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Factors affecting the measurement of soil pH buffer capacity - approaches to optimise the methods

Running title: Method to measure soil pH buffer capacity

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Summary

An accurate measurement of soil pH buffer capacity (pHBC) is essential to estimate lime requirement and predict changes in soil pH due to acid addition from farming practices. The acid-base titration using a 1:5 soil solution ratio has been commonly used to determine pHBC. However, no standardized equilibration time, type of acid and alkali, or concentration of electrolyte has been recommended. This study aimed to establish a standard procedure which is relatively method-independent and reflects the actual soil pH buffering ability. Nineteen soils with a wide range of pH, clay and organic carbon contents were collected. Soil pHBC increased with increasing ionic strength of the suspension solutions for most soils. Measuring pHBC in a 0.01 M $CaCl_2$ suspension minimized the effects of both ionic strength and cation valence following addition of different types and rates of alkalis and acids. The time required to reach near equilibrium varied from 1 hour to more than 28 days. Inclusion of sonication pre-treatment greatly expedited the pHBC determination. For soils with high carbon content, biological reactions alone could elevate soil pHBC and addition of biocides was essential during pHBC measurement. When compared with two commonly used methods: 1:1 soil:water extraction and field moist incubation, the 1:5 $CaCl_2$ method was less affected by ionic strength and biological activity, and more suitable for use as a standard method. Based on the results, we recommend using a 1:5 soil to 0.01 M $CaCl_2$ solution ratio, with addition of HCl and NaOH or $Ca(OH)_2$, and chloroform, followed by sonication.

Keywords: acidification, lime requirement, neutralizing capacity, organic matter

Introduction

Soil pH buffer capacity (pHBC) is an intrinsic soil property that quantifies the ability of a soil to resist a change in soil pH. It is usually defined as the amount of acid or base required to change the pH of the soil suspension by one unit. An accurate measurement of soil pHBC is essential when it is used to estimate the lime requirement to return acid soils to a desired pH and to predict changes in pH associated with the net acid addition rate of farming practices. Soil pH buffer capacity can be measured directly or predicted through the use of pedotransfer functions. Direct measurement methodologies include incubation and titration methods (Aiken *et al.,* 1990; Dunn, 1943). Pedotransfer functions have been based on correlations between pHBC and other soil properties such as organic matter and clay contents (Nelson, 2010; Noble *et al.,* 1997; Xu *et al.,* 2012).

The main reactions that buffer soil pH include ion exchange, protonation and deprotonation of weak acids, and dissolution and precipitation reactions (Conyers, 2000; Helyar *et al.,* 1995). Organic anions, negatively-charged clay minerals and Al hydroxyl ions are commonly recognized as important sinks for applied H^+ (Bloom, 1999). At pH<5, when Al hydrolysis becomes less significant, exchange of H^+ and Al^{3+} with base cations on permanent charge sites in silicate clays is an important mechanism for soil pH buffering (Aiken *et al*., 1990; Bloom, 1999). Under very acid conditions (pH<4.5), buffering of soil pH upon addition of acid increases due to dissolution of primary minerals, oxides or release of organic bound Al^{3+} by H^+ (Bloom, 1999; Lesturgez *et al.,* 2006). On the other hand, exchangeable Al^{3+}, Al-hydroxyl cations and H^+ ions on permanent charge sites of 2:1 clay minerals, non-exchangeable Al^{3+} and H^+ ions that are tightly bound by organic matter, H^+ associated with variable charge sites such as the edges of aluminosilicate clay minerals or Fe and Al oxides, provide the main pH buffering against increases in soil pH in acid soils (Bloom, 1999). At soil pH above 7, the precipitation and dissolution of $CaCO_3$ are the main reactions affecting the soil pHBC (Singh *et al.,* 2003).

Worldwide, methods developed for soil pHBC determination have focused on measuring the soil pH buffering to added alkalis to calculate lime requirement. For instance, the incubation of the soil with either $Ca(OH)_2$ or $CaCO_3$ at field capacity for several months has been considered as a reliable method to estimate the lime requirement of an acid soil (Barrow & Cox, 1990; Loynachan, 1981). However, this method is laborious and time-consuming, and considered impractical for routine soil test. The direct titration technique which involves incubating soil with dilute alkalis such as 0.022 M $Ca(OH)_2$ at 1:1 soil solution ratio for up to 4 days has been used as a reference method (Liu *et al.,* 2004; Tunney *et al.,* 2010). A modification of this method was to add alkali to a 1:5 soil:water suspension to facilitate the soil solution mixing and pH measurement (Aitken *et al.,* 1994; Dolling & Porter, 1994). In the USA, buffer solutions are popular methods for estimating lime requirement, since they require only minutes to react with soil acidity. With the increasing demand for a simple method, a single addition of 0.022 M $Ca(OH)_2$ with 30-min equilibration time has been developed (Liu *et al.,* 2005). Nevertheless, the short equilibrium time is likely to result in incomplete reactions and underestimate the soil pHBC (Thompston *et al.,* 2010).

The acid-base titration using a 1:5 soil to solution ratio has been commonly used to determine the pHBC in Australia (Aitken *et al.,* 1994; Dolling & Porter, 1994; Nelson

& Su, 2010). Nevertheless, no standardized equilibration time, type of acid and alkali, or concentration of electrolyte has been recommended. For example, studies have used HCl and NaOH or H_2SO_4 and $Ca(OH)_2$ as acid and alkali (Nelson & Su, 2010; Ritchie & Dolling, 1985), equilibration times from 1 hour to several days and suspension solutions including water and 0.002 to 0.01 M $CaCl_2$ (Aitken *et al.,* 1994; Dolling & Porter, 1994; Nelson & Su, 2010; Ritchie & Dolling, 1985). Soil pH buffering reactions such as dissolution/precipitation and protonation/deprotonation could be affected by suspension valence, ionic strength and equilibrium time (Aitken & Moody, 1994; Bloom & Erich, 1987), which means that determination of soil pHBC would also depend on these factors. Additionally, biological reactions that may occur during the incubation could influence soil pH, ionic strength and pH buffering reactions (Marschner, 2000; Liu *et al.,* 2008). Incorporation of microbiocides to minimize or inhibit biological process has been arbitrarily and inconsistently used among studies (Aitken *et al.,* 1994; Dolling & Porter, 1994; Nelson & Su, 2010).

This study aimed to evaluate effects of acid and base type, ionic strength, equilibrium time and biological control on soil pHBC measurement based on 1:5 soil: $CaCl_2$ (0.01 M) extraction method (1:5 $CaCl_2$). An attempt was made to establish a method with a specified equilibrium time, type of acid or alkali and ionic strength, which could provide a pHBC value close to the "true value". The validation of the 1:5 $CaCl_2$ method was carried out by comparison with the two commonly used methods: field moisture incubation (FMI) and 1:1 soil:water extraction (1:1 W) (Barrow & Cox, 1990; Liu *et al.,* 2004; Tunney *et al.,* 2010). In addition, the effect of sonication was evaluated to assess whether its inclusion could reduce the equilibrium time required to complete pHBC measurements.

Materials and Methods

Nineteen soils were used in this study. These soils were collected from the 0-10, 10-30 or 30-60 cm layers of ten soil types located in Victoria and New South Wales, Australia. These soils represented a wide range of soil properties in terms of pH, clay and organic carbon (C) content (Table 1). All soils were air-dried and sieved to less than 2 mm prior to analysis. Initial soil pH was measured in 0.01 M $CaCl_2$ (1:5 soil solution ratio, 1 hour end-over-end shaking). Total soil organic C was determined by dry combustion using a CHNS Analyser (PerkinElmer EA2400). Prior to organic C analysis, all soils were ball-milled and tested for the presence of $CaCO_3$. Only Vertosol showed visible effervescence in a fizz test and was pre-treated with H_2SO_3 to remove the inorganic C before organic C analysis (Schmidt *et al.,* 2012). Particle size analysis was conducted by Laser Particle Size Analyser (Malvern Mastersizer 2000, Worcestershire, UK). After size separation, clay mineral types were identified by X-ray powder diffraction (Siemens Kristalloflex Mod, Germany) and dominant clay types were assigned using Diffrac.EVA software (Bruker, Madison WI). The relative percentage of major clay mineral types was calculated according to their relative peak intensity. Selected properties of each soil are given in Table 1.

