.05) value of 0.75

* Means followed by a common small letter in a column and a common capital letter in a row are not significantly different by least significant difference (LSD) test at 5% level of probability using two way ANOVA.

Changes in the amount of P in the equilibrium solution with added P were examined statistically for each soil. Regression analysis indicated that the increase in adsorbed P content in the soil with the addition of increasing amounts of P was linear in all seven soils (Table 5.3).

Table 5.3 Regression equations and R² values relating added P and P remaining in the equilibrium solution for the results shown in the Table 5.2.

Soil	Regression equation	R ² Value
Camden	y = - 3.004 + 0.0624x	0.967***
Griffith	y = - 0.564 + 0.0066x	0.850*
Narrabri	y = - 2.058 + 0.0404x	0.961**
Rutherglen	y = - 1.533 + 0.0786x	0.992***
Wagga Wagga	y = - 1.592 + 0.0722x	0.987***
Wee Waa	y = - 0.846 + 0.0744x	0.989***
Yanco	y = - 2.309 + 0.0355x	0.926**

*** = significant at 0.01 level; ** = significant at 0.05 level; * = significant at 0.10 level

**CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND
THE INFLUENCE OF BACTERIA ON ITS DESORPTION**

5.2.3.3 Phosphorus adsorbed to soils after treatment with potassium dihydrogen phosphate

The P adsorption to seven soils with varying levels of added P is shown in Table 5.4. Levels varied widely in the seven soils. The interaction effect of added P level and soil was significant at all P levels. The highest P adsorption (466 mg P kg⁻¹ soil) occurred with the Griffith soil when it was treated with 500 mg P kg⁻¹ soil.

Table 5.4 The amount of P adsorbed to soils after treatment with varying amounts of P in solution.

Soil samples were treated with up to 500 mg P kg⁻¹ soil for 24 h at room temperature by mixing the soils samples with KH₂PO₄ in 0.01 M CaCl₂ solution. The soil samples were separated by centrifugation and filtration, and assayed for P content (5.2.2.1).

Added P (mg kg ⁻¹ soil)	Adsorbed P (mg kg ⁻¹ soil)						
	1 Camden	2 Griffith	3 Narrabri	4 Rutherglen	5 Wagga	6 Wee Waa	7 Yanco
100	80.14 eC	99.25 eA	89.89 eB	50.90 dD	51.28 eD	54.65 eD	96.25 eAB
200	127.29 dD	197.38 dA	156.52 dC	76.32 cF	87.94 dE	92.06 dE	167.26 dB
300	162.45 cD	292.13 cA	213.05 cC	94.61 bG	120.10 cE	103.24 cF	238.16 cB
400	179.99 bD	380.14 bA	255.33 bC	98.66 bG	130.52 bE	112.49 bF	279.19 bB
500	196.04 aD	466.65 aA	306.61 aC	117.70 aF	140.58 aE	142.44 aE	324.60 aB

Soil and added P rate interaction was significant at F probability level of <0.001 with LSD (0.05) value of 8.27.

* Means followed by a common small letter in a column and a common capital letter in a row are not significantly different by least significant difference (LSD) test at 5% level of probability.

Changes in the amount of P adsorbed with added P were examined statistically for each soil. Regression analysis indicated that the increase in adsorbed P content in the soils with the addition of increasing amounts of P was linear in all seven soils (Table 5.5).

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

Table 5.5 Regression equations and R² values relating added P and adsorbed P for the results shown in Table 5.4.

Soil	Regression equation	R ² Value
Camden	y = 63.829 + 0.2845x	0.945**
Griffith	y = 11.841 + 0.9176x	0.999**
Narrabri	y = 44.603 + 0.5323x	0.993**
Rutherglen	y = 40.853 + 0.1559x	0.953**
Wagga Wagga	y = 39.730 + 0.2212x	0.920**
Wee Waa	y = 42.170 + 0.1960x	0.941**
Yanco	y = 50.505 + 0.5686x	0.984**

** = significant at 0.01 level.

The percentage of the added P adsorbed to the soils is shown in Table 5.6. For all soils, at increasing P concentrations there was a decrease in the percentage of the available P adsorbed. The percentage P adsorption is higher at all levels of added P for the Griffith soil.

Table 5.6 Adsorbed P as a percentage of the available P in solution.

P added (mg kg ⁻¹ soil)	P adsorbed as % available P in solution						
	1	2	3	4	5	6	7
	Camden	Griffith	Narrabri	Rutherglen	Wagga	Wee Waa	Yanco
100	80.14	99.25	89.89	50.90	51.28	54.65	96.25
200	63.65	98.69	78.26	38.16	43.97	46.03	83.63
300	54.15	97.38	71.02	31.54	40.03	34.41	79.39
400	45.00	95.04	63.83	24.67	32.63	28.12	69.80
500	39.21	93.33	61.32	23.54	28.12	28.49	64.92

5.2.3.4 Relationship of adsorbed phosphorus and some physico-chemical properties of seven soils

The relationship between the mean adsorbed P for the seven soils and some physico-chemical properties was examined. The mean adsorbed P was the average of the P adsorbed by the soils at each P treatment concentration (100 to 500 mg kg⁻¹ soil). For this analysis the physico-chemical properties CEC, OM, pH and clay content were considered and the relationships are shown in Figs 5.1a –Fig 5.1d.

