

# MAXIMISING TRIAL INTERPRETATION BY MAPPING SOIL HETEROGENEITY

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## Abstract

Plant growth is influenced by the physical and chemical properties of the soil. These properties vary horizontally and vertically and, as a result, increase the variability between field trial plots and thus the precision of field trials. A study was undertaken to assess the feasibility of mapping the distribution of soil factors to identify heterogeneous areas. The data were collected from the sixth ratoon crop of a nematicide x variety trial on the North Coast of KwaZulu-Natal. The trial comprised nematicide treated and untreated plots of six cane varieties, N12, N16, N17, N23, N24 and NCo376, each replicated six times. When the cane was nine months old, soil samples were collected from the 0-20 and 20-40 cm layers. The soil from the two layers was analysed for pH, P, K, Ca, Mg, Na, Zn, Al, Mn, Fe, % clay, % silt, % fine sand, % medium sand and % coarse sand. ADE4 software was used for the principal component analysis (PCA) and Statview for the analysis of variance (ANOVA).

The PCA of the data from the topsoil identified two areas of, for the most part, contiguous plots with contrasting physical and chemical characteristics. Two similar areas could be distinguished from the analysis of the 20-40 cm layer. Analysis of the change per plot in soil characteristics with depth also identified an area that was not only different on the surface, but also in the deeper layer. Identification of plots located on different soils meant that those with a different set of characteristics could be removed before performing the analysis of variance to compare treatments. Without this adjustment three varieties, N12, N16 and N23, when treated with a nematicide, were statistically better than varieties N17, N24 and NCo376. However, after removing the 23 plots with the different topsoil, or after removing the 10 plots with a different topsoil and a different subsoil layer, N12, N16 and N23 were no longer statistically different from N17 or NCo376 (all treated with nematicide). This technique offers an unbiased method of eliminating replicates in a trial. However, reducing replicates decreases the strength of the statistical analysis. A better approach would be to recalculate yields according to the average content of the main soil components that induce the soil heterogeneity. This would not only reduce within-site variability but would maintain the full complement of replicates and allow the use of the Fisher block ANOVA.

*Keywords:* sugarcane, abiotic soil characteristics, yield analysis

## Introduction

Planting trials in commercial fields to compare agronomic treatments, variety performance or response to treatment with nematicide is a common practice in the South African sugar industry. Trial sites are always carefully selected to avoid soil variability. However, it is not always possible to find uniform fields and even so, there are always small variations within the soil elements. These small differences between interacting soil elements, although not significant on a mathematical or a soil science scale, could nevertheless influence plant growth.

The identification or mapping of these areas within the trial could allow the elimination or readjustment of the yield of the affected plots before comparing treatments with the analysis of variance. This paper demonstrates one of the statistical techniques that could be used to reach this goal.

