

# Rapid colorimetric methods for analysis of pH, extractable aluminium and Colwell phosphorus in soils

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## ABSTRACT

**Context.** Analytical procedures and technologies for soil analyses can be prohibitively expensive for small laboratories and researchers in developing countries. There is a need for low cost and high-throughput methods for assaying pH, extractable aluminium and phosphorus when conducting field trials on acid soils. **Aims.** We investigated methods to develop rapid yet inexpensive colorimetric assays for the assay of pH, extractable aluminium and Colwell phosphorus in soil extracts. **Methods.** We developed a colorimetric method to measure soil pH enabling pH to be quantified in a high-throughput assay. Similarly, two existing methods for extractable aluminium and Colwell P were modified for high throughput assays also using microtiter plates. **Key results.** All three methods yielded linear relationships when using absorbance to quantify the parameters with the high throughput methods. Furthermore, there was a strong correlation between pH values of the soil samples obtained with the colorimetric assay and pH values measured with a glass electrode. **Conclusions.** We demonstrated that the rapid assays for all three methods can be implemented to characterise field sites through the mapping of distributions for extractable Al, Colwell P and pH. **Implications.** The high-throughput methods described here will be useful for researchers who conduct field trials to map variations in soil pH, soluble Al and Colwell P. Although the focus of the current work was on acid soils, the colorimetric pH and Colwell P methods can also be applied to non-acid soils.

**Keywords:** acid soils, aluminium toxicity, colorimetric soil analysis, colorimetric soil pH, Colwell-P, pyrocatechol violet method, soil acidity, soil tests, stratified pH.

## Introduction

Soil analysis is an important component of farm management and research in agriculture as virtually all calories consumed by humans are derived from the land (Pimentel and Burgess 2013). Soils have been evolving for millions of years under various weather, bedrock, chemical and physical conditions. Natural and human processes have created soil constraints on plant production in agriculture. Soil acidity is one of the major constraints world-wide causing aluminium (Al) toxicity and impeding the uptake of the phosphorus (P) required for effective plant growth (von Uexküll and Mutert 1995). Soil analyses are essential for conducting effective field trials with the aim of developing solutions to mitigate acidity.

Analytical procedures and technologies for soil analyses have improved in recent years. However, many technologies and methods are prohibitively expensive for small laboratories and researchers in developing countries. Furthermore, some analytical procedures require special training and equipment that are beyond the budget of small laboratories. Soil analyses of many samples, as is required for large field trials, can be prohibitively costly even for well-funded projects.

Soil pH is one of the most common and useful parameters to characterise soils used in agriculture and is commonly measured using water or CaCl<sub>2</sub> extractions. The effect of soil to water ratio on soil pH was extensively investigated in the development of methods for measuring soil pH (Wilcox 1936). Among others, an important factor in measuring soil

pH is the salt content of soil (Thomas 1996). Today, a 1:5 ratio of soil to solution has been adopted by many researchers as a standard for the measurement of soil pH (Rayment and Lyons 2011). Currently, the most widespread method to measure pH is electrometrically/potentiometrically using glass electrodes. However, measuring soil pH with an electrode can be time-consuming. Although automated pH metres are available (see for example: <https://hudsonrobotics.com/products/other-products/ph-meter-probe/>) high initial and ongoing costs are prohibitive for small laboratories.

The notion of measuring pH in a solution using colorimetric methods with indicator dyes as an alternative to the electrode method has been proposed by numerous researchers (Pierre 1925; Snyder 1935; Wilcox 1936; Raupach and Tucker 1959). Snyder (1935) provided a comprehensive review of early colorimetric methods of soil pH measurements and their applications. The assays were based on estimation of pH by visual assessment of dye colours and were not quantified methods. In more recent years, there has been a renewed interest in colorimetric methods to measure pH in a wide range of media (Newman *et al.* 2012; Kirkwood *et al.* 2014; Mito *et al.* 2016). These methods were developed for samples such as ground water and protein buffers and are not suitable for soil suspensions (Kirkwood *et al.* 2014; Mito *et al.* 2016). Finding a suitable indicator or mix of indicators for soil suspensions can be difficult. Dyes can be absorbed by soil particles and colour reactions can vary in different soils as observed by Raupach and Tucker (1959). Raupach and Tucker (1959) were successful in identifying indicator dyes that were consistent in their reactions with various soils and could be used to estimate soil pH. However, their assay was based on a visual assessment of colours and was not based on a quantified measurement of absorbance.

In contrast to soil pH, there are established methods for quantifying soluble Al ( $\text{Al}^{3+}$ ) and phosphate in soils using colorimetric methods. A colorimetric method using pyrocatechol violet termed the 'PCV method' to estimate  $\text{Al}^{3+}$  in solution was developed by Anton (1960). The PCV method was subsequently modified to suit various applications and situations (Henriksen and Bergmann-Paulsen 1975; Samaritan *et al.* 1993) including the estimation of  $\text{Al}^{3+}$  in soil solutions (Ares 1986; Bromfield 1987; Kerven *et al.* 1989; Conyers *et al.* 1991; Barton and Carr 1996).

The plant-available P in soil comprises mainly of orthophosphate species ( $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$ ). Orthophosphate can form a blue phosphomolybdenum complex with molybdate reagent in a colorimetric reaction. However, the amount of 'available P' in soil solutions can vary according to the extractant used. Olsen *et al.* (1954) introduced a P extraction method using 0.5 M  $\text{NaHCO}_3$  solution at pH 8.5. The sodium bicarbonate method was modified by Colwell (1963) to determine P fertiliser requirements in Australian soils. This method can be applied to a wide range of acid and alkaline soils (Irving and McLaughlin 1990). The colour development of the phosphomolybdenum complex in soil solutions can be

assayed with various reagents. One such reagent is malachite green and a method describing the use of malachite green is described by Irving and McLaughlin (1990).

