Influence of Agricultural Lime on Soil Properties and Wheat (*Triticum aestivum* L.) Yield on Acidic Soils of Uasin Gishu County, Kenya

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Authors’ contributions

This work was carried out in collaboration between all authors. Author MAO study conception and design performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JRO, WKN and JOO managed the analyses of the study. Authors JOO and VSO interpretation of data, authors COO, BL managed the literature searches. All authors read and approved the final manuscript.

Research Article

**ABSTRACT**

A study was carried out to investigate the influence of agricultural lime (21% CaO) from Koru, Kisumu on soil properties and wheat yield on acidic soils of Uasin Gishu county. Field trials were conducted at Chepkoilel University College farm and in Kipsangui area of Uasin Gishu county. Soils were analyzed to determine their pH, available P and other nutrient levels before treatment application. The experiment was a split plot arrangement with two wheat varieties as the main plots and the lime treatments as the subplots. The two varieties compared were ‘Njoro BW 2’ and ‘KS Mwamba’ characterized as tolerant and moderate tolerant to soil acidity, respectively. Phosphorus and nitrogen were applied as a blanket treatment at the rates of 40 kg P₂O₅ /ha and 46 kg N/ha respectively. Lime was applied at the rates of 0.0, 0.5, 1.0, 1.5 and 2.0 t/ha. Soils from the two sites were acidic with low to moderate P and K levels and high Fe, Al, and Mn levels. Lime increased the pH, available P and K, exchangeable Ca and Mg, organic C and N levels. The wheat variety ‘Njoro BW 2’ was more responsive to lime than ‘KS Mwamba’. Wheat yield was significantly increased with lime application. Therefore, it is recommended that farmers in the area use agricultural lime to improve the chemical properties of acidic soils and to increase wheat yield.
moderate available P for Chepkoilel and Kipsangui sites respectively. Soil pH and soil available P increased with the increase in the rate of lime addition. Wheat grain yield increased significantly (p=0.05) due to soil acidity amendment above the control. There was a high positive correlation between wheat yields and soil available P at both sites at harvest. High cost of inorganic inputs, low wheat grain prices and the effects of the erratic rains made the majority of the treatments economically unviable for adaptation by farmers. However, the most profitable treatment was 2 t/ha of lime in Njoro BW 2 at Kipsangui site. There was no viable treatment at Chepkoilel site. Higher wheat yields may probably be achieved from rates of lime above 2 t/ha.

Keywords: Lime; nitrogen; phosphorus; soil acidity; wheat.

1. INTRODUCTION

Wheat (Triticum aestivum L.) is the first most important cereal cultivated in the world. It is the second most important cereal crop grown in Kenya after maize [1]. In contrast to sustained increases in wheat productivity in other parts of the developing world, per capita production in Kenya continues to stagnate while the consumption has been on the increase [2]. Uasin Gishu county is a major wheat producing county of Kenya, producing more than one-third of the total wheat produced in the country [3]. However, over the recent years there has been a general decline of crop yields due to unpredictable weather particularly rainfall, declining soil fertility, diminishing land parcels as the consequence of rapid population growth, low and unsustained market prices for the produce and poor crop husbandry [4]. High costs of inputs mainly Di-Ammonium Phosphate and Calcium of Ammonium nitrate, diseases, weeds and poor crop husbandry, also contribute significantly to low grain yields in the county. In addition to the above constraints, the wheat crop in this county is mainly grown on ferralsols which are characterized by low pH (soil acidity) and low nutrient levels [5]. These ferrous impervious soils also result in frequent water stagnation [6]. Although ferralsols have good internal drainage; water stagnation in this county is caused by soil compaction and crusting as a result of the use of farm machinery. Nitrogen, calcium and magnesium deficiencies and toxicities of aluminum and manganese, which characterize these acidic soils, also limit crop production in this county [7]. Further, continuous use of acidifying fertilizers like Di-Ammonium Phosphate and urea, worsen an already bad situation [8].