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

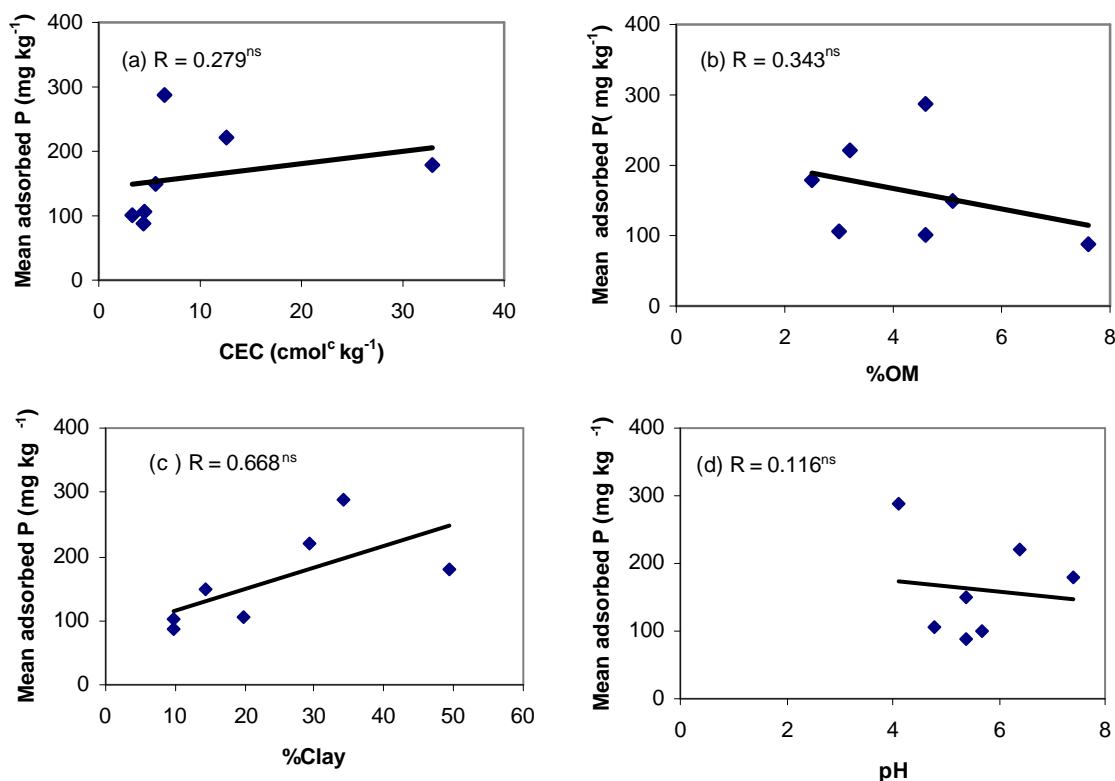


Figure 5.1 The relationship between mean adsorbed P and physico-chemical properties of soils; (a) CEC (b) OM (c) clay and (d) pH.

Mean P adsorbed was calculated as the average value of P adsorbed by soil samples treated with 100 to 500 mg P kg⁻¹ soil. ns = not significant at 0.05 level.

There was no statistically significant correlation between any of these parameters and the mean P adsorbed, although the relationship between clay content and adsorbed P does show an increase in adsorbed P at higher percentage clay content.

5.2.3.5 Adsorption Isotherms for the interaction of KH₂PO₄ with seven soil samples

The data shown in Table 5.2 for the adsorption of P to seven soils have been examined using the Langmuir, Freundlich and Temkin equation. The results of these analyses are presented in Figure 5.2 (Langmuir equation), Figure 5.3 (Freundlich equation) and Figure 5.4 (Temkin equation).

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

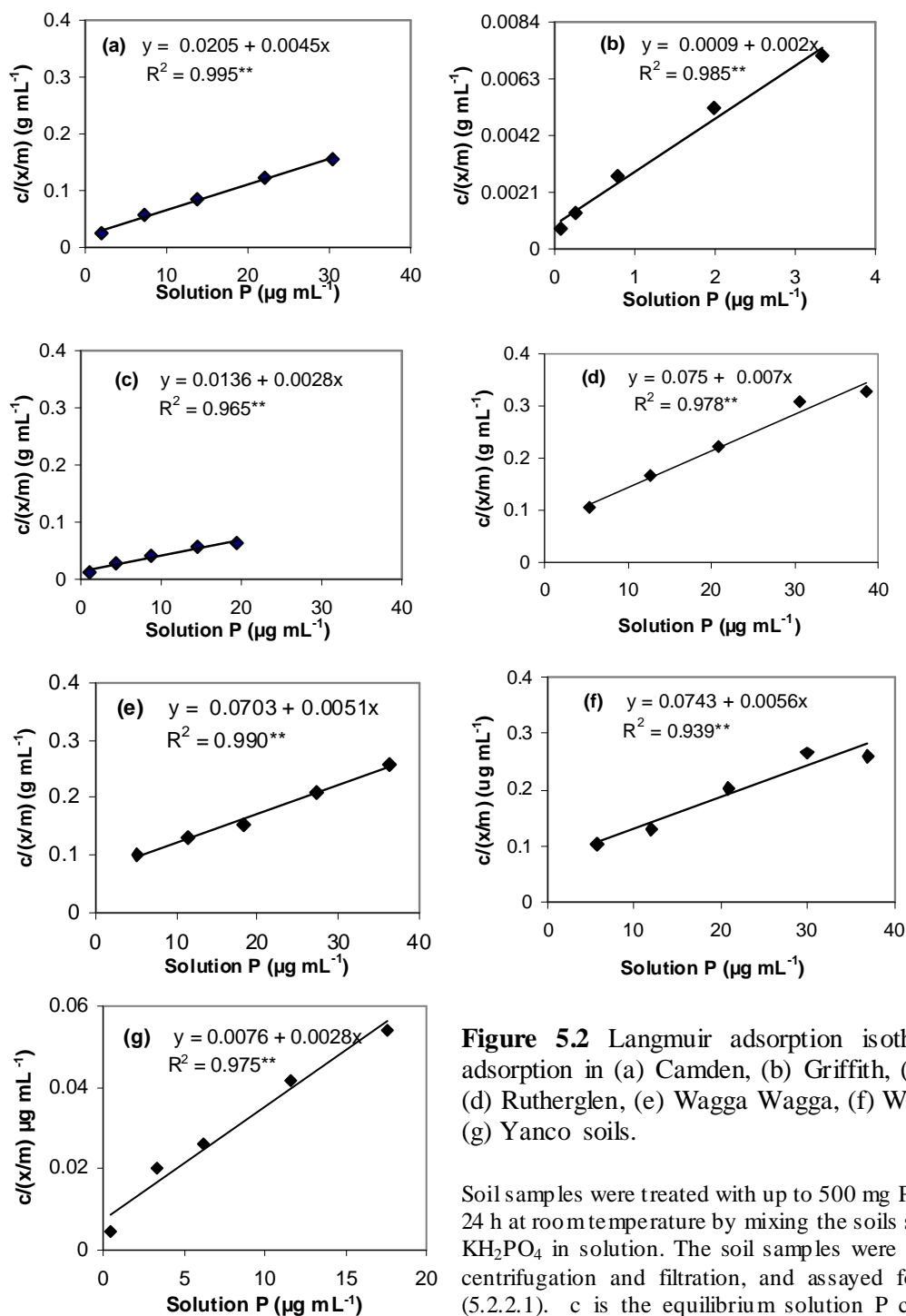


Figure 5.2 Langmuir adsorption isotherm for P adsorption in (a) Camden, (b) Griffith, (c) Narrabri, (d) Rutherglen, (e) Wagga Wagga, (f) Wee Waa, and (g) Yanco soils.

Soil samples were treated with up to 500 mg P kg⁻¹ soil for 24 h at room temperature by mixing the soils samples with KH₂PO₄ in solution. The soil samples were separated by centrifugation and filtration, and assayed for P content (5.2.2.1). c is the equilibrium solution P concentration after treatment, and x/m is the mass of P adsorbed per unit mass of soil.