Here, we present three low-cost and high-throughput methods to assay pH, Colwell P and extractable  $\text{Al}^{3+}$  in soil using small volumes of extracts in microtiter plates. A colorimetric method to measure soil pH was developed based on a commercially available kit that relies on a visual assessment of soil pH. We adapted the method to enable pH to be quantified in a high-throughput assay while existing methods for extractable Al and Colwell P were modified for high throughput assays also using microtiter plates. We show that these methods can be applied to map the distribution of pH, soluble  $\text{Al}^{3+}$  and Colwell P as is required for field trials. Although the methods were developed primarily for analysis of acid soils, they are applicable to a range of soil types.

## Materials and methods

### Soil samples

All soil samples were air dried, crushed and passed through a 2 mm sieve before they were used for the various analyses.

Soils used as standards for the colorimetric pH method were collected from four locations in Australia. The soils were either unmodified or limed to obtain particular pH values (Table 1). As a part of soil characterisation, soil organic carbon and total nitrogen content were analysed by dry combustion with a Leco-CNS 895 analyser (Leco Corporation, MI, USA), .

To test the colorimetric method for soil pH, samples were taken in August 2020 from a field trial that had been limed by deep ripping 5 T of lime  $\text{ha}^{-1}$  to 15–25 cm after surface application in late May 2020 at the Boorowa Agriculture Research Station, NSW (−34.46°S, 148.70°E). A total of 69 soil samples were collected at 0–5, 5–10, 10–20, 20–30 and 30–40 cm depths from 15 locations on the trial.

To test the soluble  $\text{Al}^{3+}$  and Colwell P rapid methods, we collected soil samples from two field trials. One trial had 210 plots of wheat located at Little Billabong 'Warrenboo' farm, NSW (−35.68°S, 147.57°E) and samples were collected in September 2017. The other trial had 220 plots of wheat in Merredin, WA (−31.48°S, 188.21°E) and samples were collected in July 2018. Both sites were initially selected because of the acidic nature of their soils. Phosphorous fertiliser was supplied as triple super phosphate at sowing with treatment rates of 0, 5, 10, 20 and 40 P  $\text{kg ha}^{-1}$  at the Little Billabong trial and 0, 7, 14, and 30 P  $\text{kg ha}^{-1}$  at the Merredin trial. A total of 630 (Little Billabong) and 660 (Merredin) samples were collected at three depths (0–5, 5–10 and 10–20 cm) from the trials.

**Table 1.** Characteristics of soil standards used in the colorimetric assay for pH.

Soil number	Soil texture/soil type	Location	Lime addition (mg kg <sup>-1</sup> )	Soil organic carbon (%)	Total nitrogen (%)	Soil pH
1	Sandy clay loam/brown chromosol	Merredin, WA, 5–20 cm (–31.48°S, 118.21°E)	None	0.22	0.05	3.9
2	Sandy clay loam brown chromosol	Merredin, WA, 5–20 cm	0.1	0.49	0.03	4.2
3	Clay loam/red-yellow podzolic	Ginninderra, ACT, 10–20 cm (–35.20°S, 149.08°E)	None	0.51	0.04	4.6
4	Clay loam/yellow chromosol	Wallaroo, ACT, 5–20 cm (–35.17°S, 149.04°E)	0.7	2.23	0.21	4.8
5	Clay loam/red-yellow podzolic	Ginninderra, ACT, 20–40 cm	None	0.17	0.00	5.2
6	Clay loam/red-yellow podzolic	Ginninderra, ACT, 60+ cm	None	0.14	0.02	5.4
7	Clay loam/yellow chromosol	Wallaroo, ACT, 5–20 cm	1.5	1.40	0.17	5.9
8	Clay loam/yellow chromosol	Wallaroo, ACT, 5–20 cm	2.0	2.09	0.20	6.0
9	Clay loam/red-yellow podzolic	Ginninderra, ACT, 20–40 cm	0.3	0.17	0.01	6.4
10	Clay loam/yellow chromosol	Wallaroo, ACT 5–20 cm	3.5	2.03	0.20	7.0
11	Clay loam/yellow chromosol	Wallaroo, ACT, 5–20 cm	5.0	2.13	0.20	7.2
12	Sandy loam/grey calcareous	Eyre Peninsula, SA, 5–20 cm (–33.77°S, 135.96°S)	None	5.28	0.19	7.7

The soil pH was determined with a glass electrode.

### Colorimetric soil pH assay

Four grams of subsamples of well-mixed samples (~50 g) were weighed into 50 mL centrifuge tubes (Cellstar #227 261, Greiner Bio-One). Soils were extracted with a 0.01 M CaCl<sub>2</sub> solution (1:5 w/v; soil: solution) by mixing for 1 h on an end-over-end rotary mixer. Extractants in 50 mL centrifuge tubes were then centrifuged for 10 min at 2093g to pellet the soil particles.

The indicator dye mix used in the method was purchased from a local hardware store as part of a soil pH measuring kit (Soil pH test kit, Model No: MTO8000, Manutec Pty Ltd, SA; available on eBay). The indicator dye was diluted to 25% by adding deionised water on the day of use. Dilution of the dye on the day of use was found to be necessary as absorbances of standard solutions would change if the diluted solution was stored for several days.