### Materials and Methods

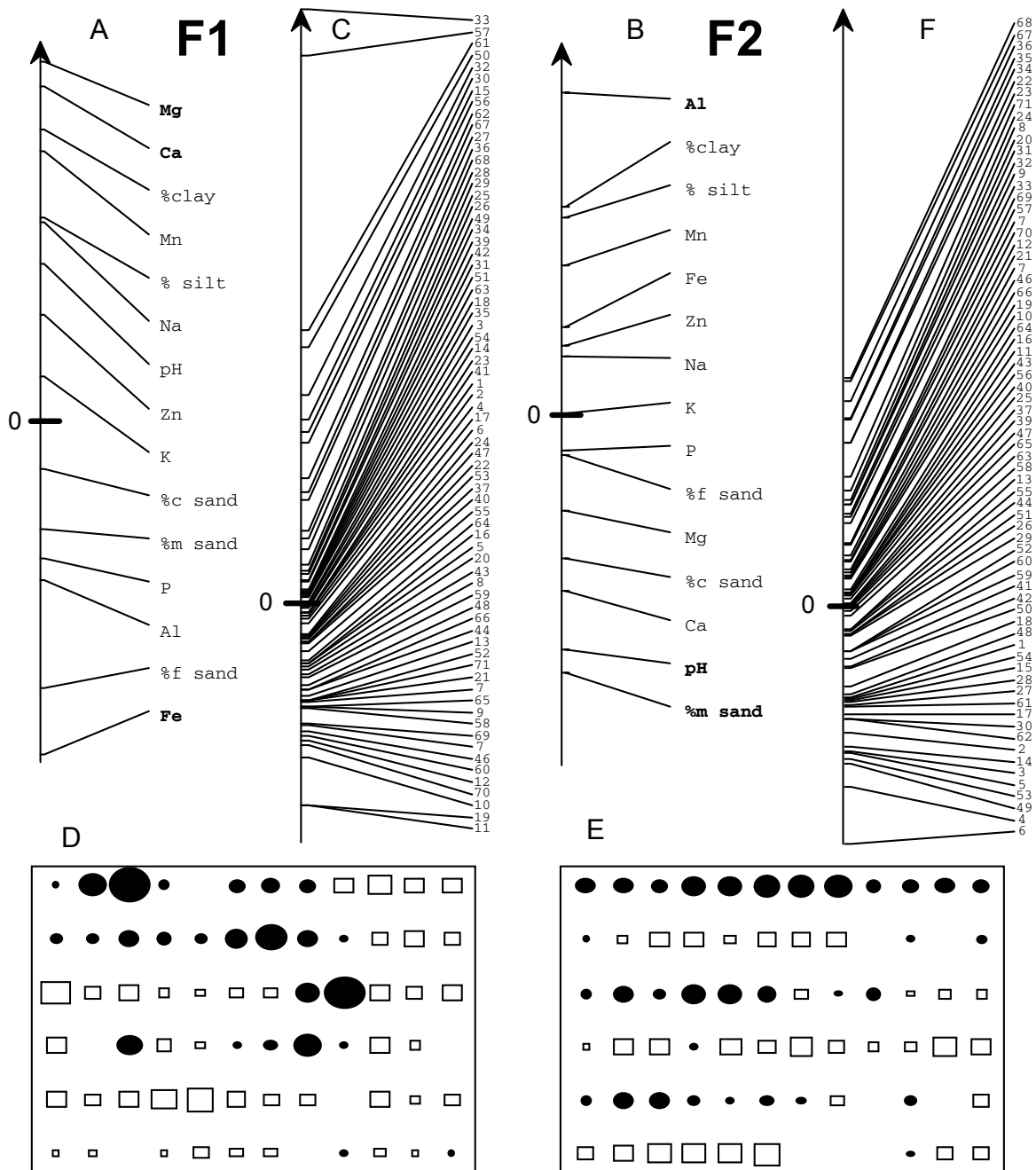
The data used in this study were collected from the sixth ratoon of a nematicide trial located on a sandy soil at La Mercy on the north coast of KwaZulu-Natal. The trial was arranged in Fisher blocks with 72 split plots, and covered an area of 4747 m<sup>2</sup> (Figure 1). Sugarcane varieties N12, N16, N17, N23, N24 and NCo376, treated and not treated with a nematicide, were compared. Row spacing was 1.2 m. Whole plots were randomised within blocks with six replicates. The nematicide, aldicarb, was applied at 3 kg/ha over the row four weeks after harvest of the previous crop. Fertiliser was applied as recommended by the Fertiliser Advisory Service of the South African Sugar Association Experiment Station. Lime had been applied to alternate blocks in the third, fourth and fifth ratoons and to all blocks in the sixth ratoon. At harvest, the yield in tons cane/ha was estimated from the weights of cane stalks taken from the four middle rows of each six-row plot. Two untreated plots of N24 were lost after the cane failed to ratoon properly following damage to the roots by nematodes.

N17 T 31	N17 C 32	N12 C 33	N12 T 34	N24 C 35	N24 T 36	N16 T 67	N16 C 68	NCo37 6 T 69	NCo37 6 C 70	N23 C 71	N23 T 72
N24 T 25	N24 C 26	NCo37 6 C 27	NCo37 6 T 28	N16 T 29	N16 C 30	N23 T 61	N23 C 62	N17 C 63	N17 T 64	N12 T 65	N12 C 66
N23 C 19	N23 T 20	N24 T 21	N24 C 22	N12 T 23	N12 C 24	N17 C 55	N17 T 56	N16 T 67	N16 C 58	NCo37 6 T 59	NCo37 6 C 60
N16 C 13	N16 T 14	N23 C 15	N23 T 16	N17 T 17	N17 C 18	NCo37 6 T 49	NCo37 6 C 50	N12 C 51	N12 T 52	N24 C 53	N24 T 54
NCo37 6 T 7	NCo37 6 C 8	N17 T 9	N17 C 10	N23 T 11	N23 C 12	N12 C 43	N12 T 44	N24 C 45	N24 T 46	N16 T 47	N16 C 48
N12 C 1	N12 T 2	N16 T 3	N16 C 4	NCo37 6 C 5	NCo37 6 T 6	N24 T 37	N24 C 38	N23 C 39	N23 T 40	N17 T 41	N17 C 42