Generalized 1:5 soil:$CaCl_2$ (0.01 M) extraction method (1:5 $CaCl_2$)

For all soils, incremental amounts of standardised 0.022 M $Ca(OH)_2$ (saturated) and 0.02 M H_2SO_4 were added to 4 g air-dried soils. Appropriate amounts of 0.01 M $CaCl_2$ were added to make up to the final volume of 20 ml. To each vial, 0.25 ml of chloroform was added to minimize the microbial activity. All suspensions were placed on an end-over-end tumbler at 25˚C and left to react for 1 h. The soil

suspension was resuspended everyday by shaking for 5 min. The pH of the suspension was measured after 7 and 14 days following centrifugation at 2000 rpm (Clements Orbital 460) (700 *g*) for 10 min. A linear regression function was fitted between soil pH (Y-axis) and the amount of H^+ or OH^- added (X-axis). The soil pHBC was determined using a pH range of approximate ± 0.5 units of the original soil pH. It was calculated as the reciprocal of the slope of the linear portion of the buffer curve for all the experiments.

Effect of equilibrium time, valence, ionic strength and biological reaction

The effect of equilibrium time on pHBC was evaluated for all 19 soils by using equilibrium times of 1 hour, and 1, 7, 14 and 28 days.

The effect of valence on soil pHBC determination was quantified for 10 soils collected from the 0-10 cm layer. $Ca(OH)_2$ and H_2SO_4 were used, respectively, as alkali and acid with divalent counter ions, whereas those with monovalent counter ions were NaOH and HCl.. Briefly, 0, 0.25, 0.5, 1, 2, 4 and 8 ml of 0.022 M $Ca(OH)_2$ and 0.02 M H_2SO_4 or 0.04 M NaOH and 0.04 M HCl were added to 4 g soil suspended in 0.01 M $CaCl_2$ to establish a pH buffer curve for each soil. The pH of soil suspension was measured after 7 days of reaction time.

The effect of ionic strength on soil pHBC was tested on five soils (Soils 1, 3, 5, 6 and 8; Table 1) with variable initial pH and texture. Four ionic strengths of 0, 0.03, 0.3 and 1.5 M were achieved by using water, 0.01 M $CaCl_2$, 0.3 M KCl (or 0.1 M $CaCl_2$) and 1.5 M KCl (or 0.5 M $CaCl_2$) as suspension solutions. Soil pH buffer curves were established by adding 0- 4 ml of 0.022 M $Ca(OH)_2$ and 0.02 M H_2SO_4 (or 0.04 M HCl), respectively, to suspension solutions. The purpose of using different combinations of suspension solution and acid was to prevent $CaSO_4$ precipitates and to avoid the confounding effect of cation valence on ionic strength .

To test the effect of biological reaction on soil pHBC, Sodosol with initial pH of 4.6, 5.6 and 6.8 were sampled from a 30-year lime trial at the University farm. A finely ground commercial compost was mixed with soil to produce the final C contents of 15, 45 and 105 g kg^{-1}. Three treatments without or with chloroform and toluene, respectively, were used to examine the influence of the biological control on pHBC. The pH was measured after 1 and 7 days of reaction time. The soil extracts at day 7 were also analysed for NH_4^+ and NO_3^- using a QuickChem 8500 Flow Injection Analyser (Lachat Instruments, Loveland, CO, USA). Overall, the experiment consisted of a full factorial design with 3 soils × 3 C contents × 3 biological control treatments and 2 replicates.

Testing mineral dissolution

A back titration procedure was adapted to test the possible occurrence of mineral or $CaCO_3$ dissolution during pHBC measurement. Reactions such as dissolution and precipitation might exhibit a hysteresis loop due to the fact that the addition of acid or alkali to soil could be rapidly consumed and would not be easily recovered following the addition of alkali or acid.

Four soils (Soils 2, 4, 5 and 7; Table 1) with nonlinear pH buffer curve at a pH range of ± 0.5 units of the original soil pH were selected for this purpose. A Sodosol with a linear titration curve was selected for a comparison. The back titration involved

adding excess amounts of acid and alkali to the soil suspension, and then titrating back with known amounts of alkali and acid, respectively. Briefly, for each soil, 4 ml of standardized 0.02 M H_2SO_4 were added to one set of 4 soil suspensions (0.01 M $CaCl_2$), while 4 ml of 0.022 M $Ca(OH)_2$ were added to another set. After a 1-h reaction time, 0, 2, 3 and 4 ml of 0.022 M $Ca(OH)_2$, and 4, 3, 2 and 0 ml of 0.02 M H_2SO_4 were added to the 8 soil suspensions, respectively. The suspension was then left to further react for 1 day before pH measurement. The standard titration using 0-4 ml of 0.02 M H_2SO_4 and 0.022 M $Ca(OH)_2$ to another set of soil suspensions, was performed for comparison.

Sonication treatment

Sonication at increasing levels of energy was tested for its ability to expedite pHBC determination. Five soil types (Soils 2, 4, 7, 9 and 15; Table 1) were selected for their high contents of clay, Fe oxides or $CaCO_3$. Briefly, acid, alkali and chloroform were added to the soil suspensions which were shaken for 1 h, and then sonicated at energy levels of 0-142 J g^{-1} soil solution for 3 min using a probe type Branson Sonifer 250 (Branson Sonic Power, Danbury, CT, USA). The pHBC was determined immediately following centrifugation at 2000 rpm, after 48 and 96 h of reaction time.

Comparison with two commonly used methods

Field moist incubation (FMI) (Aitken *et al.,* 1990)

For Ferrosol (Soils 1 and 2), Dermosol (Soils 3 and 4) and Organosol (Soil 5), four rates of $Ca(OH)_2$ powder were mixed in water and then added to 80 g soil as suspension. For other soils with relatively low pHBC, four incremental amounts of 0.022 M $Ca(OH)_2$ and 0.02 M H_2SO_4 were added to 80 g soil, respectively. All soils were incubated at field capacity at 25°C for 100 d. At the completion of the incubation, the soils were dried at 40°C, sieved through < 2 mm and measured for pH (1:5 0.01 M $CaCl_2$).

1:1 soil slurry (water) incubation (1:1 W) (Dunn, 1943)

Thirty grams of each of the 19 soils was weighed into 120 ml polypropylene vials. Water was added together with incremental amounts of 0.022 M $Ca(OH)_2$ and 0.02 M H_2SO_4 to make a final volume of 30 ml. Chloroform (0.25 ml) was added to minimize microbial activity. After thoroughly mixing, the soil slurry was covered with Parafilm to reduce evaporation. A 10-mm long slit was cut through the film for air exchange. A glass stirring rod was inserted through the opening for mixing the soil twice daily. The soil samples were incubated at 25°C for 4 days. Soil suspension pH was then measured while being stirred.

Statistical analysis

The effect of biological reactions on soil pHBC was subjected to a two-way ANOVA, followed by the multiple comparisons with the least significant difference by using Genstat (11th version) for windows. One-way ANOVA was used to test the effect of reaction time and differences among the methods. Single and multiple-linear regression analyses were used to determine the relationships between soil properties and soil pHBC. Using the stepwise selection routine, only variables with significant contribution were included into the regression equations.

Results

Soil properties

Soil pH ranged from 3.8 to 7.1 (Table 1). Clay and total organic C content varied greatly from 2 to 57% and 2 to 69 g kg^{-1}, respectively. Illite was the dominant clay mineral in all the soils except the Ferrosol and Dermosol which contained higher amounts of Vermiculite than Illite (Table 1). Most soils had a mixed clay mineralogy consisting of Illite and Kaolinite.

Soil pHBC measured by 1:5 soil $CaCl_2$ (0.01 M) extraction method

The soil pHBC varied from less than 20 $mmol_c\ kg^{-1}\ pH^{-1}$ for Podosol and Tenosol to more than 100 $mmol_c\ kg^{-1}\ pH^{-1}$ for Ferrosol and Dermosol (Table 2). Notably, the pHBC of Ferrosol, Organosol and Vertosol was higher when the acid than when the alkali was added (Figure 1). Correspondingly, during the back-titration, the soil solution pH was not reversibly recovered following alkali addition for all three soils (Figure 1).

The single regression model revealed that pHBC correlated with soil organic C content ($R^2 = 0.51$, $P < 0.001$), the proportion of vermiculite ($R^2 = 0.79$, $P < 0.001$) and clay content ($R^2 = 0.24$, $P < 0.05$). The multiple linear regression model with total organic C content, proportion of vermiculite and clay content as independent variables explained nearly 90% of the variation in soil pHBC (y, $mmol_c\ kg^{-1}\ pH^{-1}$):

$$y = 0.34 + 0.852 \text{ organic C } (g\ kg^{-1}) + 1.15 \text{ Vermiculite\%} + 0.82 \text{ Clay\%}.$$

Based on the model fitted, organic C described 51% of variation in soil pHBC, while proportion of vermiculite and clay content explained additional 32% and 7%, respectively.