Soil pH was measured with a Mettler Toledo Seven Compact pH metre with three calibration points that was attached to a Mettler Toledo InLab Exper Pro-ISM probe (Order No. 30014096). Prior to measuring a set of soil extractants, the metre was calibrated daily with pH buffers of 4.01, 7.01 and 10.01 (Hanna HI7004, 7007 and 7010). The stability criterion of the metre was set to the standard level. Values of pH were recorded when measurements had fully stabilised and the metre had locked in. It took an average of three and a half minutes per extracted soil sample to measure the pH with this instrument.

For the colorimetric pH assay, 200 µL of soil extractant in 0.01 M CaCl<sub>2</sub> was pipetted into the wells of a 96-well clear flat-bottomed microtiter plate (Greiner Bio-One # 655101).

The first row of each plate (A1 to A12) was allocated to extractants of soil standards. Diluted indicator dye (10 µL) was then pipetted into each well with colour development occurring essentially instantaneously. Absorbance of the solutions in the wells of the microtiter plates were measured at 590 nm using a Bio Tek Power Wave HT microplate spectrophotometer with KC4 V3.0 software (Millennium Science Pty Ltd, Vic, Australia). A calibration curve was constructed using the soil standards by plotting the measured soil pH using the pH metre against the absorbances using the colorimetric method. Soil pH values of test samples were obtained from the linear relationship between measured pH and absorbance as obtained from the calibration curve.

### Extractable Al<sup>3+</sup> and Colwell P

The same CaCl<sub>2</sub> extractant as used for pH measurements was used for Al<sup>3+</sup> assay by the PCV method based on that described by Conyers et al. (1991).

For Colwell P analysis, a subsample of 0.5 g of soil was weighed into a 50 mL centrifuge tube to which 50 mL of 0.5 M NaHCO<sub>3</sub> (pH 8.5) was added. The soil suspension was mixed on an end-over-end rotary mixer for 16 h at 25°C before tubes were centrifuged at 2093g for 10 min (Colwell 1963).

### PCV assay

The working standard of Al<sup>3+</sup> solution 5 µg mL<sup>-1</sup> (185 µM) was prepared by dilution with deionised water of a 10 mg mL<sup>-1</sup> (370 mM) of metal Al in 10% HNO<sub>3</sub> (Choice Analytical, Cat

# 10M1-1) stock solution. An  $\text{Al}^{3+}$  standard curve was constructed to cover the range 30.9–185.2  $\mu\text{M}$  by addition of the standard  $\text{Al}^{3+}$  solutions in 60  $\mu\text{L}$  final volume into each well. Blank wells comprised of 60  $\mu\text{L}$  of 0.01 M  $\text{CaCl}_2$ . It was convenient to use the first row of the microtiter plate for the standards and the soil extractants (60  $\mu\text{L}$ ) were added to the remaining wells.

If the extractable  $\text{Al}^{3+}$  concentration was too high (absorbance exceeded highest standard), samples were diluted with deionised water. Ten  $\mu\text{L}$  of mix of hydroxylamine (0.1% w/v) plus ortho-phenanthroline (10% w/v) was added to each well followed by 10  $\mu\text{L}$  of pyrocatechol violet (0.1% w/v) and finally 120  $\mu\text{L}$  of ammonium acetate (pH 7.0, 10% w/v). After addition of each reagent, solutions were pipetted in and out to ensure adequate mixing. The use of multi-well pipettors enabled rapid addition and mixing of the various reagents. The plate was left for 30 min for colour development and absorbance was measured at 580 nm (Conyers *et al.* 1991) using the microtiter plate spectrophotometer described above.

### Colwell assay

A working standard of 2  $\mu\text{g mL}^{-1}$  (64.5  $\mu\text{M}$ ) of  $\text{NH}_4\text{H}_2\text{PO}_4$  for the Colwell P assay was prepared from a 10 mg  $\text{mL}^{-1}$  (322.6 mM) stock solution of  $\text{NH}_4\text{H}_2\text{PO}_4$  in 0.05%  $\text{HNO}_3$  (Choice Analytical Cat# 10M39-1). A P calibration curve was developed by adding 100  $\mu\text{L}$  of solutions prepared to cover the range of 1.6–29.0  $\mu\text{M}$  and included 100  $\mu\text{L}$  of  $\text{NaHCO}_3$  as the matrix common to all samples. Absorbance of the blank was corrected by subtracting the value obtained when using the extractant solution of  $\text{NaHCO}_3$ .

For soil extractants, the pH was adjusted to values between 5 and 7 by adding a required amount of 1.8 M  $\text{H}_2\text{SO}_4$ . The amount of acid to add was determined by measuring the pH values of a subset of samples. The addition of  $\text{H}_2\text{SO}_4$  was carried out using 96 well plates (Greiner Bio-One Cat#780201) in order to make it easier to take multiple sub-samples if required with multi-well pipettors for colorimetric P analysis.

A previously described method (Irving and McLaughlin 1990) was slightly modified as the basis for the assay with volumes adjusted to be suitable for a microtiter plate. Using the nomenclature of Irving and McLaughlin (1990), 40  $\mu\text{L}$  of Reagent 1 was added to all sample wells and left for 20 min. Reagent 2 (40  $\mu\text{L}$ ) was then added to all sample wells and left for a further 20 min before absorbance at 630 nm was measured using the microplate spectrophotometer described above.