**Figure 1. Map of the trial (the missing N24 plots are Nos. 38 and 45).  
Block No. 1 comprises the 12 plots at the bottom of the figure.**

Soil samples were collected eight months after nematicide application, at depths of 0-20 and 20-40 cm, for physico-chemical analysis.

The following soil characteristics were analysed: pH (water) and phosphorus, potassium, calcium, magnesium, sodium, zinc, aluminium, manganese and iron (in ppm), and the percentages of clay, silt, fine, medium and coarse sand. The files with the soil analyses for the two depths each had 15 columns that corresponded to the above 15 soil factors, and 70 rows corresponding to the six replicates for the six varieties, treated with nematicide and untreated and excluding the two plots originally planted to N24. Statistical analyses and mapping were done with the ADE4 software (Thioulouse *et al.*, 1997) and with Statview (ANOVA).



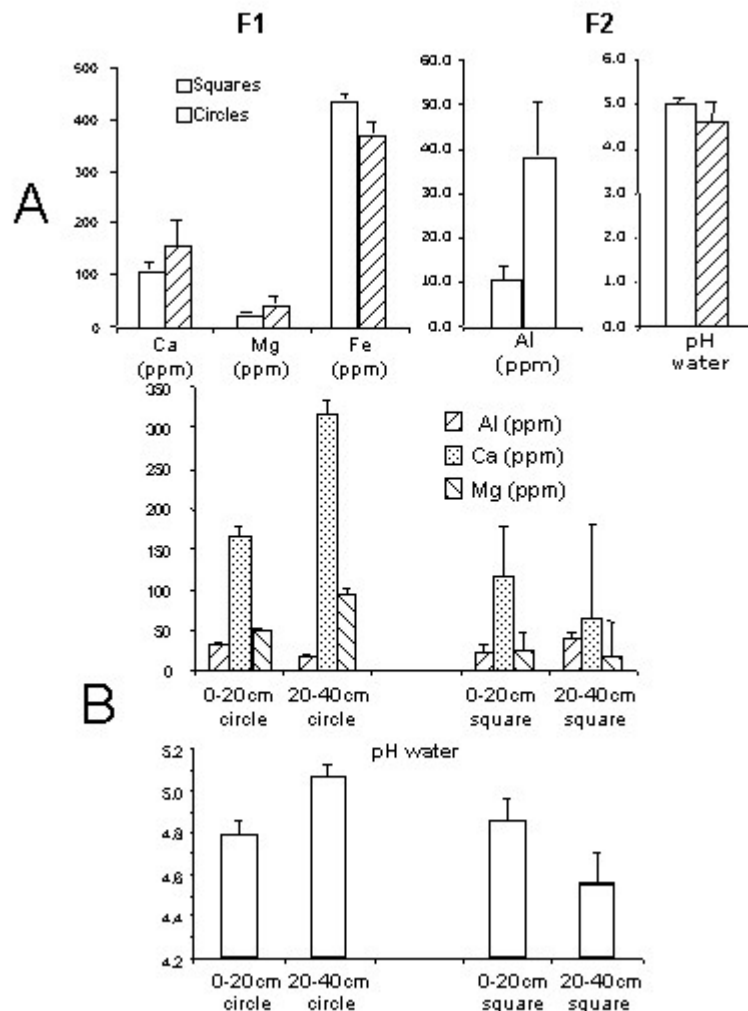
**Figure 2. Principal component analysis of the (0-20 cm) topsoil physico-chemical characteristics. The first two factors were projected separately for variables and individuals corresponding to the plots of the trial (F1 A, C and F2 B, F). D and E: projection of the factorial values on the map of the trial. (circles = positive factorial values; squares = negative factorial values).**

## Results and Discussion

### Topsoil (0-20 cm) analysis

A principal component analysis (PCA) was performed on the topsoil data (Figure 2). The two first factors represented 48% of the variability. Ca and Mg characterised the positive part of the first factor, and Fe the negative part (Figure 2A). To a lesser extent, this axis also described a particle size gradient from the positive (% clay and silt) to the negative part (% sand). This relationship with particle size also appeared on the F2 axis (Figure 2B). Taking into account all the soil elements, the PCA calculated for each factor a single value that characterised each plot. This single index represents the soil of the plot. These values can be projected on an axis with the corresponding plot numbers (Figure 2C and F).