Factors affecting soil pHBC determination

Valence

The pHBC determined in 0.01 M $CaCl_2$ using $Ca(OH)_2$ and H_2SO_4 did not differ from that using NaOH and HCl for most soils when pHBC was less than 60 $mmol_c\ kg^{-1}\ pH^{-1}$ (Figure 2). However, the pHBC measured using $Ca(OH)_2$ and H_2SO_4 was higher than the pHBC using NaOH and HCl for soils with higher pHBC (Figure 2). Notably, if soil pHBC was determined by the addition of alkalis only, $Ca(OH)_2$ gave higher value than NaOH for all soils at OH^- addition rates of more than 20 $mmol_c\ kg^{-1}$ (Figure 3). This range was normally not included in the calculation of pHBC for soils with low pHBC. On the other hand, the type of acid had less impact on the pHBC except for the Ferrosol which showed a higher pHBC determined by H_2SO_4 than HCl (Figure 3).

Ionic strength

Regardless of types of suspension solution and acid, pHBC increased with increasing ionic strength from 0 to 0.3 M with the exception of Sodosol (Figure 4). Moreover, the increase in pHBC due to increased ionic strength mainly occurred where acid was added (data not shown).

Equilibrium time and sonication effect

The end point was defined as the time when further reaction time had little influence on soil pHBC. For most soils, pHBC reached a near equilibrium state within 14 days of reaction time (Table 2). However, the Podosol with less than 5% clay reached the equilibrium point within one day. In contrast, it took 28 days or longer for the Ferrosol, Dermosol and Vertosol to achieve equilibrium (Table 2).

Sonication with increasing energy greatly accelerated pH buffering reactions. Where the highest sonication energy was used, the Sodosol and Calcarosol reached the equilibrium point within one hour, compared with 48 hours without sonication (Figure 5). The pHBC of Ferrosol measured after 96 hours, at the highest sonication energy, was very close to that measured at 28 days without sonication. .

Biological control

There was no difference between the effect of chloroform and toluene on soil pHBC, so only the results of the chloroform treatments are presented (Table 3). Soil pHBC was greatly affected by the presence of biocides for soils with relatively higher initial pH (>5.6) or higher organic C (>45 g kg^{-1}). For example, at Day 7, the addition of chloroform did not affect pHBC of soils with 15 g C kg^{-1} and initial pH below 6, but decreased pHBC by 8-14% in soils with 105 g C kg^{-1} regardless of initial soil pH.

In the absence of biocide, suspension pH remained constant during the incubation when C content was 15 g kg^{-1} (Figure 6). However, soil suspension pH increased by an average of 0.35 pH units from the initial soil pH when C content was 105 g kg^{-1} (Figure 6). Moreover, increases in soil pH with time due to biological reactions were not the same at different rates of acid and alkali addition. For example, soil suspension pH increased by 0.40 units at the highest rates of acid addition but only by 0.31 units at the highest rates of alkali application (Figure 6). Ammonification and nitrification are normally considered as the primary H^+-consuming and H^+-producing biological reactions that would affect soil pH during incubation. Correspondingly, the final NH_4^+ concentration in the soil suspension decreased gradually from 32 to 25 mg N kg^{-1} soil from acid addition to alkali addition (data not shown).

Comparison between methods

Although highly correlated, the pHBC determined by the 1:1 W method was 18-68% lower than that using the 1:5 $CaCl_2$ method (Figure 7). The extreme cases were for Ferrosol, Dermosol and Vertosol, where pHBC measured by 1:1 W was over 60% lower than those measured using the 1:5 $CaCl_2$ method.

Fifteen out of 19 soils conformed to a strong 1:1 linear relationship between the 28-day 1:5 $CaCl_2$ and 100-day FMI methods (R^2=0.99). The exceptions were soils with the highest organic C contents and free $CaCO_3$ (Figure 7). All these soils demonstrated slightly higher pHBC values with the FMI than the 1:5 $CaCl_2$ method (Figure 7).

Discussion

This study demonstrated that factors such as suspension valence, ionic strength, reaction time and biological processes greatly affected the determination of soil pHBC. Also, these impacts on pHBC determination varied among the soils. The major soil properties affecting soil pHBC included organic C, clay contents and type of clay minerals. When compared with two commonly used methods (1:1 W and FMI), the 1:5 $CaCl_2$ method appeared to be more reliable for the pHBC determination. An improved method which can be used as a reference and routine method for future pHBC determination is recommended.

Factors affecting soil pHBC measurement

Valence and ionic strength

Only alkali type showed an apparent effect on soil pHBC determination, with divalent cations giving higher pHBC than monovalent. This was possibly attributed to: 1) the stronger affinity of divalent Ca^{2+} than monovalent Na^{+} for binding sites and effective displacement of H^{+} and Al^{3+} from the negatively-charged surfaces; and/or 2) higher surface charge density at the variable charge site in the presence of divalent Ca^{2+} than monovalent Na^{+} (Singh and Uehara 1986). While clay minerals and organic matter of most soils were negatively charged, anion valence had little impact on soil pHBC in our study. With the only exception of Ferrosol, specific adsorption of SO_4^{2-} onto the surface of Fe or Al oxides possibly increased soil pH buffering in response to the applied acids (Aitken & Moody 1994; Bloom & Erich 1987). Thus, using HCl rather than H_2SO_4 as the acid type could avoid undesired effects of specific adsorption or precipitation of SO_4^{2-} with soil minerals. On the other hand, if pHBC was determined to estimate lime requirement for ameliorating soil acidity, $Ca(OH)_2$ should be used over NaOH.

Using 0.01 M $CaCl_2$ as the suspension solution instead of water reduced the effect of cation valence of alkali on soil pHBC determination. In this study, little difference was detected between $pHBC_{Ca(OH)_2}$ and $pHBC_{NaOH}$ when the addition rates of $Ca(OH)_2$ were less than 10 $mmol_c\ kg^{-1}$. Aitken & Moody (1994) found that $pHBC_{Ca(OH)_2}$ was 1.3 to 7.5 times higher than $pHBC_{NaOH}$ for 92 out of 100 soils, when water was used as the suspension solution. This indicates that pHBC of most soils would be affected by the alkali valence when soils were suspended in a more diluted salt solution or water. On the other hand, using 0.01 M $CaCl_2$ as the suspension solution notably minimized the impact of increased ionic strength on pHBC, due to incremental additions of alkali or acid. In water suspensions, soil pHBC was underestimated in the acid direction while overestimated in the alkaline direction, mainly attributed to the acute pH decrease from increased ionic strength following addition of acid and alkali. In most cases, the relationship between soil pH and added H^{+} / OH^{-} in water suspension was not linear. Therefore, when determining soil pHBC using the titration method, water or more dilute salt solution than 0.01 M $CaCl_2$ is not recommended as the suspension solution.

Soil pHBC increased with increasing ionic strength of the suspension solutions but the responses differed between the soils. The different responses may be explained by the different pH buffering mechanisms. Soil pH buffering reactions such as dissolution/precipitation and protonation/deprotonation are highly dependent on ionic strength of the soil solution (Aitken & Moody, 1994). Several studies showed that the dissolution rate of clay minerals increased with increasing ionic strength (Aitken & Moody, 1994; Bloom & Erick, 1987). Thus, the pHBC of Ferrosol, Dermosol and Vertosol was expected to increase with increasing ionic strength due to the apparent pH buffering from mineral dissolution. In addition, for soils containing variable charge sites, such as Ferrosol and Organosol characterized with high FeO_x and organic matter, respectively, increased ionic strength in the soil suspension could increase charge density, and consequently induced higher pHBC (Aitken & Moody, 1994). On the contrary, Sodosol that was dominated by permanent charge sites of illite and showed no mineral dissolution due to acid addition, would be less affected by the ionic strength of the soil solution.

Ideally, soil pHBC measurement should be relatively method-independent and reflect the actual soil pH buffer ability under field conditions. Efforts to find a standard and

universal suspension solution for soil pHBC measurement need to consider at least two factors. Firstly, the ionic strength of suspension solution should simulate the field condition. Secondly, any variation in valence or ionic strength due to addition of different types and rates of acid and alkali should be minimized by the suspension solution adopted. Although soil suspended in 0.002 M $CaCl_2$ could represent the ionic strength in soil solution of many soils (Aitken *et al.,* 1994; Bruce *et al.,* 1989), it could be easily modified by the addition of acids and alkalis. Using 0.01 M $CaCl_2$ appeared to provide the reduced effects of both alkali valence and ionic strength on the pHBC measurement. Moreover, it is commonly used for soil pH measurement.