** significant at 0.01 level

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

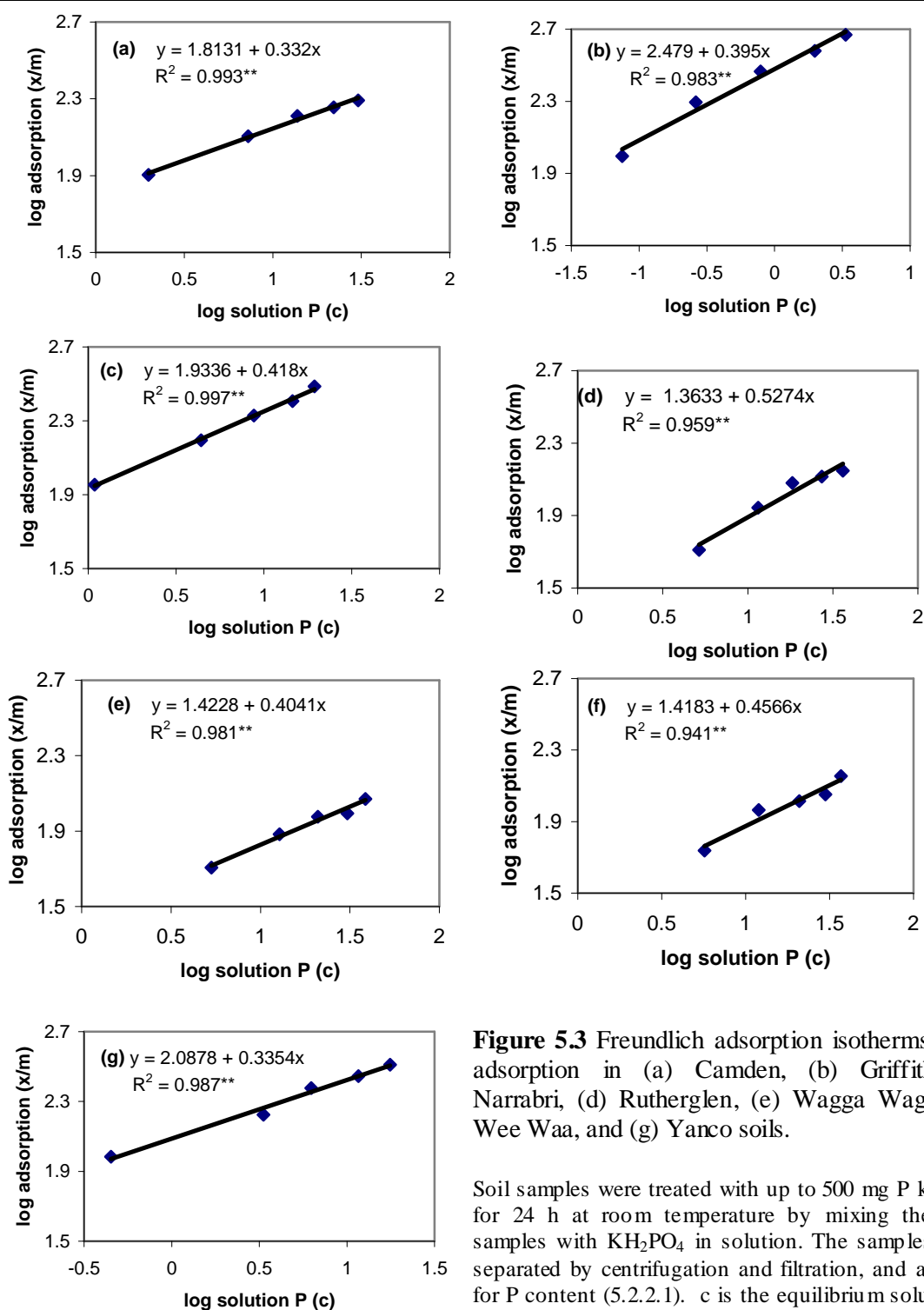


Figure 5.3 Freundlich adsorption isotherms for P adsorption in (a) Camden, (b) Griffith, (c) Narrabri, (d) Rutherglen, (e) Wagga Wagga, (f) Wee Waa, and (g) Yanco soils.

Soil samples were treated with up to 500 mg P kg⁻¹soil for 24 h at room temperature by mixing the soils samples with KH₂PO₄ in solution. The samples were separated by centrifugation and filtration, and assayed for P content (5.2.2.1). c is the equilibrium solution P concentration after treatment, and x/m is the mass of P adsorbed per unit mass of soil.