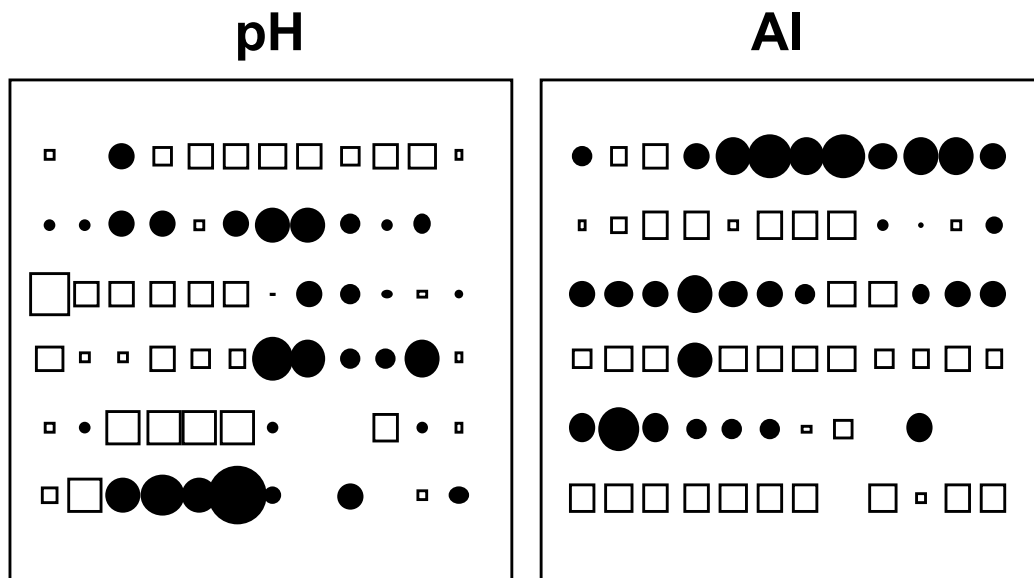
For the first factor, plots 33 and 57 had the highest positive factorial values, and plots 10, 11 and 19 the greatest negative values. The situation in plots 33 and 57 resulted from higher levels of Ca, Mg and % clay, all with positive F1 values, and lower levels of Fe, fine sand and other variables with negative factorial values (Figure 2A and C). The opposite is true for plots 11, 19 and 10. Corresponding tendencies were found for all plots having positive or negative factorial values, following a decreasing gradient from the extremities of the axis to the origin.



**Figure 3. Changes for the most important soil elements corresponding to circles or squares for the topsoil, the deeper soil layer or the plots with contrasting subsoil.**

Instead of using an axis, these factorial values can be projected on the map of the trial (Figure 2D). The positive factorial values, represented as circles, are mostly grouped in the same area on the top left of the trial, describing a soil heterogeneity. In the area with negative factorial values (i.e. the plots with squares in Figure 2D), levels of Ca, Mg and clay content were lower (Figure 3A). The reverse was true for Fe content, which increased by almost 22%.