Biological control

In this study, biological reactions were thought to be responsible for the higher soil pH and pHBC in the absence than the presence of biocides. For example, increased soil pH could be attributed to H^+-consuming reactions like ammonification and decarboxylation of organic compounds (Rukshana et al. 2014; Yan et al. 1996). Rapid ammonification and decarboxylation were observed following rewetting of air-dried soil with high level of organic C (Conyers *et al.,* 2000). Greater increases in soil pH in the absence of biocide could also be explained by the reduction of Fe or Mn minerals under anaerobic incubation conditions (Ponnamperuma, 1972), but this should be less prominent due to the periodic shaking of soil suspension during incubation. In the absence of biocides, part of soil organic matter may be mineralised. A decline in soil pHBC is expected due to decreased organic matter. However, the increased soil pHBC would suggest that changes in H^+ budget altered soil pHBC more than loss of organic matter.

It is assumed that pH change due to biological activity alone was the same at different addition rates of acid and alkali (Kissel *et al*., 2012; Thompson *et al*., 2010). However, in this study, the increase in soil pH due to biological reactions were more apparent at lower than higher equilibrium pH. This was accompanied by a decreased NH_4^+ concentration with increasing equilibrium pH (data not shown). It has been well recognized that low soil pH has a less effect on ammonification, a H^+-consuming reaction, than nitrification, a H^+-producing process (Fu *et al.,* 1987). The lower NH_4^+ concentration and lesser pH increase at higher than lower equilibrium pH possibly indicate an increase in soil nitrification. Moreover, it is likely that soils with a high initial pH were more susceptible to biological attack, and thus exhibited more rapid and pronounced responses to biocide treatment than soils with low initial pH. Routinely, the determination of soil pHBC aims to measure the non-biological reactions. Thus, addition of biocides should be considered as an essential procedure during pHBC measurement, especially for soils with relatively high initial pH and organic C content.

In addition to pHBC, to calculate lime requirement, one might need to consider acidity or alkalinity produced from biological processes. However, a short-term analysis in the laboratory cannot account for a long-term C or N cycling and other uncertainties such as NO_3^- leaching, cropping practice or N fertilizer which also contribute to H^+/OH^- production, under field conditions. Moreover, temporal changes in pHBC would occur due to changes in the amount and/or nature of organic matter. Thus, any soil pHBC values obtained from laboratory analysis only represent the non-biological soil pH buffering ability at a specific sampling time. A discrepancy

between the measured soil pHBC and actual lime requirement is expected in the field where biological reactions occur.

Equilibrium time

The time required for added acid or alkali to equilibrate with the soil solution depended on soil properties and particular pH buffering reactions. Cation exchange, and protonation/deprotonation reactions are expected to occur rapidly, but non-exchangeable Al^{3+} or $Al(OH)^{2+}$ or $Al(OH)_2^{+}$ bonded with organic matter or trapped in the interlayers of 2:1 clay minerals, can only be slowly neutralized (Brady, 1990). In the current study, a long equilibrium time was expected for the soils dominated by 2:1 clay minerals or with high organic C. In addition, dissolution of Fe oxides, minerals and encapsulated $CaCO_3$ are slow reactions (Qiu *et al.,* 1998), and was likely to have accounted for equilibrium times of more than 14 days for soils such as Ferrosol, Dermosol and Vertosol in the current study. In particular, the Vertosol contained some acid-resistant carbonate such as dolomite (Gardner, 2004) and did not reach the equilibrium within 28 days. Given that these slow reactions could highly buffer soil pH (McBride, 1994; Lesturgez *et al.* 2006), much longer equilibrium time was shown for soils with high than low pHBC (Table 2).

Equilibrium/reaction time adopted by various methods for measuring soil pHBC varied from few minutes to more than 3 months. For example, incubation of the soil with either $Ca(OH)_2$ or $CaCO_3$ at field capacity required months, in contrast to the direct titration technique and buffer solutions methods which used only days and hours, respectively (Conyers *et al.,* 2000; Dunn, 1943; Liu *et al*., 2005). Recent studies (Liu *et al.,* 2005; Kissel *et al.,* 2007) suggested that 30-min equilibration/reaction between soil and a single addition of $Ca(OH)_2$ could provide an accurate estimation of pHBC. However, other studies reported that the time required to reach equilibrium was at least 3 to 7 days for soils with high clay contents (Thompson *et al.,* 2010). In our study, an equilibrium/reaction time of one hour could underestimate pHBC for all the soils except the Podosol. Apparently, short-term equilibration/reaction excluded the slow reactions contributing to soil pH buffering and could underestimate the soil pHBC by 30 to 70% in this study. We consider that any new or quick method should be compared or calibrated with the methods using a long reaction time before applied to a routine soil test. Recently, calibration models have been developed to predict soil pHBC at equilibrium using the pHBC from a 30-min equilibration (Kissel *et al*., 2012; Thompson *et al*., 2010).

If the buffering reactions are mainly limited by the access of acids or alkalis to the reaction sites, dispersing soil particles could help expose these sites to the acid and alkali. Using a sonication treatment appeared to be efficient in dispersion of soil particles and thus acceleration of buffering reactions, particularly for the Sodosol and Calcarosol where 1- to 7-day reaction time could be shortened to one hour. For Ferrosol, where dissolution of minerals was found to take part in the acid buffering, sonication also greatly expedited these reactions. As incomplete dispersion by sonication might have occurred for Ferrosol and Vertosol with relatively strong binding agents such as free iron oxides and $CaCO_3$, respectively (Turchene & Oades, 1978), a reaction time of more than one hour was still required for these soils. Alternatively, dispersion of soil particles contribute little to the dissolution of some acid-resistant carbonate in Vertosol, and it might take longer for reactions such as mineral dissolution or Al hydrolysis in the interlayer space to complete for Ferrosol

dominated by vermiculite and with much higher pHBC. Nevertheless, pre-treatment with sonication could alleviate the underestimation of pHBC for soils dominated by 2:1 clay minerals and involved with mineral dissolution.

Soil properties affecting soil pHBC

The significant correlation between soil pHBC and organic C found in this study agrees well with other studies (Aitken *et al.,* 1990, Dolling *et al.,* 1994; Nelson, 2010; Weaver *et al.,* 2004). Soil organic matter with multiple individual acidic functional groups is able to buffer pH over a wide range of pH values (Aitken, 1992; Magdoff & Bartlett 1985). Moreover, among all solid soil components, soil organic C had a higher capacity in pH buffering (2000 mmol kg^{-1}) (McBride, 1994). Moore *et al*. (1998) found that the pHBC of soils in Western Australia with low clay content could be estimated accurately from their organic matter content alone. In this present study, soils with the highest organic matter content, such as Organosol, Ferrosol and Dermosol (topsoil) showed the highest pHBC.

The importance of clay mineralogy on soil pH buffering was highlighted by the strong correlation between the proportion of vermiculite and soil pHBC. Vermiculite with the highest charge density (1500-2000 $mmol_c$ kg^{-1}) among all silicate clays (Borggaard, 1983; McBride, 1994), should be responsible for the high pHBC of Ferrosol and Dermosol at 10 to 30 cm layers. Ferrosol was also characterized by high amounts of free iron oxides, but their capacity in buffering soil pH is much lower (50-400 $mmol_c$ kg^{-1}, Borggarrd, 1983) than Vermiculite. Qiu *et al.* (1998) and Xu *et al.* (2012) also found that the most effective factor in determining pHBC for soils in tropical and subtropical regions is the content of montmorillonite and vermiculite, respectively. While certain soil properties were frequently used for predicting soil pHBC, additional information on clay mineralogy should provide a more accurate estimation.

Without being isolated from the effect of organic C and clay mineralogy, soil clay content was less related to soil pHBC (P=0.04) in this study. When all these factors had been accounted for, the contribution of clay content to soil pHBC became more significant (P=0.005), with about 7% contribution. This indicates that organic C and clay mineralogy were more important factors than clay content in determining soil pHBC. Other studies have also found that clay content was poorly correlated with pHBC (Singh *et al.,* 2003; Weaver *et al.,* 2004). However, inclusion of clay content was expected to improve the estimate of soil pHBC at similar organic C content and clay mineralogy. In this study, the lowest pHBC was invariably shown for soils such as Podosol and Tenosol with the lowest clay content. It is likely that organic C contributed more to pH buffering of surface soils while clay content and clay mineralogy was more important in determining pHBC of subsoils where organic C was low.