** significant at 0.01 level

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

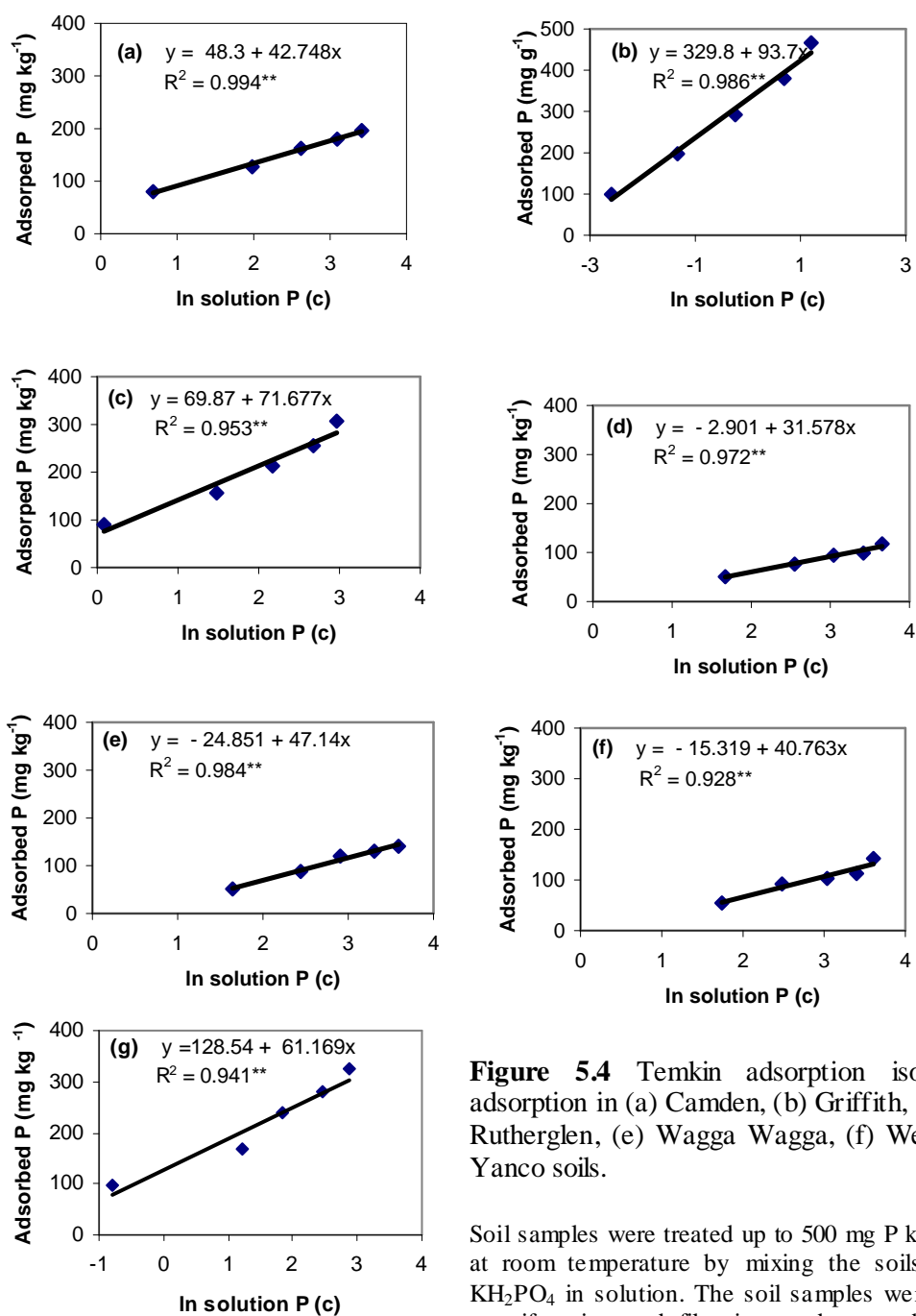


Figure 5.4 Temkin adsorption isotherms for P adsorption in (a) Camden, (b) Griffith, (c) Narrabri, (d) Rutherglen, (e) Wagga Wagga, (f) Wee Waa, and (g) Yanco soils.

Soil samples were treated up to 500 mg P kg⁻¹ soil for 24 h at room temperature by mixing the soils samples with KH₂PO₄ in solution. The soil samples were separated by centrifugation and filtration, and assayed for P content (5.2.2.1). After treatment the remaining solution P was converted into ln value, and then the value was correlated with adsorbed P (x/m) (mg kg⁻¹) in adsorption isotherm

** significant at 0.01 level

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

The data for P adsorption (Table 5.2 and 5.4) fitted the Langmuir, Freundlich and Tempkin equation and these data were used to calculate maximum adsorption capacity, constant of energy adsorption, and buffering capacities for P in the seven soils using the Langmuir and Temkin equations. The results are presented in Table 5.7. The highest maximum P-adsorption and P-buffering capacities were obtained for the Griffith soil. The highest constant of energy adsorption was obtained for the Yanco soil.

Table 5.7 Maximum adsorption capacity, constant of energy of adsorption and buffering capacity for P of seven soils.

Soil	Maximum P adsorption capacity ^a (mg kg ⁻¹)	Constant of energy of adsorption ^b (k value)	Buffering capacity ^c (mg kg ⁻¹)
Camden	222.2	0.220	42.7
Griffith	500.0	0.222	93.8
Narrabri	357.1	0.003	71.7
Rutherglen	142.9	0.093	31.6
Wee Waa	178.6	0.075	40.8
Wagga Wagga	196.1	0.073	47.1
Yanco	357.1	0.368	61.2

^aCalculated b value from the Langmuir equation (Equation 5.1).

^bCalculated k value from the Langmuir equation (Equation 5.1).

^cCalculated b value from the Temkin equation (Equation 5.4).

The relationship between P adsorption and the P-buffering capacity of the soils using the data from Table 5.7 is shown in Fig 5.5.

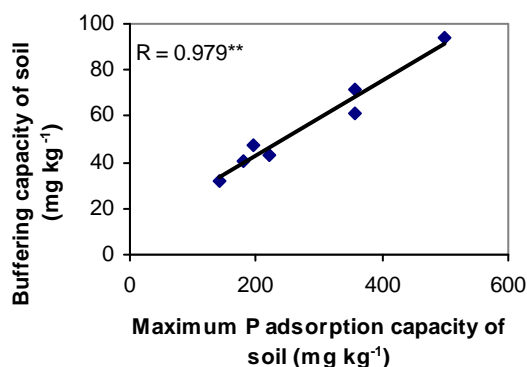


Figure 5.5 Relationship between maximum P adsorption capacity and the P-buffering capacity of soils.

Maximum P adsorption capacity and P-buffering capacity were calculated from the Langmuir and Temkin equations (Table 5.7). ** significant at 0.01 level.

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

The P adsorption data for all soils fitted well to the Langmuir (Figure 5.2), the Freundlich (Figure 5.3), and the Temkin adsorption equations (Figure 5.4). Coefficients of determination (R^2 value) were significant at the 1 per cent level of probability for all three adsorption equations in all seven soils, indicating they were of similar utility in describing the adsorption process. The Langmuir and Temkin equations were best fitted for the Camden soil, while the Freundlich equation was best fitted for the Narrabri soil. The highest maximum P adsorption capacity obtained from the b value of the Langmuir equation was 500 mg kg^{-1} in the Griffith soil whereas it was the lowest (143 mg kg^{-1}) in the Rutherglen soil (Table 5.7). The constant k (obtained from the Langmuir equation), which is a measure of energy of adsorption was the highest (0.368) for the Yanco soil followed by the Griffith soil (0.222) and the Camden soil (0.220) while it was the lowest for the Narrabri soil (0.003). The P-buffering capacity (mg kg^{-1}) (obtained from the b value of the Temkin equation) was the highest in the Griffith soil followed by the Narrabri and the Yanco soils, respectively, whereas the lowest P-buffering capacity was obtained in the Rutherglen soil (Table 5.7).

In this experiment it was found that these three adsorption isotherms (Langmuir, Freundlich and Temkin) are applicable for analysis of P adsorption in the experimental soils. Application of adsorption isotherms for explaining phosphorous adsorption behaviour in the soil matrix is well established (Barrow, 1978; Akhtar *et al.*, 2003). Using the data from Table 5.7 it was found that the P adsorption capacity is strongly correlated ($R = 0.979$) to the P-buffering capacity of the soils (Figure 5.5).

5.3 Experiment on phosphorus mobilisation from two soils by two bacterial isolates

5.3.1 Materials

5.3.1.1 Soil samples

Two soils (Griffith and Narrabri, 5.2.1.1) were used in this experiment. The Griffith soil was a sandy clay loam, with total P 240 mg kg^{-1} and available P $11.3 \text{ (mg kg}^{-1})$. It had a CEC 6.4 cmol kg^{-1} , pH 4.1 and contained 4.6 per cent OM. It contained 56.0 per cent sand, 9.8 per cent silt and 34.2 per cent clay. The Narrabri soil was a clay soil

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

with total P 280 mg kg⁻¹ and 11.1 mg P kg⁻¹ of available P. The CEC was 32.9 cmol⁺kg⁻¹, pH 7.4 and contained 2.5 per cent OM. It contained 33.1 per cent sand, 17.3 per cent silt and 49.5 per cent clay. Total P and available P were similar in these soils (Table 5.1). The soils were not sterilized.