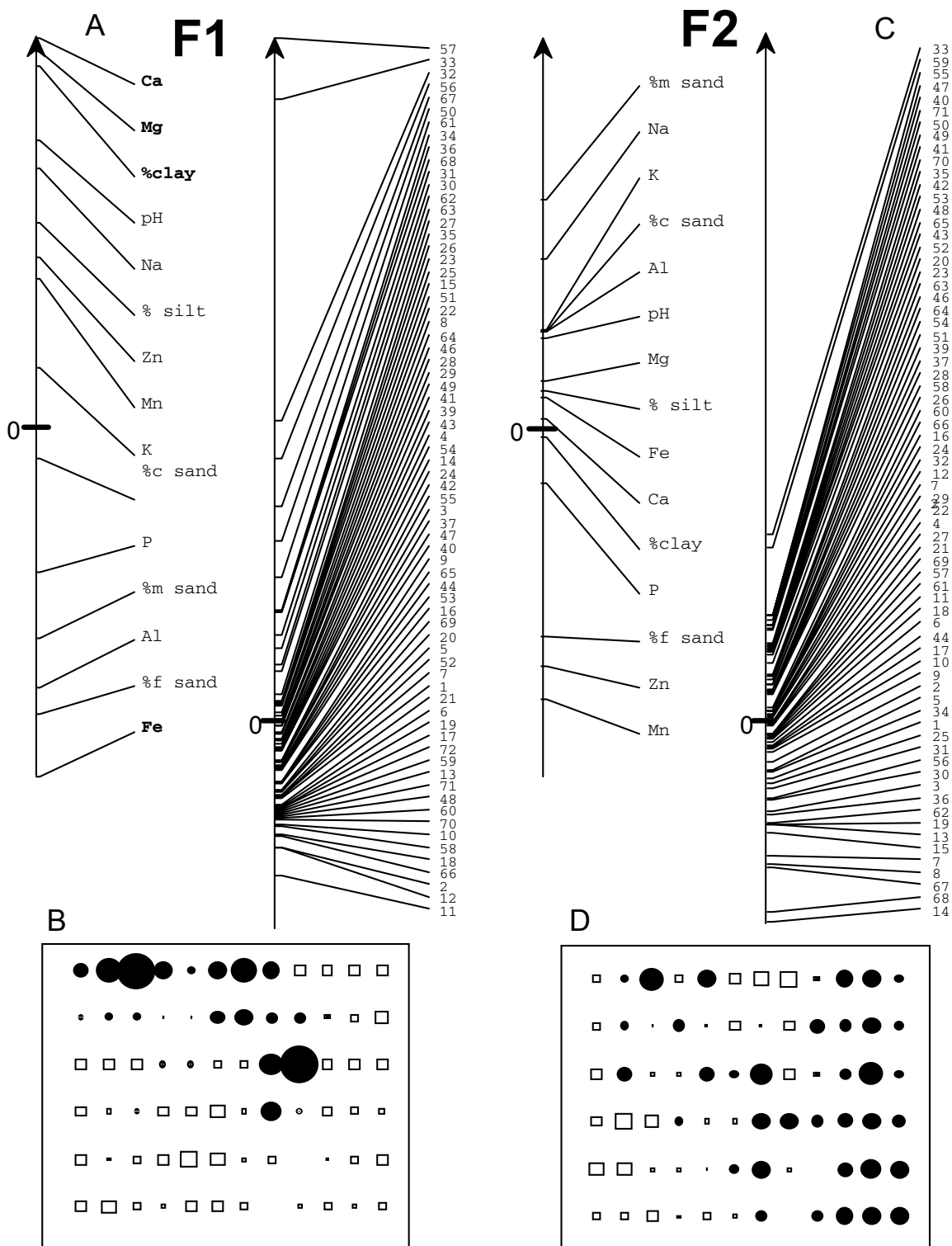
For the second factor, the projection of the factorial values on the map revealed a predominance of squares in blocks 1, 3 and 5, while circles were concentrated in the remaining blocks (Figure 2E). In the circle delimited strips, the soil elements correlated with the positive part of F2 and correspond to places with higher levels of Al in particular. The opposite was true for the square delimited areas corresponding to the negative part of F2 and representing mainly a higher pH and greater proportions of medium sand (Figure 2B, E and F). The higher levels of aluminium in blocks 2, 4 and 6 correspond to those that did not receive lime in the previous three crops. Lime was required, and had been recommended, for the whole trial but was restricted to three alternate blocks for experiment purposes. The analysis shows clearly the effect of the lime by taking into account all soil factors. Using actual levels of pH alone or Al alone does not give as clear a picture of the effect of lime (Figure 4).