Soil acidity is attributed mainly to abundance of hydrogen (H\(^+\)), aluminum (Al\(^{3+}\)) and manganese (Mn\(^{2+}\)) cations in soils at levels that interfere with normal plant growth. Soil acidity has a negative effect on crops mainly through P unavailability from P fixation in soils whereby the Fe and Al soil components (sesquioxides) fix sizeable quantities of P. Excess Al\(^{3+}\) ions, from soil acidity, tend to accumulate in plant roots and thereby prevent P, Mo and other ions translocation to the tops from the roots, as evidenced by the inhibition of root elongation and overall retarded crop development [9,10,11]. The detrimental effect of H\(^+\) ions is not as distinct as that of Al\(^{3+}\) cations, but excess of H\(^+\) ions in acid soils affects plant root membrane permeability and therefore interferes with ion transport [11].

In acid soils and P deficient tropical soils where the plant capacity to scavenge the native or use added P with efficiency is critical [12], correcting soil acidity and P fertilizer addition are important. Lime is widely known as the effective way of correcting soil acidity or inputs recommended for amelioration of acid soils [13]. Its direct effect is soil pH increase (14). In Kenya, management of soil acidity through liming is highly recommended [11,15]. Lime
reduces Al, H, Mn, and Fe toxicities and increases P, Mg, Ca and Mo availability in acidic soils [11,16,17]. Therefore, in P fixing acid soils, combined lime and P application is necessary for increased availability of the applied P for plant uptake. Although, not permanent the direct effect of lime lasts longer than any other amendment, such as organic materials. The most popular varieties of wheat grown in Uasin Gishu county are Njoro BW 2 and KS Mwamba because of their outstanding characteristics. Both wheat varieties are high yielding and tolerant to diseases such leaf and stem rusts, however the yields in the county have remained low even with proper crop husbandry. Thus the objectives of this study were to (1) To investigate the effects of agricultural lime (21% CaO), on wheat production in acid soils of Uasin Gishu county. (2) To determine the response in terms of changes in pH and available P (3) To evaluate the economic returns of the two wheat varieties from amelioration of depleted soils by application of lime.

2. MATERIALS AND METHODS

2.1 Uasin Gishu County

The county lies between longitudes 34º 50’ and 35º 37’ East and latitudes South and 0º 55’ North. It is a highland plateau. It’s terrain varies greatly with altitude which ranges between 1500 and 2100 metres above sea level. Eldoret Town, the capital of Uasin Gishu county, which is at an attitude of 2085 metres above sea level, marks the boundary between the highest and the lowest altitudes of the county. The county’s general landscape is that of an undulating plateau with no significant mountains or valleys. The average rainfall is between 900 mm -1200 mm per annum. Due to high altitude in the county, temperatures are relatively low. The highest is 24ºC and the lowest is about 8.8ºC. Humidity is moderate, averaging 56%. The average temperatures in the county are 18ºC during the wet season with a maximum of 26.1ºC during the dry season [18].

2.1.1 Study sites

2.1.1.1 Chepkoilel University College

The field experiment was conducted at the Crop Seed and Horticultural Science Department Field, Chepkoilel University College, Moi University in Moiben division of Uasin Gishu county. The soils of Chepkoilel belong to a group of soils found on plateaus and high level structural plains. The soils are of igneous origin, acidic (pH: 4.5-5.0), and low in fertility and are underlain with murrum. They are classified as Rhodic Ferralsols according to the FAO/UNESCO classification and Oxisols according to the USDA classification [5,19].

2.1.1.2 Kipsangui

The experiment was also concurrently conducted at Mrs Selina Maswai’s farm in Kongasis location, Kipsangui sub-location, Soy division of Uasin Gishu county. The area receives a unimodal rainfall distribution pattern. The average amount of rains received in the area for the last ten years is 1171 mm per annum. According to National Agricultural and Livestock Extension Programme Broad Based Survey (NALEP-BBS) report of 2011, the soils are sandy loam. They are classified as Rhodic Ferralsols according to the FAO/UNESCO classification and as Oxisols according to the USDA classification [5,19].
2.1.1.3 Experimental design and treatment

Lime was applied three weeks prior to planting at the rates of 0.0, 0.5, 1.0, 1.5, and 2.0 ton/ha (quantity) as CaO from Koru, Kisumu. It was broadcast evenly within the plots on a fine seedbed and then incorporated into the soil using a hoe. DAP fertilizer was applied as a blanket treatment at rate of 40 kg P\textsubscript{2}O\textsubscript{5}/ha (17.6 P kg/ha). N- was applied at the rate of 46 kg N/ha. To supplement the N in the DAP, urea foliar feed was applied at the rate of 28 kg/ha before boot stage. Experimental arrangement was a split plot with varieties namely Njoro BW 2 and KS Mwamba forming the main plot and lime levels as the sub plot. Liming materials were obtained from Koru in Kisumu county, Kenya which contains about 21% CaO and of 100 mesh. The sub plot size was 4 m * 4 m.