The Griffith soil adsorbed 466 mg P kg⁻¹ soil, while the Narrabri soil adsorbed 306 mg P kg⁻¹ soil when treated with 500 mg P kg⁻¹ soil in solution (Table 5.4). The adsorption capacity calculated from the Langmuir equation was 500 mg P kg⁻¹ soil for the Griffith soil, and 357 mg P kg⁻¹ soil for the Narrabri soil (Table 5.7).

5.3.1.2 Preparation of soil samples with adsorbed phosphorus

Air dried soil samples (2 g) were placed in screw-capped polypropylene centrifuge tubes (acid washed in 2 M HCl overnight). One hundred mL of KH₂PO₄ solution in 0.01 M CaCl₂ (pH 5.76) was added containing 200 mg P (100 mg P kg⁻¹ soil) and shaken using a head to head shaker at 140 rpm for 24 h so that the soil attained an equilibrium situation for P adsorption (5.3.2). The soil was centrifuged at 20,000 g (Sorvall RC 5C; Heraeus: Model-930437) for 10 min and the supernatant filtered through Whatman No. 42 filter paper. The soil samples were dried in an oven at 40°C to provide air dried soil. Using the known clay content in the soils, field capacity was estimated as 28 per cent for the Griffith soil and 40 per cent for the Narrabri soil (<http://www.bettersoils.com.au>, 2005).

5.3.1.3 Bacterial samples

Seven bacteria with P-mobilizing ability isolated from three soil samples have been tentatively identified (2.4). The two strains FA001 and FA010 that had the greatest capability for mobilizing P in liquid culture (3.4), tentatively identified by conventional and molecular techniques as *Pantoea ananatis* and *Pantoea agglomerans*, were selected for investigation of their P-mobilising capacity in soil.

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

5.3.1.4 Preparation of bacterial suspensions for inoculation of soils

Ampoules of freeze-dried bacterial strains (FA001 and FA010) were opened aseptically and the strains cultured on nutrient agar plates (3.2.1.2) for 24 h. Sterile deionised water was used to prepare bacterial suspensions. The 24 h bacterial cultures were scraped from the surface using a Pasteur pipette bent by flame, washed with 3-4 mL sterile water, and using another sterilized Pasteur pipette transferred into glass bottles. Bacteria per mL suspension were determined by plate counting (3.2.2.2).

5.3.2 Methods

5.3.2.1 Establishment of soil and bacterial treatments

For each soil three sets of six cultures were set up. The sets of six cultures were divided into two groups of three cultures which were treated differently in the assays for solubilised P.

The triplicate sets of cultures containing soil and FA001 were called Treatment 1 (T₁) and Treatment 2 (T₂). The triplicate sets containing soil and FA010 were Treatment 3 (T₃) and Treatment 4 (T₄). The triplicate sets of soil without added bacteria were T₅ and T₆ (Table 5.8).

Table 5.8 Soil and bacterial treatments set up to examine P-mobilisation from the Griffith and Narrabri soils by FA001 and FA010 bacteria.

Soils were treated with 100 mg P kg⁻¹ soil (5.3.1.2). Bacterial strains FA001 and FA010 were identified as potentially useful P-mobilising bacteria (3.3.1).

Treatments	Components of the cultures
T ₁	Soil and bacteria (FA001)
T ₂	Soil and bacteria (FA001)
T ₃	Soil and bacteria (FA010)
T ₄	Soil and bacteria (FA010)
T ₅	Soil and sterile water
T ₆	Soil and sterile water

For the Griffith soil the cultures were labelled T_{1G} to T_{6G}. For the Narrabri soil the cultures were labelled T_{1N} to T_{6N}

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

Bacterial suspensions (5.3.1.4) were added to the 2 g samples of P-treated air dried soil in screw-capped polypropylene centrifuge tubes. For the Griffith soil with a field capacity of 28 per cent, (5.3.1.2), 0.56 mL bacterial suspension was added. For the Narrabri soil with a field capacity of 40 per cent (5.3.1.2), 0.8 mL bacterial suspension was added. To provide conditions in the soil similar to rhizosphere, where organic substances are excreted, 4 mg powdered glucose (2 g kg^{-1} soil) was added to all samples at the time of inoculation and then samples were vortexed for 1 min. For the uninoculated control samples sterile water was used (0.56 mL and 0.8 mL for the Griffith and Narrabri soils) and 4 mg glucose was added. The samples were capped and incubated in an oven at 28°C for three days.

5.3.2.2 Determination of mobilised phosphorus

After three days of incubation, three mL of chloroform were added to the triplicate samples of the treatments T_{1G} , T_{1N} , T_{3G} , T_{3N} , T_{5G} and T_{5N} to lyse bacteria. The capped tubes were mixed by vortexing for three 10-sec intervals over a period of 30 min at room temperature (24°C) (Brookes *et al.*, 1982). The tubes remained capped for about 4 h to allow lysis of bacteria. The caps were then removed to allow evaporation of the CHCl_3 at 24°C over 16 h.

Calcium chloride (0.01 M) solution was added to all of the T_1 , T_2 , T_3 , T_4 , T_5 and T_6 replicates at the rate of 10 mL g^{-1} soil. The tubes were shaken by an end to end shaker (50 rpm) overnight (16 h) at room temperature (24°C). The soil suspension was centrifuged at $20,000 \text{ g}$ ($5\text{-}10^{\circ}\text{C}$; Sorvall RC 5C; Heraeus, Model- 930437) for 12 min and filtered through Whatman No. 42 filter paper (Agbenin, 2003) using vacuum filtration to obtain the CaCl_2 extraction. Then the moist soil samples were extracted a second time with 0.5 M NaHCO_3 (pH 8.5) at 1:50 ratio ($2 \text{ g soil } 100 \text{ mL}^{-1}$) (Colwell, 1963). Shaking rate, time, temperature, centrifugation, and filtering were the same as for the extraction with 0.01 M CaCl_2 .

Inorganic P was determined in the 0.01 M CaCl_2 and 0.5 M NaHCO_3 extracts by the method of Murphy and Riley (1962).

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

5.3.2.3 Statistical data analysis

Data were analysed using statistical software GenStat (ver 7.0); using two way analysis of variance (ANOVA) (Payne *et al.*, 2003).

5.3.3 Results

5.3.3.1 Phosphorus extraction by 0.01 M CaCl₂

The P extracted into 0.01 M CaCl₂ from cultures of Griffith and Narrabri soils (with adsorbed P) treated with P-mobilising bacteria (FA001 and FA010) is shown in Table 5.9.

Table 5.9 Phosphorus extracted from cultures of Griffith and Narrabri soils with and without P-mobilising bacteria by 0.01 M CaCl₂ with and without treatment with CHCl₃.

Soils were treated with 100 mg P kg⁻¹ soil (5.3.1.2). Bacterial strains FA001 and FA010 were identified as potentially useful P-mobilising bacteria (3.3.1).