Reagent 1 was prepared by adding 17.55 g  $\text{L}^{-1}$  of ammonium molybdate (Sigma-Aldrich #A7302) to 3.1 M  $\text{H}_2\text{SO}_4$  and Reagent 2 prepared by adding 3.5 g  $\text{L}^{-1}$  of polyvinyl alcohol (Sigma-Aldrich #341583, Mw 89000–98000, 99+% hydrolysed) to 0.25 g  $\text{L}^{-1}$  of malachite green

carbinol hydrochloride (Sigma-Aldrich #213020) all dissolved in DI water.

## Results

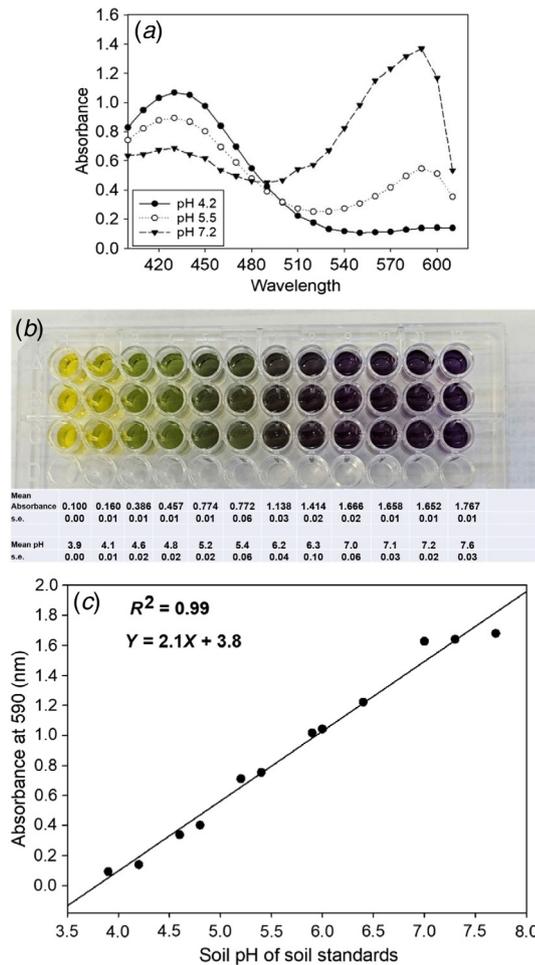
### Colorimetric assay for pH

We assessed the dye reagent from a commercially available kit describing a colorimetric assay for soil pH. The kit provides a visual estimate of pH using a soil paste and we adapted the dye reagent to develop a quantified assay to measure pH values of a soil extracted with a 0.01 M  $\text{CaCl}_2$  solution. We determined the absorbance spectrum of the dye reagent that had reacted with soil extractions over this pH range, which was of primary interest to our research. Fig. 1a shows that absorbance measured at about 590 nm gave the greatest discrimination between the various pH values of the soil extracts and we subsequently used 590 nm for routine measurements. Fig. 1b shows the colour development and absorbance at 590 nm by extracted soils that had pH values over the range of pH 4.0–7.0. Fig. 1c shows there was a strong linear relationship between pH measured with a glass electrode and the absorbance of solutions that had reacted with the dye reagent. These data indicated that the dye reagent was suitable for quantifying the pH of soil extracts over the range of pH 4.0–7.5. The data also show that the dye mix included in the commercial kit was robust in quantifying pH in a range of soils that varied in their properties.

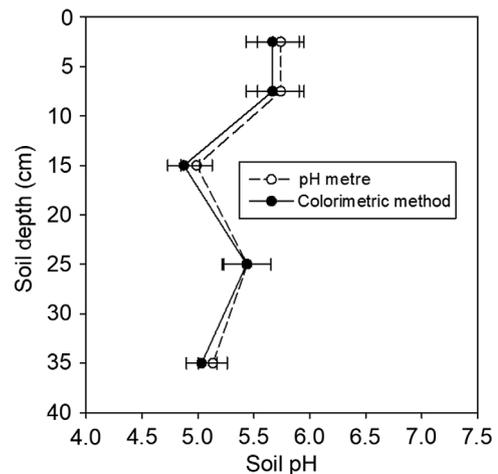
To demonstrate the utility of the method, we measured pH values of soil extracts using the colorimetric assay and compared it to pH values measured with a glass electrode. The soil samples were collected from a field site on an acid soil that had been treated with lime by incorporation to about 20 cm. Soil samples were taken down the profile to determine if the liming had increased the pH relative to soil deeper down the profile. The two methods showed the same pattern down the profile (Fig. 2) and indicated that the pH of the surface layers were increased (0–5 cm layer and 5–10 cm layer both pH  $5.7 \pm 0.17$ ;  $n = 15$  at each depth, mean  $\pm$  s.e.) compared to samples taken from the surface layers of an adjacent un-limed site (0–5 cm layer pH  $5.1 \pm 0.14$ ; 5–10 cm layer pH  $4.5 \pm 0.25$ ;  $n = 3$  at each depth, mean  $\pm$  s.e.). The pH values of the soil samples obtained with the colorimetric assay were strongly correlated with the pH values measured with a glass electrode (Fig. 3).