This study revealed a higher pHBC when acid rather than when alkali was added for soils with low (< 4.2) and high initial pH (> 7.0), such as Ferrosol and Vertosol, respectively, indicating the occurrence of mineral dissolution upon acid addition. This was confirmed by the hysteresis loops exhibited for these soils during the back-titration procedure due to the fact that consumed acid by mineral or $CaCO_3$ dissolution was not recovered by the following addition of alkali. Lesturgez *et al.* (2006) found that the dissolution of clay minerals following acid addition could make

the pHBC of soils with low initial pH (pH 4) become infinite. Similarly, Singh *et al.* (2003) showed that pHBC of Vertosol (pH above 7) was mainly controlled by the dissolution and precipitation reactions of the carbonate minerals.

Comparison with two commonly used methods

Soil pHBC determined by the 1:1 W method was 18-68% lower than that using 1:5 $CaCl_2$. Although representing a soil solution condition closer to field conditions, the 1:1 W method was more sensitive to changes in ionic strength from the addition of acid and alkali, compared with the 1:5 $CaCl_2$ method. The final pHBC measured using the 1:1 W method may essentially be an artefact caused by the inconsistent ionic strength from addition of acid and alkali, especially for soils with low ionic strength. Moreover, the relatively short reaction time of 4 days might also result in incomplete reactions and underestimate the soil pHBC for most soils.

In this study, discrepancies between the FMI and 1:5 $CaCl_2$ methods were found for soils with high organic matter (~60 g kg^{-1}) and high $CaCO_3$. It has been suggested that FMI could overestimate the actual pHBC due to biological reactions favoured by its incubation conditions (Liu *et al.,* 2008). Conyers *et al.* (1995; 2000) found that the increase in soil pH due to biologically H^+-consuming reactions even negated the effect from addition of acids after 30 days of incubation at field capacity. This agreed well with our findings that biological reactions alone could greatly elevate soil pH and pHBC for soils with high C content. For this reason, the 1:5 $CaCl_2$ method with relatively low soil: solution ratio, shorter reaction time and biocide addition was expected to provide a more accurate pHBC measurement than the FMI method.

An improved method

Based on the results of the present study, we recommended an improved method. The procedure of the method is as follows. Use 4 g soil at a 1:5 soil to 0.01M $CaCl_2$ solution ratio, with acid and base added as HCl and NaOH or $Ca(OH)_2$, respectively, at rates less than 20 $mmol_c\ kg^{-1}$ (less effect from the base valence). After the addition of 0.25 ml chloroform or toluene, soil suspensions are shaken end-over-end for 1 hour, then sonicated for 3 minutes in ice water at an energy level of 125-150 J g^{-1} soil solution, and centrifuged at 2000 rpm (700 g) for 10 min before determining the supernatant pH.

Acknowledgements

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746 **Table 1.** Selected physical and chemical properties of 19 soils used in this study.

Soil No	Soil type (Isbell 2002)	FAO-UNESCO (1976)	Depth (cm)	pH (0.01 M $CaCl_2$)	EC ($\mu S\ cm^{-1}$)	Clay content (%)	Organic C ($g\ kg^{-1}$)	Organic N ($g\ kg^{-1}$)	Relative proportion of three major clay minerals		
									Vermiculite (%)	Illite (%)	Kaolinite (%)
1	Ferrosol	Ferralsol	0-10	4.2	24	32	61.9	3.1	56	36	8
2	Ferrosol	Ferralsol	10-30	4.2	27	55	12.3	0.7	57	37	8
3	Dermosol	Planosol	0-10	4.3	57	33	69.2	2.8	55	36	9
4	Dermosol	Planosol	10-30	4.3	32	46	25.2	1.4	56	36	10
5	Organosol	Histosol	0-10	4.1	89	11	65.2	4.4	15	60	25
6	Vertosol	Vertisol	0-10	7.1	134	37	15.9	1.5	0	88	12
7	Vertosol	Vertisol	10-30	7.1	136	55	9.1	0.8	0	86	14
8	Sodosol	Solonetz	0-10	4.6	131	36	12.5	1.1	0	70	30
9	Sodosol	Solonetz	10-30	5.3	121	41	7.8	0.7	0	75	25
10	Sodosol	Solonetz	30-60	5.2	400	57	5.9	0.5	0	80	20
11	Kandosol	Arenosol	0-10	5.7	154	12	13.4	1.1	0	73	27
12	Kandosol	Arenosol	10-30	5.5	71	23	8.6	0.8	0	74	26
13	Chromosol	Luvisol	0-10	4.6	49	15	15.8	1.4	0	90	10
14	Chromosol	Luvisol	10-30	4.4	70	26	7.2	0.7	0	91	9
15	Calcarosol	Calcisol	0-10	6.6	135	44	19.0	1.6	0	85	15
16	Podosol	Podozol	0-10	3.8	59	3	13.8	0.6	0	65	35
17	Podosol	Podozol	10-30	4.4	50	2	2.7	0.2	0	63	37
18	Tenosol	Cambisol	0-10	4.7	122	13	10.7	1.0	0	73	27
19	Tenosol	Cambisol	10-30	6.0	41	9	1.9	0.2	0	74	26

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Table 2. pH buffer capacity ($mmol_c\ kg^{-1}\ pH^{-1}$) determined at different reaction time using 1:5 soil 0.01 M $CaCl_2$ incubation method (1:5 $CaCl_2$) for 19 soils.

Soil No	Soil type	Sampling depth (cm)	Reaction time (1:5 soil 0.01 M $CaCl_2$ incubation)					
			1 hour	1 day	7 days	14 days	28 days	LSD (*P*=0.05)
1	Ferrosol	0-10	71	99	114	125	132	10
2	Ferrosol	10-30	77	74	98	111	129	14
3	Dermosol	0-10	66	97	110	124	145	12
4	Dermosol	10-30	62	70	91	115	127	15
5	Organosol	0-10	44	63	74	82	96	9
6	Vertosol	0-10	24	32	61	65	79	16
7	Vertosol	10-30	26	30	73	78	85	12
8	Sodosol	0-10	13	16	17	22	22	4
9	Sodosol	10-30	13	14	16	22	22	4
10	Sodosol	30-60	18	19	22	37	44	7
11	Kandosol	0-10	10	13	23	24	24	5
12	Kandosol	10-30	9	11	18	22	24	4
13	Chromosol	0-10	13	14	21	24	26	4
14	Chromosol	10-30	9	12	17	19	20	4
15	Calcarosol	0-10	13	15	22	26	27	6
16	Podosol	0-10	15	16	16	17	17	4
17	Podosol	10-30	8	9	9	11	11	3
18	Tenosol	0-10	10	11	13	16	16	4
19	Tenosol	10-30	5	5	6	6	6	1

Table 3. Effect of biological control (addition of chloroform) on soil pH buffer capacity ($mmol_c\ kg^{-1}pH^{-1}$) of a Sodosol with different initial pH (4.6, 5.6 and 6.8) and different C contents (15, 45 and 105 g kg^{-1}) after 1- and 7-day incubation. Different initial pH was due to different rates of lime application at a 30-year lime trial and various initial C contents were achieved by addition of various amounts of compost.
n.s., *, ** and *** represent P > 0.05, < 0.05, < 0.01 and < 0.001, respectively.

Initial soil pH	Treatment	Initial C (g kg^{-1})		
		15	45	105
		Day 1		
4.8	-Biocide	18	33	83
	+Biocide	19	35	78
5.6	-Biocide	12	33	78
	+Biocide	13	28	69
6.8	-Biocide	15	64	121
	+Biocide	14	47	87
LSD (*P*=0.05)		1	7	10
Treatment		n.s	**	**
Initial soil pH		***	***	**
Treatment × Initial soil pH		n.s	*	*
		Day 7		
4.8	-Biocide	22	47	101
	+Biocide	22	41	88
5.6	-Biocide	21	43	109
	+Biocide	19	39	94
6.8	-Biocide	26	134	175
	+Biocide	22	107	161

LSD (*P*=0.05)	2	10	5
Treatment	**	***	***
Initial soil pH	***	***	***
Treatment × Initial soil pH	*	***	n.s