2.1.1.4 Soil sampling

Prior to treatment application surface (0-15) cm depth soil samples were randomly taken using a soil auger from several points at the experimental sites and thoroughly mixed together to make a composite sample. Six weeks after planting and during harvesting composite samples were again taken from each plot at the depth of 0-15 cm to monitor changes in soil available P and pH.

2.1.1.5 Field procedures

The seeds were drilled by hand at the recommended spacing row of 25 cm. The placement within the row by hand was predetermined prior to planting by running a mechanical drill planter and observing /noting the closeness of the spacing. The depth of placement was established at 2.5 cm - 3 cm deep. After sowing, seeds were covered with top soil and slightly compressed to ensure close seed-soil contact. This was done to ensure a rapid and even germination [20]. The seed rate was 125 kg /ha. Pesticide, O, O-Dimethyl S-(N-methylcarbamoylmethyl phosphorodithioate (56)(Dimethoate) was used to control pests. Herbicide known as 3, 4-methylenedioxyxymethamphetamine (Puma Complete) was sprayed a month after planting i.e. 4 leaf stage at the rate of 0.75 litres/ha. Fungicide, Tebuconazole - (RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-methyl)pentan-3-o (Nativo 300 SC) at 0.75 litres/ha was also applied at 4 weeks after planting and urea foliar was sprayed just before booting stage. Harvesting of wheat was done at physiological maturity. It was done on centre rows of each plot at final harvest by discarding outer rows per sub plot. Thus the inner rows were harvested by hand giving an effective area of 14 m\textsuperscript{2}. The ends of plots plants were discarded. In the harvested area, total heads, fresh weights and sub samples weights were taken and recorded. The wheat was threshed manually (by beating using a stick). The sub-samples were dried in the greenhouse and their weights recorded for estimates of dry weights. The straw within the harvested area was cut at ground level at harvest and its weight taken. Sub-samples from the straw were taken randomly from each plot and cut into small pieces and mixed thoroughly. All samples were air dried (in the absence of oven) and their fresh (initial) and dry weights recorded and used to compute yields per plot (grain and straw). These samples were then ground (0.02 mm) for plant tissue analysis to determine N and P contents. Yield was calculated using the relationship.

\[
\text{Yield/plot} = \frac{\text{Total fresh weight}}{\text{sample fresh weight}} \ast \text{sample dry weight}
\]
2.1.1.6 Laboratory analysis

All the soil samples were air-dried and sieved through 2 mm mesh. Soil samples were analysed for soil pH (Soil H$_2$O: 1:2.5), texture (dispersion method), total carbon (C) % (Walkley-Black method), total N (%) (Kjeldahl distillation method) and available P (Olsen method). Samples taken after treatment application were also analysed for pH (1:2.5, water) available P, plant N and P (Kjeldahl distillation method) and their concentration determined by spectrophotometer.

2.1.1.7 Economic analysis

The most economically acceptable were treatments were determined by partial budgeting analysis to estimate the gross value of grain by using the adjusted yield at the market value of grain inputs during the cropping year. In partial budgeting only costs that vary from the control are used referred to as total costs that vary (TCV). The prices of lime, DAP, urea foliar fungicide and pesticide, bags for storing wheat, transport and wheat grain were determined through market survey at each of the two sites during the research period. labour wage rates for applying lime, fertilizers and shelling of the grain were also determined through market survey to estimate the labour costs that vary. Yield data were adjusted downward by 10% since research has found out that farmers using the same technologies would obtain 10% yield lower than those obtained by researchers. The discounted rate of capital was determined at the rate of 10 and 20% per season and year, respectively and was applied to cash costs only. The discounted rate reflects the farmer’s preference to receive benefits as early as possible and to postpone costs. All costs and benefits were converted to monetary values in Kenya Shilling (Ksh) and reported on a per hectare basis.