All P values are the mean of three replicates

Treatments	P (mg kg ⁻¹ soil) extracted into 0.01 M CaCl ₂ solution	
	Cultures T _{1G} to T _{6G} (Griffith soil)	Cultures T _{1N} to T _{6N} (Narrabri soil)
T ₁ Soil + FA001 + (CHCl ₃)	0.006 a B*	0.492 a A
T ₂ Soil + FA001	0.003 a B	0.270 c A
T ₃ Soil + FA010 + (CHCl ₃)	0.003 a B	0.365 b A
T ₄ Soil + FA010	0.001 a B	0.277 c A
T ₅ Soil + CHCl ₃	0.011 a B	0.259 cd A
T ₆ Soil	0.006 a B	0.236 d A
Soil- types mean	0.005 B	0.317 A

F probabilities for soil and soil x treatments interaction were <0.001 and <0.001, respectively. The lsd values for soil and soil x treatments interaction were 0.012 and 0.029, respectively at 0.05 level of probability.

* Means followed by a common small letter in a column and a common capital letter in a row are not significantly different at 0.05 level by lsd.

These results indicate that the strains FA001 and FA010 bacteria were able to desorb some P from the Narrabri soil. There was no significant desorption of P from the Griffith soil. In the Narrabri soil cultures T_{2N} and T_{4N}, the P desorbed was not significantly different with the FA001 and FA010 bacterial strains, but was significantly greater than in the without bacteria, non-CHCl₃ treated T_{6N} culture. The highest P desorption was found in lysed soil inoculated with the strain FA001 (T_{1N}),

**CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND
THE INFLUENCE OF BACTERIA ON ITS DESORPTION**

significantly greater than with the strain FA010 (T_{3N}) and significantly greater than the culture without bacteria (T_{5N}).

5.3.3.2 Phosphorus extraction by 0.5 M NaHCO₃

The P extracted into 0.5 M NaHCO₃ from cultures of Griffith and Narrabri soils (with adsorbed P) treated with P-mobilising bacteria (FA001 and FA010) is shown in Table 5.10.

Table 5.10 Phosphorus extracted from cultures of Griffith and Narrabri soils with and without P-mobilising bacteria by NaHCO₃ with and without treatment with CHCl₃.

Soils were treated with 100 mg P kg⁻¹ soil (5.3.1.2). Bacterial strains FA001 and FA010 were identified as potentially useful P-mobilising bacteria (3.3.1).

All P values are the mean of three replicates.

Treatments		P (mg kg ⁻¹ soil) using 0.5 M NaHCO ₃ solution	
		Cultures T _{1G} to T _{6G} (Griffith soil)	Cultures T _{1N} to T _{6N} (Narrabri soil)
T ₁	Soil + FA001 + (CHCl ₃)	57.42 abA*	57.42 aA
T ₂	Soil + FA001	56.67 bA	50.68 bcB
T ₃	Soil + FA010 + (CHCl ₃)	59.67 aA	59.67 aA
T ₄	Soil + FA010	58.17 abA	52.92 bB
T ₅	Soil + CHCl ₃	58.17 abA	49.18 cB
T ₆	Soil	51.42 cA	47.68 cB
	Soil-types mean	56.92 A	52.92 B

F probabilities for soil and soil x treatments interaction were <0.001 and <0.001, respectively. The lsd values for soil and soil x treatments interaction were 1.19 and 2.92, respectively at 0.05 level of probability.

* Means followed by a common small letter in a column and a common capital letter in a row are not significantly different at 0.05 level by lsd.

For all cultures P extracted into NaHCO₃ was at least 100-fold greater than into CaCl₂. The mean P desorbed in the six Griffith soil cultures (T_{1G} to T_{6G}) was significantly greater than in the six Narrabri soil cultures (T_{1N} to T_{6N}). For the Griffith soil cultures containing bacteria, desorbed P was very similar with or without CHCl₃ treatments (Table 5.10). For the cultures without added bacteria the P desorbed with CHCl₃ treatments (T_{5G}) was similar to that in T_{1G} to T_{4G}, and significantly greater than in T_{6G}. For cultures containing the Narrabri soil, extractable P values in the T_{1N} and T_{3N} cultures were statistically greater than from the T_{2N} and T_{4N} cultures (without

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

CHCl₃ treatments). The cultures T_{5N} and T_{6N} contained the lowest levels of extractable P and were not significantly different.

5.4 Discussion

The varied physico-chemical properties of the seven soils studied in this experiment are shown in Table 5.1. Total P (mg kg⁻¹) ranged from 150 mg kg⁻¹ in the Camden and Wagga Wagga soils to 380 mg kg⁻¹ in the Yanco soil. Available P (varied from 4 to 30 mg kg⁻¹) was not correlated with total P. The low total P soils contained 3.9 and 17.0 mg kg⁻¹ of available P in the Camden and Wagga Wagga soils, and 18.1 mg kg⁻¹ in the high total P Yanco soil. Total N (mg kg⁻¹) varied from 890 mg kg⁻¹ in the low total P Wagga Wagga soil to 2600 mg kg⁻¹ in the Rutherglen soil. The other low total P soil, from Camden, contained 1900.0 mg N kg⁻¹. The OM in these soils ranged from 2.5 per cent to 7.6 per cent; pH ranged from 4.1 to 7.4 and CEC (cmol⁺kg⁻¹) from 4.4 to 32.9 cmol⁺kg⁻¹. The Narrabri soil was the only soil described as a clay soil, with 49.5 per cent clay and only 33.1 per cent sand. The Wee Waa soil was mostly sand (83.0 per cent) and the other soils were sandy loam or sandy clay loam.

After treatment of the soils with a range of P-concentrations in 0.01 M CaCl₂ solution P remaining in the equilibrium solution increased significantly with addition of P up to 500 mg P kg⁻¹ in all soils, except the Griffith soil. This soil did not show any significant increase up to 300 mg P kg⁻¹ soil, indicating its high P adsorption capability (Table 5.2). On treatment with 500 mg P kg⁻¹ soil the equilibrium solution contained 3.34 mg kg⁻¹ for the Griffith soil and from 17.62 to 38.64 mg kg⁻¹ for the other six soils. In the samples with no P addition, the equilibrium solution P concentration in the Wee Waa soil was significantly higher than for the other soils. This is probably because the CaCl₂ solution had extracted some of the available P from this very sandy soil (Table 5.1). In the 100 mg P kg⁻¹ soil treatments, the highest equilibrium P concentrations were in the equilibrium solutions with the Wee Waa, Rutherglen and Wagga Wagga soils whereas the lowest equilibrium P concentrations were with the Griffith and Yanco soils (Table 5.2). The highest and the lowest equilibrium P concentrations were obtained with the Rutherglen and the Griffith soils respectively with all other treatments (200, 300, 400, 500 mg P kg⁻¹ soil).