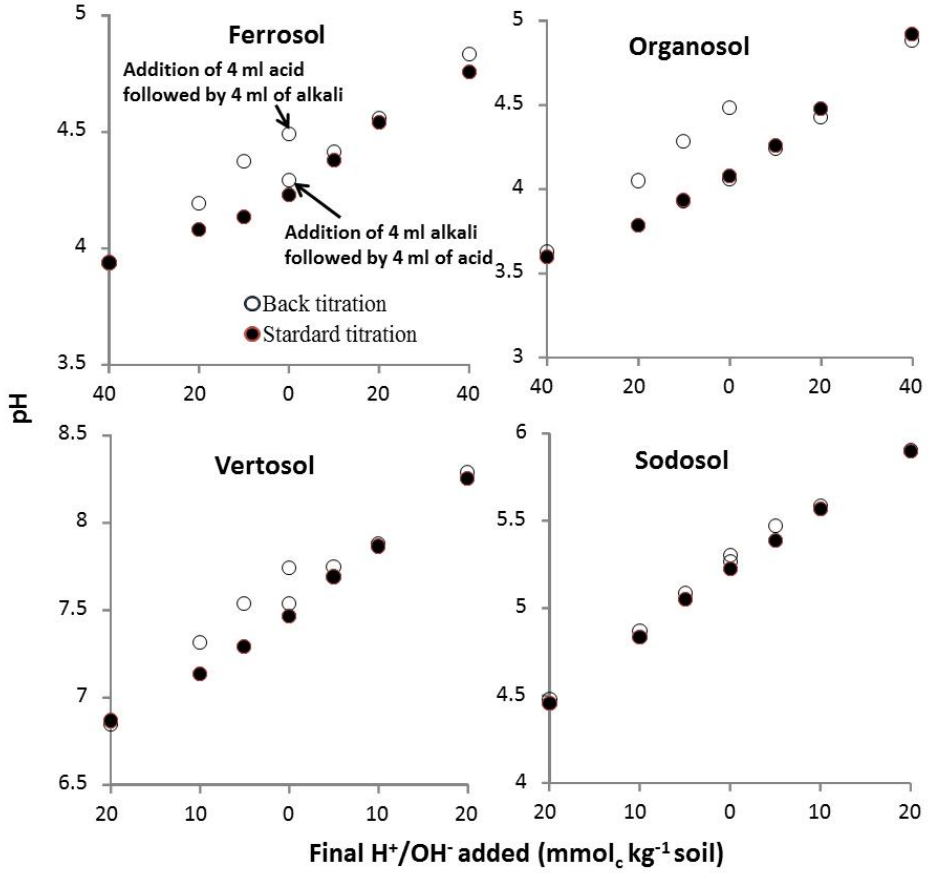

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Figure 1. Comparison of back titration (8 points) with standard titration (7 points) for Ferrosol (Soil 2), Organosol (Soil 5), Vertosol (Soil 7) and Sodosol (Soil 9). The back titration involved adding excess amounts of acid and alkali to the soil suspension, and then titrating back with known amounts of alkali and acid, respectively, giving the final addition of H^+ and OH^- as same as that of the standard titration.

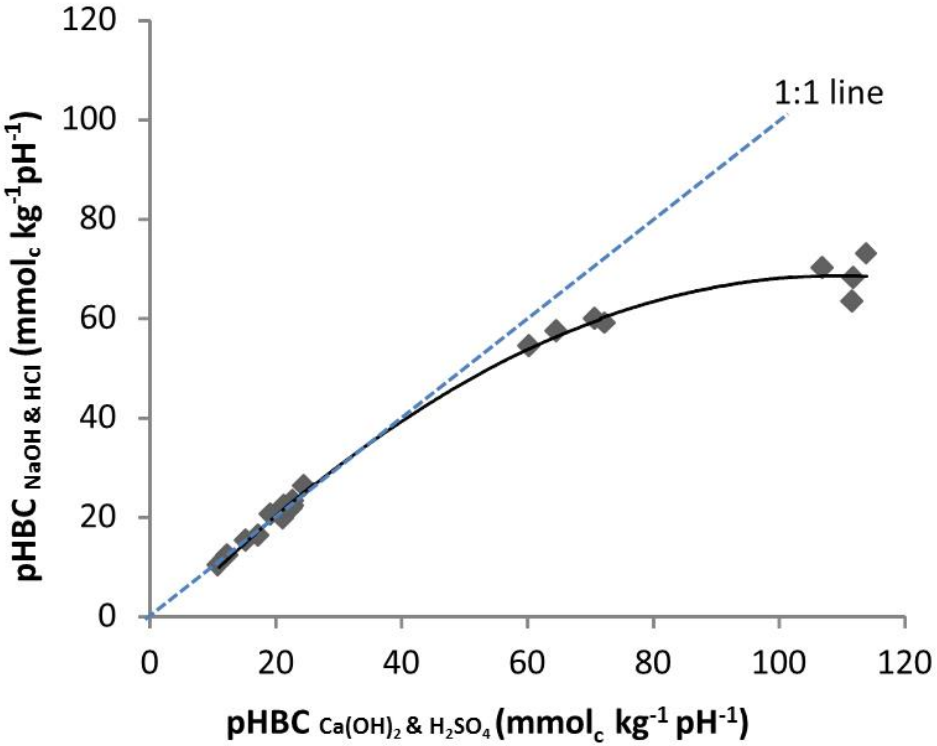
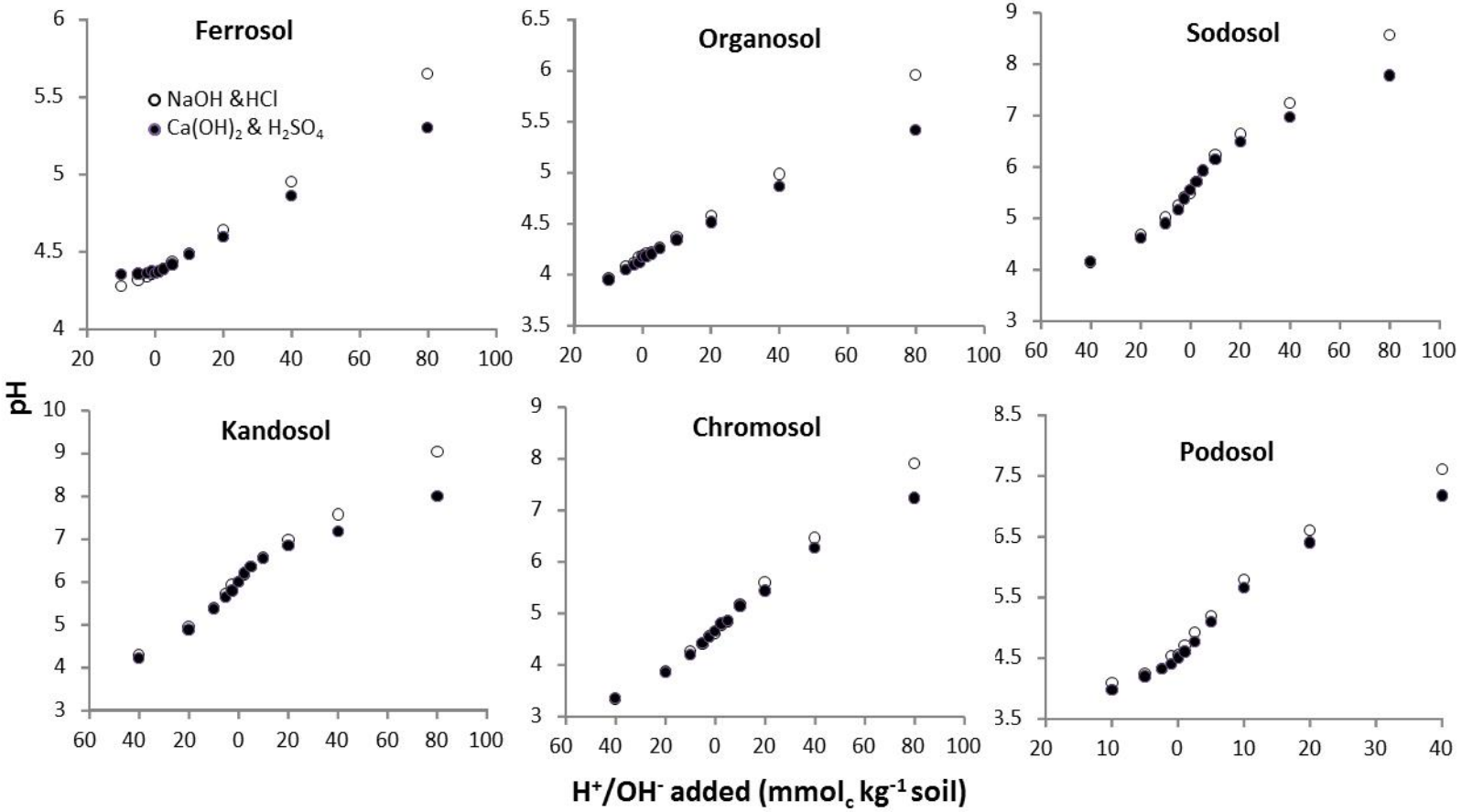


Figure 2. Relationship between pH buffer capacity ($mmol_c\ kg^{-1}\ pH^{-1}$) determined in 0.01 M $CaCl_2$ using divalent $Ca(OH)_2$ and H_2SO_4, and monovalent NaOH and HCl.