The net accrued net financial benefits (NFBs) and TCV were then compared across the treatments dominance analysis the formula shown below.

\[
NFB = (Y \times P) - TCV
\]

Where \(Y \times P\) = Gross Field Benefit (GFB), \(Y\) = Yield per ha and \(P\) = Field price per unit of the crop.

Treatment with less than or equal to treatment with lower TCV are dominated and were marked a “D” while ones with higher NFB than the treatments and lower TCV are undominated. The marginal rate of return (MRR) analysis was carried out on the dominated treatments.

\[
MRR(\%) = \frac{\text{Change in } NFB (NFB_b - NFB_a) \times 100}{\text{Change in } TCV (TCV_b - TCV_a)}
\]

where \(NFB_a\) = NFB with the immediate lower TCV, \(NFB_b\) = NFB with the next higher TCV, \(TCV_a\) = the immediate lower TCV & \(TCV_b\) = the next highest TCV
Change in NFB and TCV also referred to marginal benefits and costs, respectively.
2.1.1.8 Statistical analysis

The generated data of wheat grain yield, soil and plant material across the experimental sites were subjected to analysis of variance (ANOVA) with standard error SE using General statistics. Means were separated using least significance difference (LSD) whenever treatment difference were significant (p=0.05).

3. RESULTS AND DISCUSSION

3.1 Soil Physical and Chemical Characteristics of the Study Sites

Table 1 shows a summary of soil parameters of the surface soils (0-15 cm) of the experimental sites determined in the laboratory before treatment application. The soils pH in the study areas was 4.92 and 5.32 for Chepkoilel and Kipsangui sites respectively. The low to moderate soil pH in both sites indicates that the acidic levels require to be amended through liming if optimum crop yield is to be achieved. According to [21], liming can neutralize soil acidity and most field crops perform best at a soil pH level between 5.5 and 6.8. The soils from Chepkoilel had organic carbon of 1.69% while those from Kipsangui had organic carbon of 2.66%. The soil available P was 9.88 mg/kg for Chepkoilel site and 10.1 mg/kg for Kipsangui site. For the soil particle size analysis, the soils from both sites were classified as sandy loam. The moderate soil available P in these soils suggested the need for supplementary P addition for increased crop yields [22]

Table 1. Soil physical and chemical characteristics of surface (0-15 cm) soils taken before planting (2009 LR) at two study sites in Uasin Gishu county, Kenya

| Particular                  | Value | Methods                              |
|-----------------------------|-------|--------------------------------------|
| Sand %                      | 60    | Hydrometer method [1]                 |
| Silt %                      | 16    |                                      |
| Clay %                      | 24    |                                      |
| **Textural class**          |       | Sandy loam                           |
| pH (1:2.5 soil: water)      | 4.92  | Glass electrode pH meter [2]          |
| %N                          | 0.17  | Kjeldahl distillation method [3]      |
| %C                          | 1.69  | Walkley-Black method [4]              |
| C:N                         | 10.1  | Olsen method                         [5]|
| Olsen P (mg/kg)             | 9.88  |                                      |

| Particular                  | Value | Methods                              |
|-----------------------------|-------|--------------------------------------|
| Sand %                      | 60    | Hydrometer method [1]                 |
| Silt %                      | 18    |                                      |
| Clay %                      | 22    |                                      |
| **Textural class**          |       | Sandy loam                           |
| pH (1:2.5 soil: water)      | 5.32  | Glass electrode pH meter [2]          |
| %N                          | 0.28  | Kjeldahl distillation method [3]      |
| %C                          | 2.66  | WALKLEY-BLACK method [4]              |
| C:N                         | 10.1  | Olsen method                         [5]|
| Olsen P (mg/kg)             | 10.37 |                                      |
3.2 Effects of Lime Additions on Soil pH during Wheat Growth