### Rapid assay for $\text{Al}^{3+}$

Here, we adapted an existing PCV colorimetric assay to a high throughput assay using microtiter plates and a multi-well pipettoman as described for the colorimetric pH assay. Fig. 4a shows there was a strong relationship between colour development and  $\text{Al}^{3+}$  even though the volumes of reagents



**Fig. 1.** Colorimetric assay for soil pH. (a) Absorbance spectrum of the indicator dye at three soil pH values. Three soil standards of pH values that spanned the range of interest (pH 4.2–7.2) were extracted in 0.01 M CaCl<sub>2</sub> solution (1:5 w/v; soil: solution) and assayed in microtiter plates. Soil pH was measured with a Mettler Toledo Seven Compact pH metre. An aliquot of 200 µL of soil extraction and 10 µL of indicator dye were pipetted into each well and absorbance over the range of ~420–600 nm was measured for each sample. An absorbance was selected for assay (590 nm) that gave the greatest discrimination between the different soil pH values. (b) Photograph of a microtiter plate with extracts of soil standards mixed with the pH indicator dye. Soil solutions were extracted in 0.01 M CaCl<sub>2</sub> solution (1:5 w/v; soil:solution) in triplicate. Soil pH was measured with a Mettler Toledo Seven Compact pH metre. An aliquot of 200 µL of soil extraction and 10 µL of indicator dye were pipetted into each well. The data below the plate show mean absorbance values at 590 nm and measured pH values. (c) Calibration curve for colorimetric assay of soil pH showing absorbance from the colorimetric assay plotted against pH as measured with a glass electrode. Soil extracts were combined with the indicator dye and absorbance measured at 590 nm. A series of soils (Table 1) that varied in pH were extracted with 0.01 M CaCl<sub>2</sub> solution and measured for pH with a glass electrode and the same sample assayed colorimetrically. Reproducibility of the graph was validated with number of extractions on different occasions with soil pH standards (data not shown). The equation of the line shown on the figure was used to calculate the pH from the absorbance.



**Fig. 2.** Soil pH down a profile measured with the colorimetric assay (590 nm) is consistent with measurement using a glass electrode. The field trial near Boorowa (Boorowa Agriculture Research Station, NSW; -34.46°S, 148.70°E) was sampled in 2020 after lime had been incorporated. Samples were taken at depths of 0–5, 5–10, 10–20, 20–30 and 30–40 cm. Values for each soil layer are means ( $n = 15$ ) and horizontal bars represent standard errors.

and samples for the assay had been scaled down to suit the microtiter plates.

To assess the utility of the PCV assay, we assayed soil samples obtained from two field trials established on acid soils. The distribution of Al<sup>3+</sup> across the site at various depths for both the Little Billabong ‘Warrenboo’ and Merredin sites indicated the presence of Al<sup>3+</sup> down the profiles (Fig. 5).

### Rapid assay for Colwell P

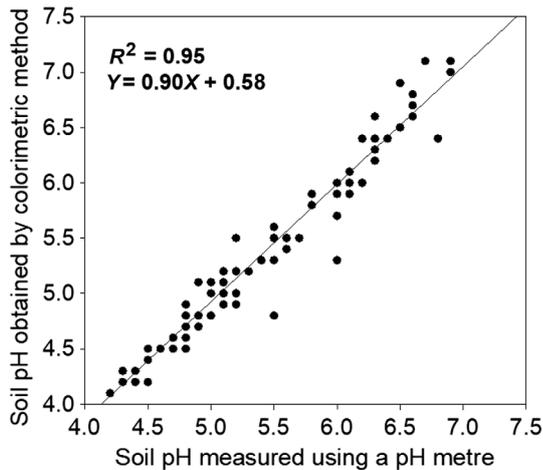
As for the PCV assay, we adapted an existing Colwell P assay for high throughput assay of soil extracts using microtiter plates and multi-well pipettors. Again, a strong correlation of absorbance at 630 nm and P was obtained with reduced volumes of reagents (Fig. 4b).

Fig. 6 shows the Colwell P distribution of soil sampled to a depth of 20 cm for two contrasting field sites.

## Discussion

### Soil pH

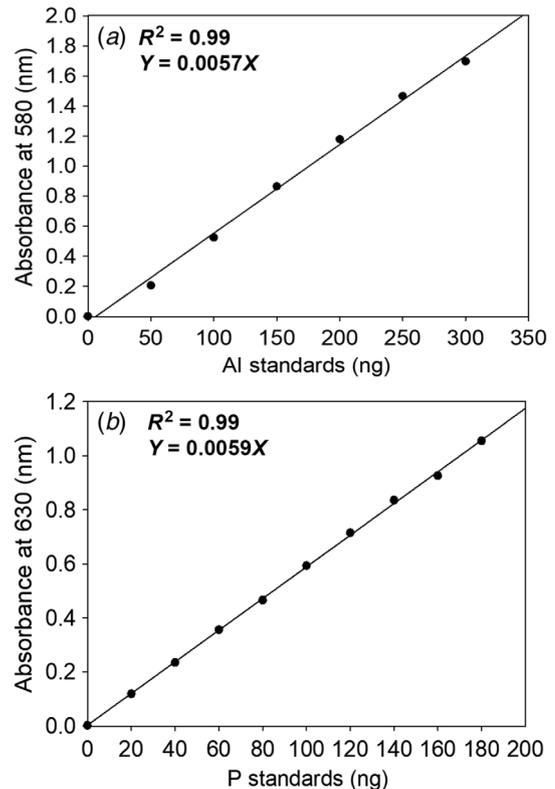
Suitable indicator dyes to measure soil pH colorimetrically should ideally absorb over a wide range of pH values and be stable regardless of soil type. The commercially available single indicator dyes typically provide good colour development over a narrow pH range for either the acid or alkaline range (data not shown). Furthermore, colour development of dyes with pH buffers can differ to the dye reactions with solutions extracted from soil even when they have similar