772 **Figure 3.** The effect of addition of H^+ and OH^- ($mmol_c\ kg^{-1}$ soil) as $Ca(OH)_2$ and H_2SO_4, and as NaOH and HCl on pH (in 0.01 M $CaCl_2$) for
Ferrosol (Soil 1), Organosol (Soil 5), Sodosol (Soil 8), Kandosol (Soil 11), Chromosol (Soil 13) and Podosol (Soil 16).
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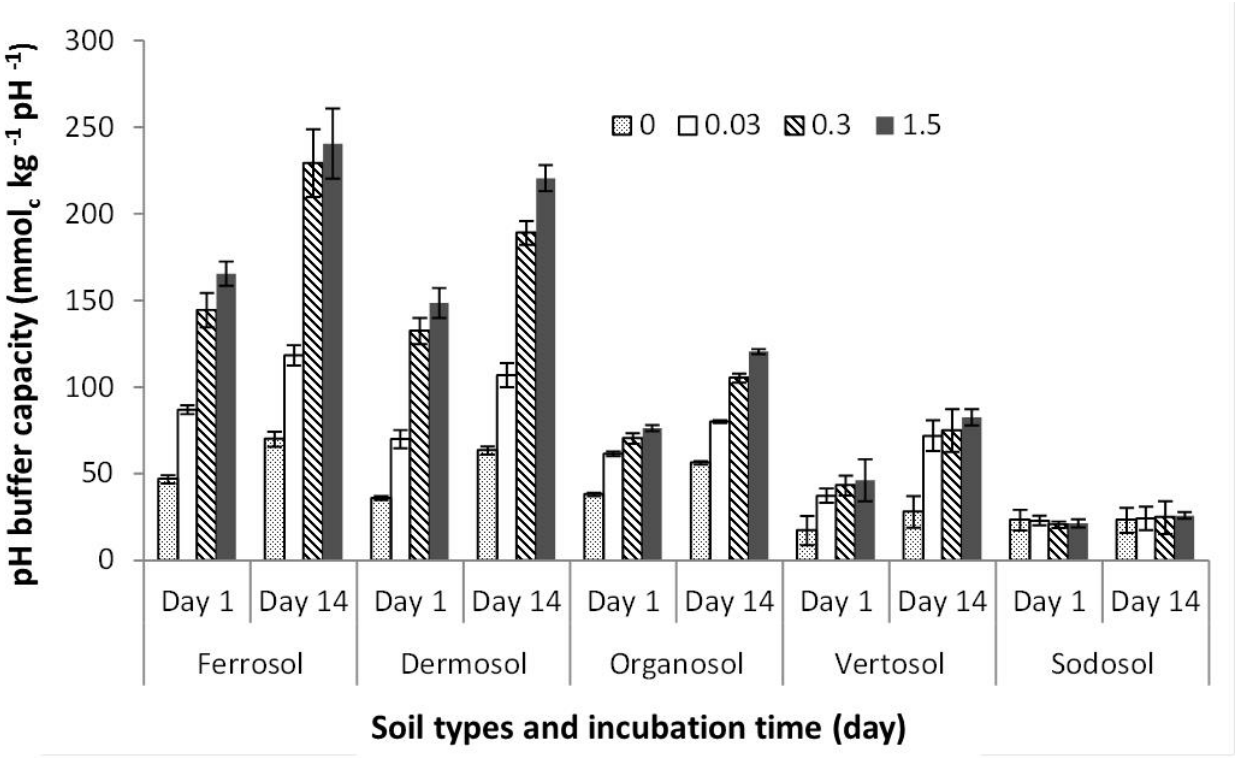

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778 **Figure 4.** Effect of ionic strength (0, 0.03, 0.3 and 1.5 M) on pH buffer capacity (mmol$_c$ kg^{-1} pH^{-1}) of Ferrosol (Soil 1), Dermosol (Soil 3) Organosol (Soil 5), Vertosol (Soil 6) and Sodosol (soil 8) after incubation for 1 and 14 days. Error bars represent standard error of means of 2
780 replicates.

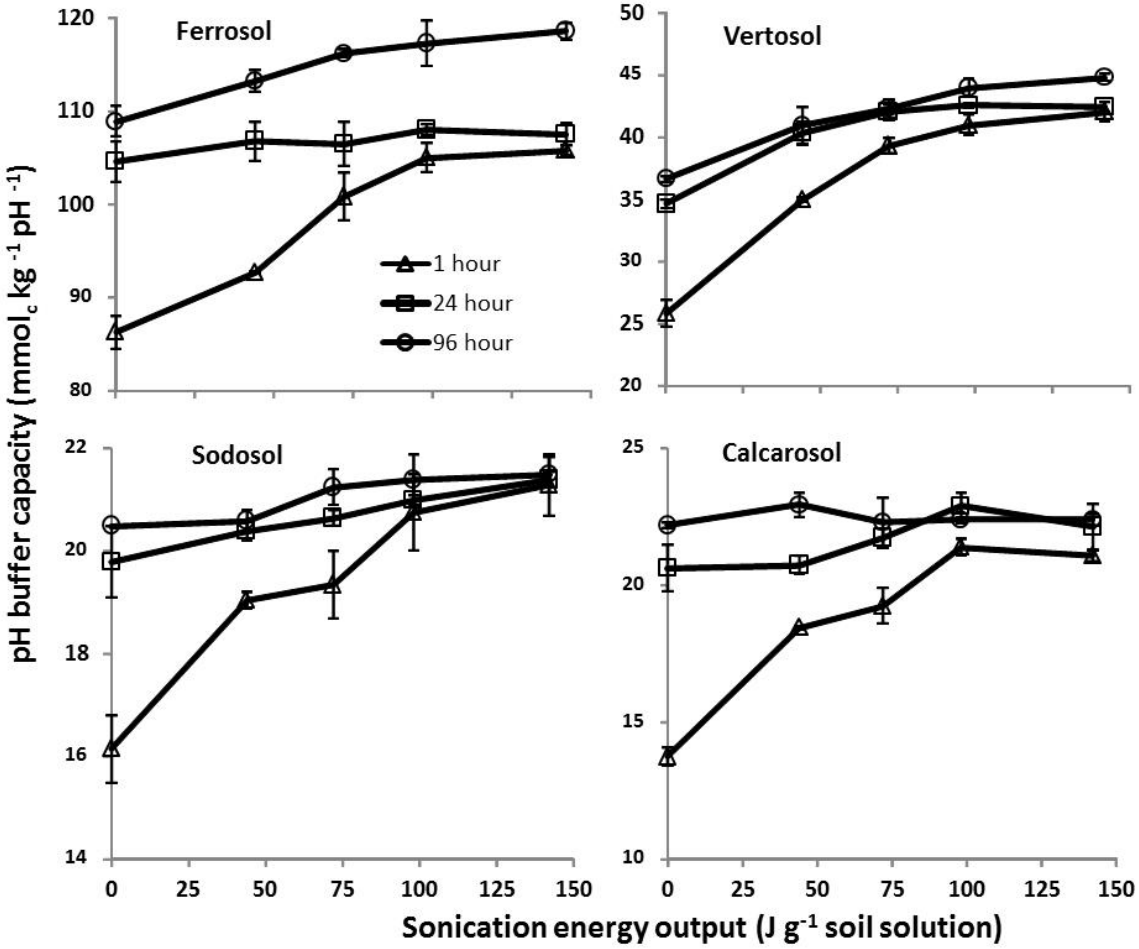

Figure 5. Soil pHBC of four soils determined at 1, 48 and 96 hours following sonication pre-treatment at increasing energy level. Four soils are Ferrrosol (Soil 2), Vertosol (Soil 7), Sodosol (soil 9) and Calcarosol (Soil 15). Error bars represent ± standard error of means of 2 replicates.