The effect of application of lime on the pH values of the soils taken at harvesting for the Chepkoilel and Kipsangui sites are given in Figs. 1 and 2. At both sites, addition of lime had significant increase \((p=0.5)\) on the soil pH above the control treatment with the lime of addition of 1.0, 1.5 and 2 t/ha. This was because lime likely increased the \(\text{Ca}^{2+}\) ions, which it contains, displaced the \(\text{Al}^{3+}, \text{H}^+, \text{Fe}^{3+}\), ions, prevalent in acid soils such as described by (14). However, there was a decrease in soil pH with lime addition of 1 t/ha in KS Mwamba wheat variety at Chepkoilel site and an increase in soil pH in the control treatment at both sites. This could be attributed to management history of the two sites. Prior management confirmed use of Single Super phosphate type of fertilizer and crop rotation, whereby the farmer alternates wheat production with maize intercrop with beans at Kipsangui site. At Chepkoilel site, the land was used by university students for research purposes and it was difficult to establish the different inorganic, organic fertilizers and soil amendment materials used. According to [23] crop rotations can lead to dramatic increases in soil fertility, help to optimize nutrient and water use by crops, and improve soil resources.

![Fig. 1. Soil pH changes during the 2009 LR season for Chepkoilel site as affected by treatment application. Error bars indicate standard error of means](image-url)
3.3 Effect of Lime on Soil Available P Taken at Wheat Harvest

The results of available P taken at harvesting for Chepkoilel and Kipsangui sites as affected by lime additions for the two wheat varieties are shown in the Fig. 3. The data were analysed across both experimental sites. There was a significant difference (p=0.05) of soil available P from treatments above the control with increase in the lime rate applied in both varieties and sites. Lime application increased both the native soil P and P fertilizer availabilities which were taken up by plant possibly decreased soil sorption capacity (not measured). Lime has been reported to reduce the P sorption in acid soils resulting in increased available P for plants [24, 25]. In highly weathered soils of the tropical and subtropical acid soils, the applied P fertilizers readily react with Al and Fe sesquioxides to form sparingly soluble P forms. This normally results in very low soil available P for plant absorption [26, 27]. The soil available P at Kipsangui site was higher than that at Chepkoilel site in both wheat varieties with the increase of lime levels. This could be attributed to the initial soil fertility status. The soil available P at both sites was lower in the control treatment when compared to all levels of lime additions. The soil available P was highest with lime addition of 2 t/ha in both varieties and sites. This could be attributed to the initial soil fertility status. Kipsangui site had more fertile soil as compared to Chepkoilel site.
3.4 Effects of Soil Amendments on Wheat Yield

Yields obtained from the test crop as a result of application of soil amendment material lime during the year 2009 LR are given in Fig. 4 for Chepkoilel and Kipsangui sites. Amendment of soil acidity with lime addition, increased grain yield significantly ($p=0.05$) in Chepkoilel and Kipsangui. The lowest grain yield in Njoro BW 2 wheat variety of 1.18 t/ha in Chepkoilel site and 1.27 t/ha in Kipsangui site were found on control treatment. The lowest grain yields in KS Mwamba variety of 0.78 t/ha at Chepkoilel and 0.84 t/ha at Kipsangui sites were also found on the control treatment. This is in agreement with [28] who stated that application of lime significantly increased grain yield in Kenya. However, there was no significant difference in the grain yield between the two varieties with addition of 1.0, 1.5 and 2 t/ha at Chepkoilel. This trend also confirms the fact that if the growth of wheat at any stage is limited by a specific factor such as nutrient supply, water, light or temperature, then the grain yield is limited irrecoverably unless it can be compensated by modifying a yield component occurring at a later stage of development [29]. During the year 2009 Kipsangui area received 1031 mm of rainfall while Chepkoilel received 817 mm. At the grain filling stage, rainfall received in 30 days at the two sites was 144.5 mm and 47.5 mm for Kipsangui and Chepkoilel respectively. The difference in the amounts of rain received at both sites is reflected in the performance in the grain yield.

Fig. 3. Changes in soil available P (mg/kg) at harvesting due to lime and P application during the 2009 LR for Chepkoilel and Kipsangui sites 125 days after application. Error bars indicate standard error of means
Fig. 4. Wheat grain yields (t/ha) per site as affected by treatment application for both sites during the long rains 2009. Error bars indicate LSD \(_{(0.05)}\).