**Fig. 3.** The colorimetric assay (590 nm) for pH based on acid-base indicator dyes is strongly correlated with pH measured with a glass electrode. Soils were collected from a field site at the Borowra Agriculture Research Station that had been limed and an adjacent site that was un-limed to yield samples that ranged from pH 4.0 to 7.0. Each point represents a different soil sample collected from the various depths described in the legend to Fig. 2. The equation describes the relationship between the two methods and the coefficient of determination ( $R^2$ ) is given.

pH values when measured with a glass electrode. We tested several pH indicator dyes including phenol red, nitrazine yellow, chlorophenol red, bromophenol blue and bromothymol blue for their suitability. We found several problems with the use of these indicators for a colorimetric assay. Apart from being only suitable for a narrow pH range, the colour development with soil standards was inconsistent (data not shown). As discussed by Raupach and Tucker (1959), this could be due to reactions of indicator dyes with soil particles.

Raupach and Tucker (1959) surveyed a wide range of acid-base indicators to identify a combination that could be used in a simple colorimetric assay of soil pH that they termed 'soil reaction'. The assay was based on mixing a sample of soil with a reagent comprising of a mixture of acid-base indicators to yield a coloured paste. The pH was estimated by comparing the colour developed to a colour chart, which allowed the pH to be estimated. Here, we developed a quantified colorimetric assay for pH that has several advantages over the use of a pH electrode. First, the assay is considerably quicker than a pH electrode when measuring many samples. For instance, it is conceivable that hundreds to thousands of samples need to be collected from field trials that aim to assess germplasm for tolerance of acid soils. Burns *et al.* (2017) proposed that samples should be assayed for pH down a soil profile and that the resolution of the soil collections in some instances require the collection of samples at 2.5 cm depth increments. Collection of samples throughout the trial in a 2Dl pattern

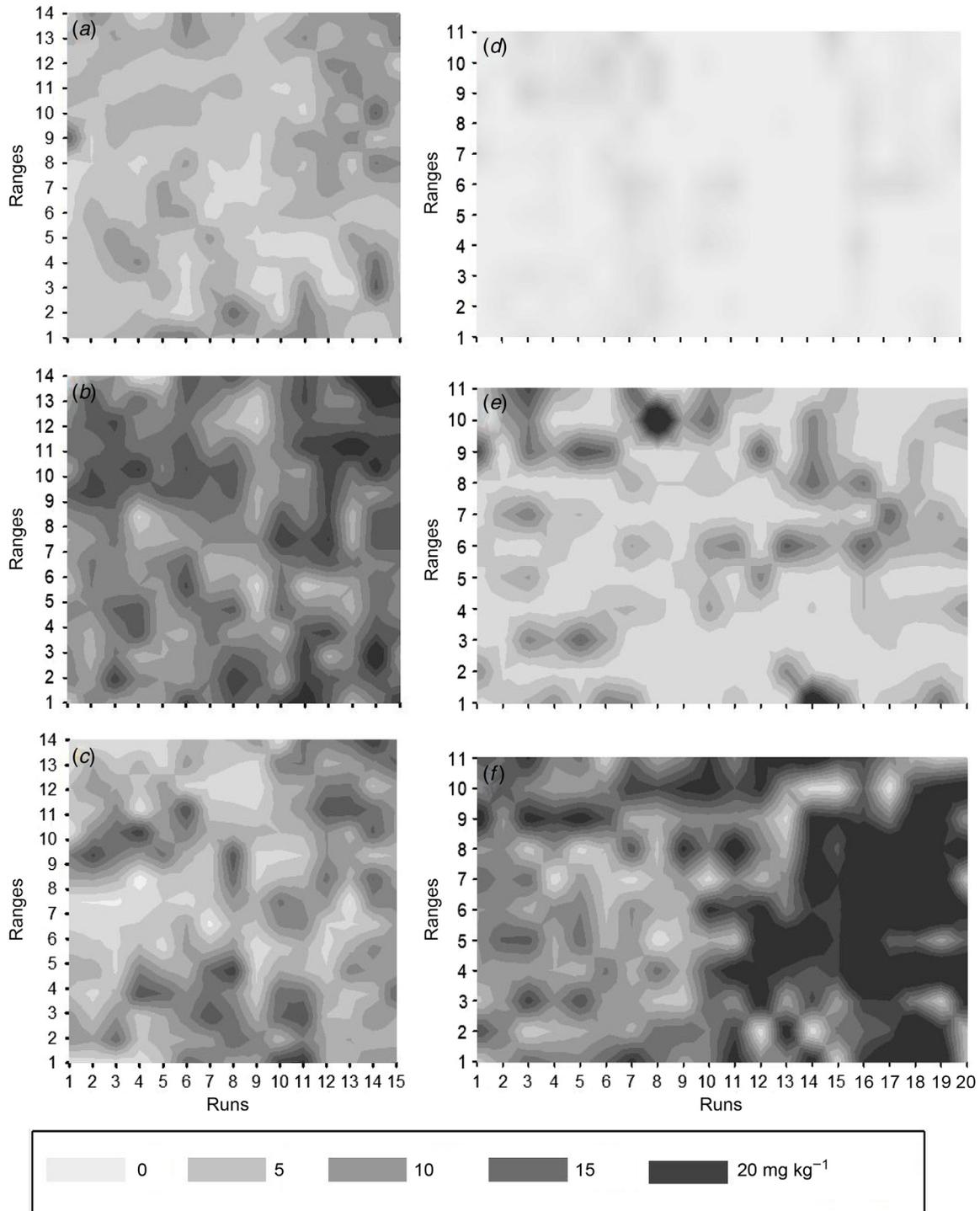


**Fig. 4.** Calibration curves for extractable Al ( $Al^{3+}$ ) using the PCV method (a) and for Colwell P (b) using methods based on microtiter plates. The absorbance was measured at 580 nm for  $Al^{3+}$  (a) and 630 nm for Colwell P (b). The equations describe the linear relationships for each method and the coefficients of determination ( $R^2$ ) are given.