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

In a complex matrix such as soil many processes may interact with humus, and soil components such as iron and aluminium, to affect P adsorption. Phosphate adsorption is associated with the occurrence of reactive surface sites in the mineral soil. It is possible that when the adsorption sites are filled by phosphate ions, the percentage of extra added phosphate that is adsorbed may decrease with the increasing level of added P. Reduced affinity for adsorption has been reported when increased amounts of adsorbed P are present in soil (Barrow, 1978). The results of this experiment are in agreement with other reports that show that P adsorption increased significantly with increased levels of added P and the percentage of added P adsorbed decreased with the increasing level of added P (Abedin and Saleque, 1998; Akhtar *et al.*, 2003).

At 100 mg P kg⁻¹ added P, the amounts of P adsorbed by the Griffith and Yanco soils were statistically similar and significantly greater than for the other five soils. At all higher levels of added P, the adsorption of P was significantly higher in the Griffith soil compared to the other soils (Table 5.4). At all levels of added P, the lowest amount of adsorption occurred in the Rutherglen soil. The percentage of the added P adsorbed by the soil decreased with the increasing level of P addition (Table 5.6) indicating that the available binding sites for P were decreasing.

Regression analysis showed that the increase in P adsorption as a result of addition of P was linear in all the seven soils studied (Table 5.5). The rate of increase was the highest in the Griffith soil followed by the Yanco and Narrabri soils. The lowest rate occurred with the Rutherglen soil. The R² values were significant at 0.01 level of probability for all the soils. Correlation analysis indicated that relationships between P adsorption with clay, CEC, OM and soil pH, were not significant (Figure 5.1) although the relationship between P adsorption and clay content was better (R = 0.668) than for the CEC, OM and soil pH relationships. The pH of the Griffith soil was lower (pH 4.1) than all other soils, and the relative acidity that indicates high aluminium and iron might have a positive impact on P adsorption because of P retention by Fe²⁺, Fe³⁺, and Al³⁺ brought into solution at low pH. Higher phosphate adsorption by soils containing higher total amounts of extractable aluminium and iron has been reported (Brady and Weil, 2002, Pant *et al.*, 2002; Agbenin, 2003; Gielser *et al.*, 2005).

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

Marked differences in P-buffering capacity were noted among the soils. While the P-buffering capacity of the Griffith soil was 94 mg kg⁻¹, it was only 32 mg kg⁻¹ in the Rutherglen soil. The term P-buffering capacity is used to describe phosphate characteristic of the soil. The significance of P-buffering capacity in characterising phosphate availability to plants has been demonstrated by Olsen and Watanabe (1963), Mattingly (1965), Barrow (1967), and Holford and Mattingly (1976). Phosphate-buffering capacity also has been related to the soils' fertiliser phosphate requirements (Ozanne and Shaw, 1968). Bhadoria *et al.* (1991) reported that in the process of diffusion, P is desorbed from the soil with a high P concentration and transported to unfertilised soil. It was found that soil P diffusion coefficients are dependent on (a) the buffer power, (b) whether P is being desorbed or adsorbed and (c) on the time available for reaction (Bhadoria *et al.*, 1991). As the P-buffering capacity was lowest in the Rutherglen soil, it is likely that the diffusion of P could be faster in this soil compared to the Griffith soil. For this reason, more P fertiliser may be needed in the Griffith soil to obtain equal plant growth.

In the second part of this study, the Griffith and Narrabri soils were treated with the bacterial strains FA001 and FA010 to define the extent of desorption possible. These two soils were chosen because they have similar total P, similar available P but different pH values (Table 5.1). For each bacterial strain and the control (without bacteria) desorbed P was extracted from cultures with and without treatment with CHCl₃. Soluble P extraction was carried out using 0.01 M CaCl₂ (pH 5.76) and 0.5 M NaHCO₃ (pH 8.5) solution.

Treatment with CHCl₃ causes lysis of bacteria in soil, whether they are endogenous strains or specific strains used for inoculation (FA001 and FA010). It would be expected that higher levels of soluble P would be extracted from cultures treated with CHCl₃ because of the release of soluble bacterial P from the lysed bacteria. However, total bacterial P is not included in extracted P because P in cell components such as membranes and nucleic acids is not solubilised by CHCl₃. Some of the organic P released from the cells may be broken down after the CHCl₃ treatment. It has been estimated that the soluble P that can be extracted from bacterial cells is about 40 per cent of the total P in the cell (Brookes *et al.*, 1982; Bliss *et al.*, 2004).

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

It was found in this study that soluble P extracted with CaCl_2 was less than one per cent of that extracted with NaHCO_3 , indicating that CaCl_2 is a very weak extractant (Brookes *et al.*, 1982). Overall, it was found that soluble P extracted in the CaCl_2 solution was higher from the Narrabri soil that had high pH (pH 7.4) and high clay content, than in the Griffith soil extract. In the Narrabri cultures, the extractable P varied significantly according to the treatment. The Narrabri soil culture containing the strain FA001 treated with CHCl_3 (T_{1N}) had significantly the highest amount of soluble P extracted by the CaCl_2 solution. For all treatments (T_{1N} to T_{6N}) the soluble P content was significantly higher in the CHCl_3 -treated cultures. The P content in the cultures amended with bacteria (T_{1N} , T_{2N} , T_{3N} and T_{4N}) was higher than the cultures without added bacteria (T_{5N} and T_{6N}). Considering soil with bacteria (not CHCl_3 -treated), there was no significant difference in extracted P between the cultures containing the strains FA001 and FA010. There was a small but significant difference between cultures containing these two strains and the cultures with no added bacteria. For cultures containing the Griffith soil, with and without bacteria, and with and without CHCl_3 treatment, negligible P was detected in CaCl_2 extracts. No significant differences between the samples were found. As the Griffith soil had low pH, and it had higher amounts of iron and aluminium cations, it is possible the added P was tightly bound with these soil cations.

In the Narrabri soil, the soluble P content without CHCl_3 treatment in cultures containing added bacteria was about 0.038 mg kg^{-1} soil greater than for the cultures without added bacteria (for FA001, $0.277-0.236 = 0.041 \text{ mg kg}^{-1}$; and for FA010, $0.270-0.236 = 0.034 \text{ mg kg}^{-1}$). This is a seven to eight-fold greater value than the total P extracted by CaCl_2 from the Griffith soil (Table 5.9). For cultures containing bacteria and treated with CHCl_3 the strain FA001 solubilised a significantly higher amount of P than the strain FA010. Phosphorus released from bacteria was 0.222 mg kg^{-1} for FA001 ($0.492-0.270 = 0.222 \text{ mg kg}^{-1}$) and 0.188 mg kg^{-1} for FA010 ($0.365-0.277 = 0.188 \text{ mg kg}^{-1}$), an average of $0.205 \text{ mg P kg}^{-1}$ soil. This is about five times the free soluble P in the soil ($0.205/0.038 \approx 5$). These cultures were incubated for three days and these results show that most of the P mobilised by bacteria was used by the bacteria in this incubation period. The significant increase in the P extracted from the T_{5N} cultures (after CHCl_3 -treatment) compared with T_{6N} culture ($0.023 \text{ mg P kg}^{-1}$

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

soil) indicated that there were some endogenous bacteria in the soil. These results indicate that the bacterial strains FA001 and FA010 were able to desorb some P from the Narrabri soil. There was no significant effect with the Griffith soil, when 0.01 M CaCl_2 was used as equilibrium solution.