3.5 Correlation between Wheat Yields and Soil Available P at Wheat Harvest

Correlation analysis was conducted between wheat yields and soil available P at harvesting and the results obtained are given in Figs. 5 and 6. There was a highly positive and significant correlation between soil available P and grain yields i.e. \( r = 0.97 \) and \( r = 0.94 \) for KS Mwamba wheat variety at Chepkoilel and Kipsangui sites respectively. The correlation between soil available P and grain yields was also positive i.e. \( r = 0.66 \) and \( r = 0.97 \) for Njoro BW 2 wheat variety at Chepkoilel and Kipsangui sites respectively. The high positive correlation is probably because lime increased the overall pH which led to the enhanced availability of phosphorus. Soil reaction (pH) affects the physical, chemical and biological properties of soils and crop yields. Soil acidity has a negative effect on crop yields mainly through reduced P availability though Fe and Al fixation of P [30]. This is in line with the findings of [31] found that the increase of soil pH resulted in a significant increase in grain yields. Grain yields responded to lime addition in acid soil but at different magnitudes. Further, according to [32] better grain yield was obtained when lime was applied in combination with fertilizer.
Fig. 5. Relationship between soil available P (mg/kg) and wheat grain yields (t/ha) as observed at harvesting during 2009 LR for Chepkoilel site for Njoro BW 2 and KS Mwamba variety.
Fig. 6. Relationships between soil available P (mg/kg) and wheat grain yields (t/ha) as observed at harvesting during 2009 LR for Kipsangui site for Njoro BW 2 and KS Mwamba.

Njoro BW 2 variety

\[ y = 0.2764x - 1.9723 \]
\[ r = 0.97 \]

KS Mwamba variety

\[ y = 0.1637x - 0.5373 \]
\[ r = 0.94 \]
3.6 Uptake of P and N in Wheat

Figs. 7 and 8 show the effect of lime on the wheat grain N and P uptake. Lime had a significant (p=0.05) effect on grain P and N uptake however, there was no significant increase on P uptake. Soil amendment (lime) increased N uptake in both wheat grain at both sites. The mean P uptake for the wheat grain in Njoro BW 2 variety was 6.39 kg P/ha and 5.35 kg P/ha for Chepkoilel and Kipsangui sites respectively means from all treatments. Increased P uptake with lime addition has been reported by other workers [33]. This has been attributed to better soil amelioration by the amendments [34]. According to [35], acidic conditions reduce the rate of release of mineral-N from organic-N. Liming however, increases the rate of mineralization and hence improves the supply of mineral-N to the plants.

![Nutrient P uptake (kg P/ha) for wheat straw as affected by treatment application for Chepkoilel and Kipsangui sites. Error bars indicate standard error of means](image)

Fig. 7. Nutrient P uptake (kg P/ha) for wheat straw as affected by treatment application for Chepkoilel and Kipsangui sites. Error bars indicate standard error of means
3.6 Economic Analysis

Tables 2 and 3 show economic analysis data of benefits, costs and marginal rate of return of treatments using partial budget techniques. The GFBs, NFBs, and TVC to investment were different among the sites. Treatments that produced lower NFBs were not worth for investment. They are known as dominated and were marked “D”.

Most of the treatments did not realize economically viable returns. However, at Kipsangui site, for Njoro BW 2 wheat variety with lime treatment of 2.0 t/ha and NFBs of Ksh 34,010 was the only viable treatment option (Table 3). At Chepkoilel site there was no viable treatment. Based on economic returns, the best treatment in Kipsangui site was Njoro BW 2 variety with 2 t/ha Koru lime. In this study the majority of the farm inputs for example DAP did not realize economically viable returns to investment due to high cost of inorganic inputs, low wheat prices offered and poor distribution of rainfall in the year 2009.
4. CONCLUSION

Soil acidity may negatively affect nutrient availability and cause Al and Mn toxicity. It is evident from this study that soil acidity problems can be corrected by the use of lime. Applications of lime improved soil conditions resulting in increase in, available P, yield and nutrient uptake (N and P). The general increase on wheat yields in both varieties with clearly indicate that the use of lime as a soil amendment material is paramount irrespective of the genetic makeup of the variety if a viable economic returns is to be realized in acidic soils. Although the combination between Njoro BW 2 variety and soil amendment at the rate of 2 t/ha lime gave the highest grain yield and thus viable economic returns, the nature of the rather low lime response of the wheat varieties to lime application, shows that there is need to apply lime above 2 t/ha rate to obtain a full response curve. Further, a long-term studies in
these soils to investigate the effects of lime on wheat yield, as a basis for fertilizer formulations and recommendation is necessary.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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