including sampling at variable depths can quickly generate large numbers. For instance, using an accurate pH probe it took 3–10 min for the pH to stabilise at each reading, which becomes a limiting factor when measuring many samples. By contrast, there is no such delay using the colorimetric assay, which based on the strong linear correlation shown in Fig. 3, has a similar level of accuracy as the pH electrode. Although soil collection, drying and preparation is unchanged with this method, time for the preparation of soil can also be curtailed by reducing the sample volumes.

### Soil $Al^{3+}$

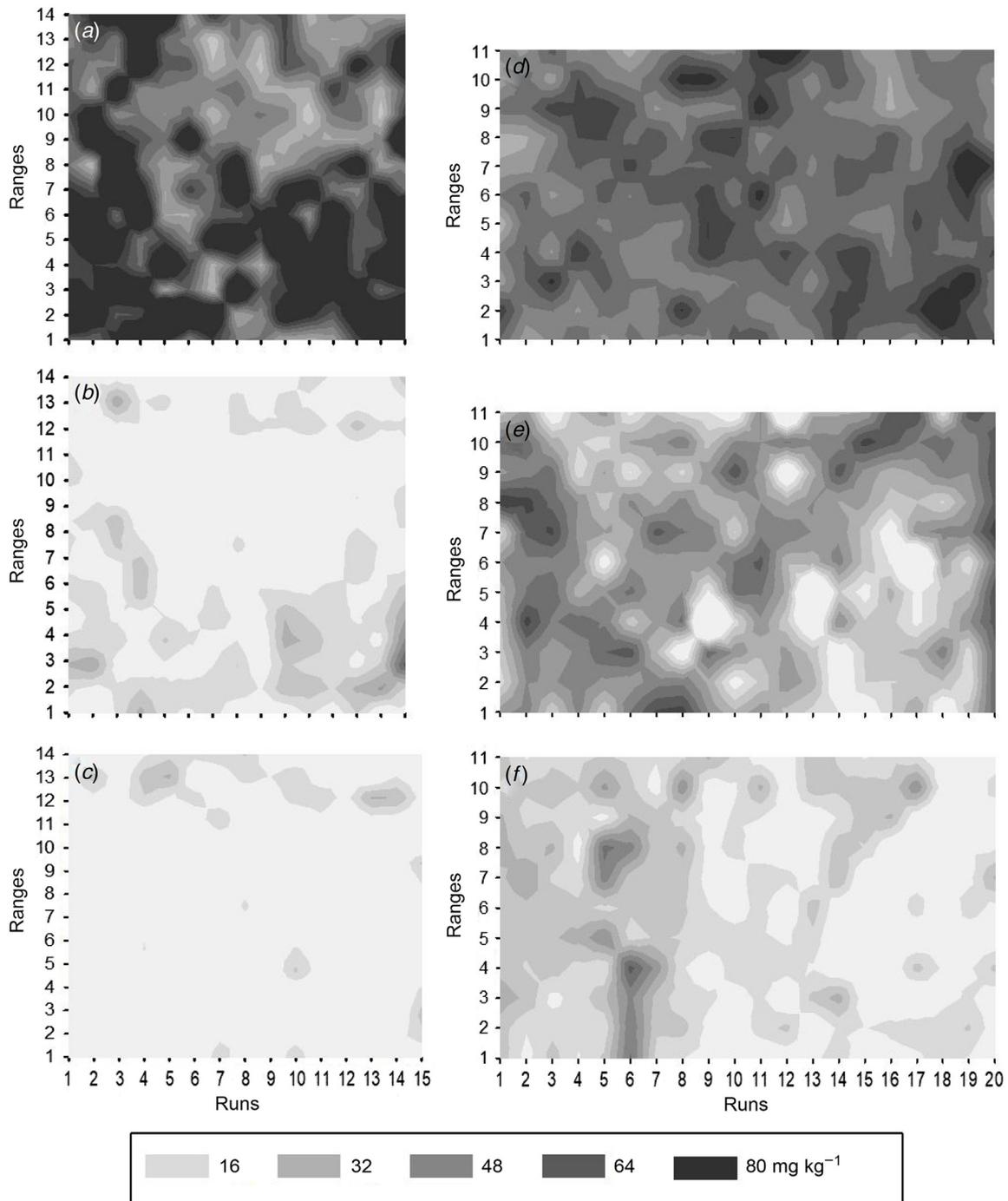
The activity of  $Al^{3+}$  increases when the soil pH drops below 5 (Parfitt *et al.* 1995). Soluble Al as  $Al^{3+}$  is the major limiting factor for crop and pasture production on acid soils (Dolling *et al.* 1991; Tang *et al.* 2002; Anderson and Bell 2019). This contrasts with plant species such as legumes that are sensitive to low pH and can be limited at soil pH values where  $Al^{3+}$  would be absent (Burns *et al.* 2017). Extractable monomeric Al, largely as the  $Al^{3+}$  ion in soil solution, is a good indicator of likely Al toxicity in plants grown on acid