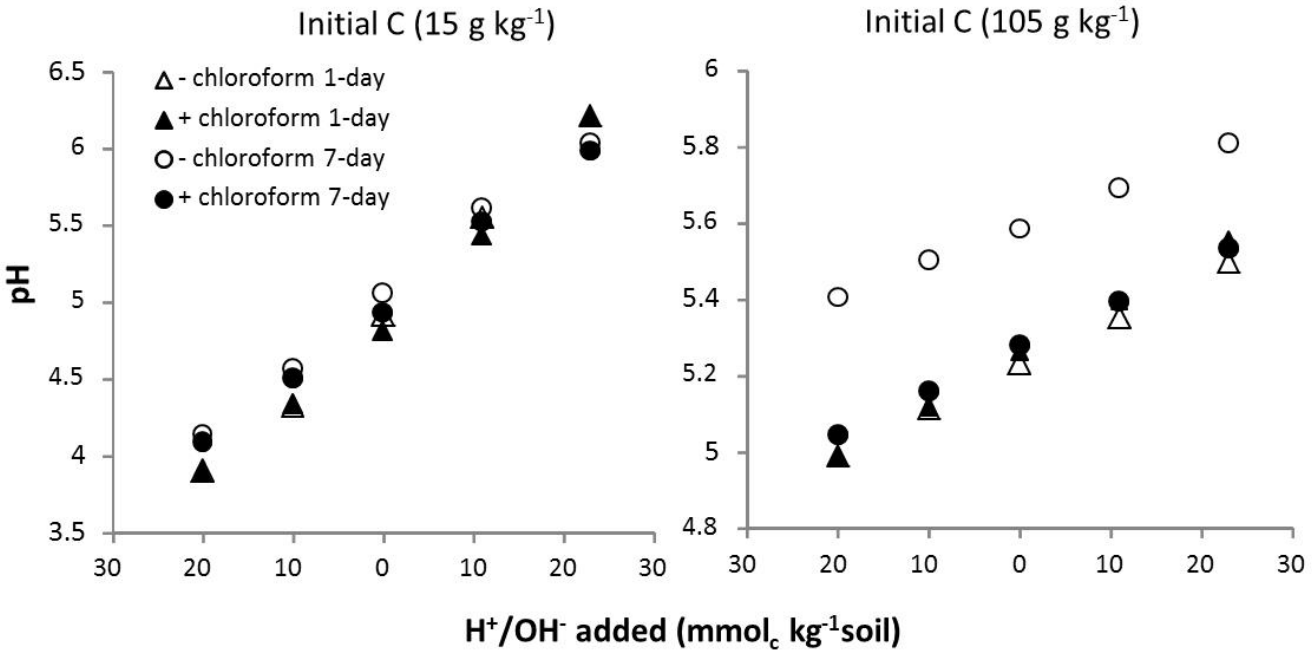

Figure 6. Effect of chloroform addition on the pH buffer curve of the Sodosol with C content of 15 and 105 g C kg^{-1} (due to compost (pH 5.3) addition) and initial soil pH of 4.8 after 1- and 7-day incubation.

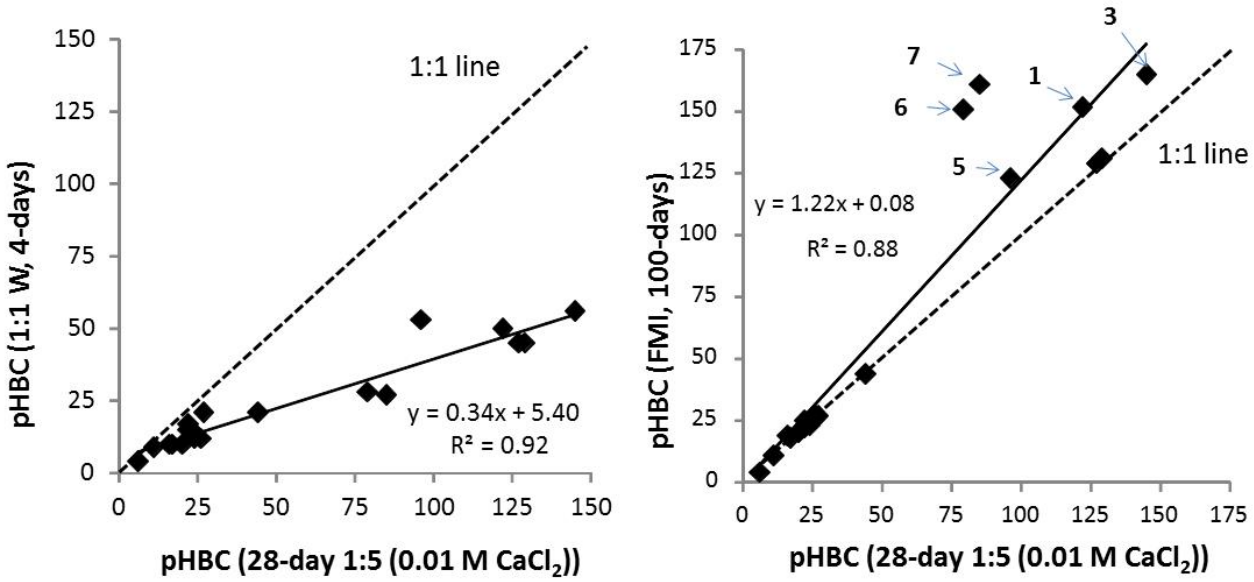

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Figure 7. Comparison of pHBC determined by 1:5 soil 0.01M $CaCl_2$ incubation (x-axis) with, 4-day 1:1 soil water (1:1 W) (left) and field
792 moisture incubation (FMI) (right). The numbers (with arrows) indicate different soils shown in Table 1.

794

Supplementary: optimized protocol for soil pH buffer capacity measurement

Reagents:

Standardized 0.04 M NaOH and 0.04 M HCl; 0.01 M $CaCl_2$; Chloroform

Procedure:

1) Weigh 4 g air-dried soil into each 50-ml centrifuge tube.
2) Add 15 ml 0.01 M $CaCl_2$ into the centrifuge tube and mix with soil.
3) Add incremental amounts of acid and alkali to the soil suspension (in 0.01 M $CaCl_2$). For soils with clay content of <30% or organic C content of <20 g kg^{-1}, add 1, 0.5, 0.25, 0 ml of 0.04 M HCl and 0.25, 0.5, 1 ml of 0.04 M NaOH into seven successive soil suspensions, respectively. For soils with higher clay or soil organic C contents, 2, 1, 0.5, 0 ml of 0.04 M NaOH and 0.5, 1, 2 ml of 0.04 M NaOH are recommended to establish a relatively linear pH buffer curve.
4) Adjust each centrifuge tube to a final solution volume of 20 ml with 0.01 M $CaCl_2$.
5) Add 0.25 ml of chloroform into each centrifuge tube to minimize the microbial activity, and shake on an end-over-end tumbler at 25 °C for 1 hour.
6) To accelerate the buffer reactions, soil suspensions may be sonicated at 125-150 J g^{-1} soil suspension for 3 minutes in ice water (e.g power output position '4', Branson Sonifer 250 probe (Branson Sonic Power, Danbury, CT, USA)).
7) If sonification is not available, soil suspensions may be left standing for at least 7 days to reach the near equilibrium state except for soils of <10% clay which require only 1 hour. During equilibrium, the soil suspension should be resuspended daily by shaking for 5 minutes.
8) After equilibrium, measure the pH of the suspension following centrifugation at 700 *g* for 10 minutes. Fit a linear regression function between soil pH (Y-axis) and the amount of H^+ or OH^- added ($mmol_c$ kg^{-1} soil) (X-axis) (Figure S1). The soil pHBC is calculated as the reciprocal of the slope of the buffer curve.

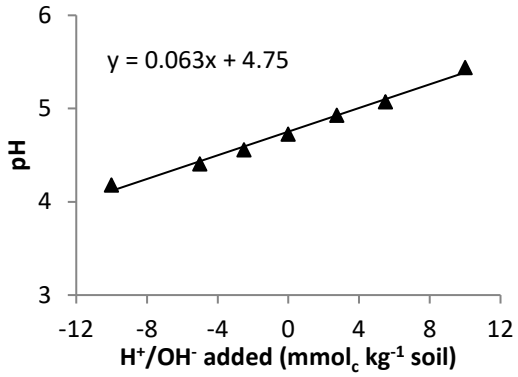

Figure S1. The effect of addition of H^+ and OH^- ($mmol_c$ kg^{-1} soil) as NaOH and HCl on pH (in 0.01 M$CaCl_2$) of a Sodosol. Negative symbols on the x-axis indicate the acid addition.

$$\text{Soil pH buffer capacity} = \frac{\Delta(H^+/OH^-)}{\Delta pH} = 1/0.063 = 15.9 \text{ mmol}_c \text{ kg}^{-1}\text{pH}^{-1}$$

0.25, 0.5 and 1 ml of 0.04 M HCl in 4 g of soil are equivalent to 2.5, 5 and 10 mmol H^+ kg^{-1} soil, respectively.

0.25, 0.5 and 1 ml of 0.04 M NaOH in 4 g of soil are equivalent to 2.5, 5 and 10 mmol OH^- kg^{-1} soil, respectively.