The physico-chemical properties of these two soils are shown in Table 5.1, showing that they contain similar amounts of endogenous available P (11 mg kg^{-1} soil). Their pH and soil textural classes were different. The initial pH values of the Narrabri and Griffith soils were 7.4 and 4.1 respectively and their textural classes were clay and sandy clay loam. Thus, it is possible that the Griffith soil's acidic pH enhanced P fixation with free ions (Fe^{3+} , Al^{3+}) or Fe and Al oxides or by forming complexes with dissolved organic compounds in the soil solution (Brady and Weil, 2002). It is possible that the bacteria that were used in the Griffith soil failed to grow substantially at the acid pH of the cultures. Soils containing large quantities of clay (more surface area exposed) will fix more P than those containing small amounts of clay (small surface area exposed) (Tisdale *et al.*, 1985). They may have been unable to readily access fixed P. The bacteria may grow better in the higher pH (pH 7.4) of the Narrabri soil. The maximum P adsorption capacities for the Narrabri and Griffith soils were 357 and 500 mg kg^{-1} soil, respectively (Table 5.7). The constants of energy adsorption of the Narrabri and Griffith soils were 0.003 and 0.222, and soil P-buffering capacities were 71.68 (mg kg^{-1} soil) and 93.78 (mg kg^{-1} soil), respectively (Table 5.7). The lower P-buffering capacity of the Narrabri soil may allow more P to diffuse to the media resulting in more soluble P in the Narrabri than the Griffith soil (Barrow, 1967; Holford and Mattingly, 1976; Choudhury and Khanif, 2000). Most importantly, the maximum P adsorption capacity and constant of energy for adsorption are relevant to the desorption of P. The Griffith soil had a greater maximum P adsorption capacity and constant of energy adsorption than the Narrabri soil (Table 5.7). The P adsorption capacity and constant of energy for adsorption relate to the degree of strength of bonds in the ecosystem. Since the pH of 0.01 M CaCl_2 solution was low (pH 5.76) and its ionic strength was low, it would not be expected to affect the soil pH. For the low pH Griffith soil extraction of soluble P by CaCl_2 solution is likely to be low. The recovery of microbial-P varied inversely with P adsorption capacity (Hedley and

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

Stewart, 1982), which might result in tighter binding of solubilised P in the Griffith soil.

The soluble P in the NaHCO₃ extracting solution for both the Griffith and Narrabri soils was about 100-fold greater than that in the CaCl₂ extracts. The mean P concentration in extracts from the Griffith soil was significantly greater than from the Narrabri soil. For the Narrabri soil the P content in the treatments amended with bacteria (T_{1N}, T_{2N}, T_{3N} and T_{4N}) was higher than the treatments without added bacteria (T_{5N} and T_{6N}). The P content was not significantly different in the extracts from T_{1N} and T_{3N} (containing strains FA001 and FA010). For both treatments the T_{1N} and T_{3N} soluble P levels were significantly greater than for the cultures without CHCl₃ treatments (T_{2N} and T_{4N}). For extracts from the Griffith soil the P concentration in the treatment T_{6G} (without bacteria and CHCl₃ treatment) was significantly lower than for the other five treatments.

The P adsorbed by the soils used in this experiment was supplied by treatment with a solution of 100 mg P kg⁻¹ soil. The Griffith soil adsorbed 99 mg P kg⁻¹ soil and the Narrabri soil adsorbed 90 mg P kg⁻¹ soil (Table 5.4). The pH of the 0.5 M NaHCO₃ extracting solution (pH 8.5) increased the pH of the Griffith soil from 4.1 to about 7.5. This increase in the pH of the soil suspension may have facilitated the dissociation of previously adsorbed P from aluminium and iron oxides (Brady and Weil, 2002). Most of the P extracted by NaHCO₃ is probably desorbed directly from the soils with only limited amounts resulting from bacterial sources.

It is possible that soil P-buffering capacity contributed to some extent to the soluble P in the CaCl₂ extractant from the Narrabri soil, even though bacteria did contribute to P desorption. The P mobilised by bacteria extracted by 0.01 M CaCl₂ was small, but it was significant with the Narrabri soil and insignificant with the Griffith soil. The small amount of P mobilized by bacteria may partially fill the requirement of plants for P. Soil microorganisms are involved in a range of processes that affect P transformation and thus influence the subsequent availability of P to plant roots (Rodríguez and Fraga, 1999; Richardson, 2001; Harris *et al.*, 2006). It seems likely that the difference in soil physicochemical properties of these two soils in their P-

CHAPTER 5 PHOSPHORUS ADSORPTION IN SOME AUSTRALIAN SOILS AND THE INFLUENCE OF BACTERIA ON ITS DESORPTION

adsorbing and P-buffering capacities affected the effectiveness of bacteria to mobilise soil-adsorbed P (Table 5.7).

5.5 Conclusion

This study has shown that increasing levels of addition of P significantly increased the P adsorption of these soils. The P-buffering capacity (mg kg^{-1}) (retention capacity of adsorbed P) and the maximum P-adsorption capacity (mg kg^{-1}) of a soil determine its P adsorbing potential. While the results show that bacterial activity can be involved in P desorption, bacterial P desorbing capability also seems to depend on P retention capacity and the maximum adsorption capacity of the soil. The difference in the response of the two soils examined also indicates that the capacity of different soils to respond to the presence of P-mobilizing strains of microorganisms also varied. The P extracted by dilute CaCl_2 solution was significantly greater from the Narrabri soil (pH 7.4) cultures than from the Griffith soil cultures (pH 4.1). The results obtained from this experiment using 0.5 M NaHCO_3 (pH 8.5) in acidic soil for P determination, suggest that the increase in pH facilitates the release of P bound to iron and aluminium. It is possible that knowledge of the P-buffering capacity (mg kg^{-1}) and the maximum P-adsorption capacity (mg kg^{-1}) of soil may be useful in determining the effectiveness of the application of microorganisms as phosphatic biofertiliser.

An experiment using the soils with the highest and lowest maximum adsorption capacity and P-buffering capacity (Table 5.7, Griffith soil and Rutherglen soil), might help to answer these questions. The effect of varying glucose concentration at fixed P control levels could be examined, as well as the use of soil with autoclaving and without autoclaving (to assess the importance of endogenous bacteria). This study has raised more questions than it has answered. Hence it supports the concept that such studies using other types of soil and different bacterial strains, using different mechanisms of P-mobilisation might enable better prediction of the response to application of biofertiliser strains.