**Fig. 5.** Distribution of  $\text{CaCl}_2$  extractable  $\text{Al}^{3+}$  ( $\text{mg kg}^{-1}$ ) in different soil depths at the 'Warrenboo' site in 2017 (a, 0–5 cm, b: 5–10 cm and c: 10–20 cm) and at the Merredin site in 2018 (d, 0–5 cm, e: 5–10 cm and f: 10–20 cm). Plots are represented on a rectangular drawing with runs denoting the designation of plots from left to right and ranges the designation of plots from the bottom to the top. This allowed any plot to be identified by its unique run and range location. The trials had 210 ( $15 \times 14$ ) and 220 ( $20 \times 11$ ) plots in 'Warrenboo' and Merredin, respectively. Grey intensity indicates the concentration of  $\text{Al}^{3+}$  as shown in the grey scale representing ranges of values of 0, 1–5, 6–10, 11–15 and 16–20 as  $\text{mg kg}^{-1}$  soil.

soil (Pavan *et al.* 1982; Kerven *et al.* 1989; Conyers *et al.* 1991). Although pH can be a good indicator of acid soils, it does not always correlate with the concentration of  $\text{Al}^{3+}$  in

soil solution or crop yield (Conyers and Poile 2018; Anderson and Bell 2019). Furthermore, it is important to know if toxic  $\text{Al}^{3+}$  is present at soil pH values that are near



**Fig. 6.** Distribution of Colwell P ( $\text{mg kg}^{-1}$ ) at various soil depths for 'Warrenboo' in 2017 (a, 0–5 cm; b, 5–10 cm; and c, 10–20 cm) and for Merredin in 2018 (d, 0–5 cm; e, 5–10 cm; and f, 10–20 cm). Plots are represented on a rectangular drawing with runs denoting the designation of plots from left to right and ranges the designation of plots from the bottom to the top. This allowed any plot to be identified by its unique run and range location. The trials had 210 ( $15 \times 14$ ) and 220 ( $20 \times 11$ ) plots in 'Warrenboo' and Merredin, respectively. Grey intensity indicates concentrations of Colwell P as shown in the grey scale and they represent ranges of values of 0–16, 17–32, 33–48, 49–64 and 65–80 as  $\text{mg P kg}^{-1}$  soil.

the limit where Al is solubilised by the acidity. For these reasons, it is valuable to couple  $\text{Al}^{3+}$  with pH assays when surveying field sites.

We modified a pre-existing PCV assay for soluble  $\text{Al}^{3+}$  (Conyers et al. 1991) and showed that it could be successfully used to profile two contrasting acid soils. The peak  $\text{Al}^{3+}$  concentration for 'Warrenboo' occurred in the 5–10 cm layer (Fig. 5a–c) whereas for Merredin, the peak  $\text{Al}^{3+}$  concentration occurred in the 10–20 cm layer (Fig. 5d–f). These findings are consistent with the Merredin site being a deep acid sand (Scanlan et al. 2017) whereas Little Billabong 'Warrenboo' typifies sites that have an  $\text{Al}^{3+}$ -toxic acid 'throttle' closer to the surface with pH increasing as the depth increases. Both pH and  $\text{Al}^{3+}$  could be assayed in the same  $\text{CaCl}_2$  extract enabling sites to be rapidly profiled for both parameters.

## Colwell P

Colwell P values provide a good estimate of the P status of soil (Bolland et al. 1995). Studies have shown that there can be a linear relationship of soil Colwell P with plant leaf tissue P concentration of the crop (Kirchhof et al. 2008). When screening germplasm for phosphorus use efficiency (PUE), there is a need to measure the soil P status of every plot and at multiple depths. The 'Warrenboo' site (Fig. 6a–c) shows a strong stratification of P down the profile with the majority of the P located in the surface soil (0–5 cm). This pattern is typical of many soils where the P remains near the surface unless it is incorporated and is a consequence of the tight binding of phosphate to positively charged soil particles. By contrast, although the P down the profile of the Merredin site was also stratified, a larger proportion leached down the profile as would be expected for a sandy soil (Fig. 6e, f).

## Concluding remarks

Here, we describe high throughput assays for soil pH,  $\text{Al}^{3+}$  and Colwell P using microtiter plates and multi-well pipettors. We developed a quantified colorimetric assay for pH, adapted existing methods for monomeric  $\text{Al}^{3+}$  and Colwell P and showed the utility of the assays in characterising field sites. The  $\text{Al}^{3+}$  and pH assays have the advantage of using the same soil extract, which is valuable for assessing field trials aimed at screening germplasm for tolerance of acid soil. Extensive soil sampling is required if researchers aim to use pH and  $\text{Al}^{3+}$  concentrations as covariates when analysing data based on plant productivity.

Foregoing soil pH electrodes means this methodology is high throughput, and comparatively accessible. However, microplate readers are expensive equipment and are also likely to be inaccessible to some laboratories. Fortunately, there has been progress in the development of 'open source'

microplate readers, which can be constructed for a tenth of the cost of commercially available units (Szymula et al. 2019).

Although we described high throughput methods, the assays can be adapted for single-sample assays where a microplate reader is not available by adjusting reagent and sample volumes to suit different size cuvettes. The reagents for the various assays are relatively cheap and the small volumes of the assays further reduces costs. The small volume of dye reagent used in the pH assay enables many thousands of samples to be assayed from a single kit. Using these colorimetric assays, it is feasible to scale down the required soil sampled to 1 g or less if adequate mixing of a larger sample is undertaken to ensure that the subsample is representative of the total.

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**Data availability.** The data that support this study will be shared upon reasonable request to the corresponding author.

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