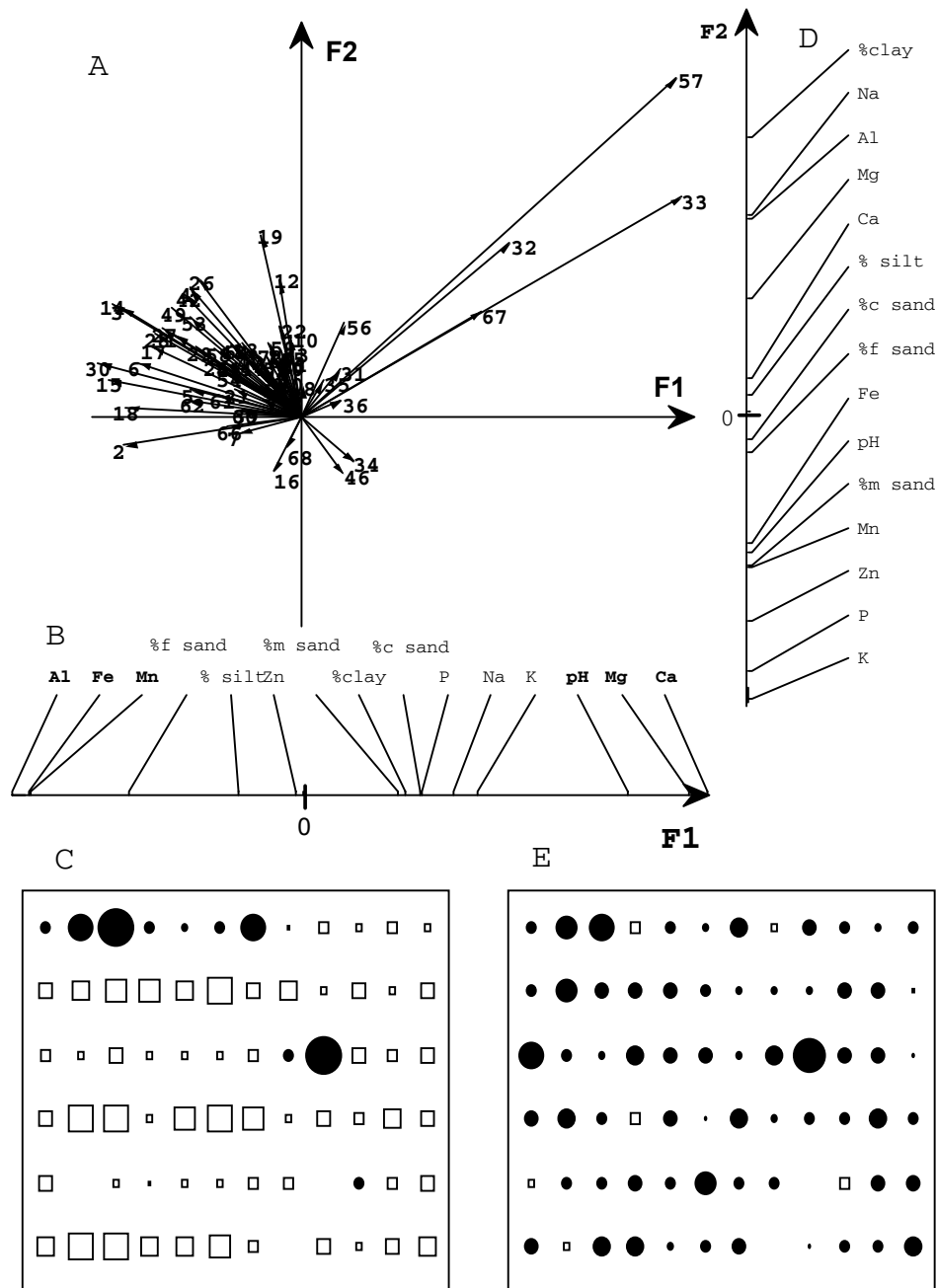
**Figure 4. Distribution of pH and Al in the different plots of the trial site. (circles = values above average; squares = values below average).**

#### *Deeper (20-40 cm) layer analysis*

The PCA performed on the soil physico-chemical characteristics in the 20-40 cm layer showed that a few plots were different from the others (Figure 5). These plots with positive F1 values had higher levels of the elements correlated with the positive part of the factor, mainly % clay (not significant;  $P < 0.05$ ), Ca and Mg, and lower levels of elements correlated with the negative part of the factor, mainly Fe (Figure 5A). When projected on the map of the trial, the circles were located in the same top left part of the site, corresponding to a large extent to the area previously identified by the topsoil analysis (Figure 5B). No structure was apparent with the second factor, indicating that the lime had not affected the deeper layer (Figure 5C and D).



**Figure 5. Principal component analysis of the physico-chemical characteristics of the deeper soil (20-40 cm). The two first factors were projected separately for variables and individuals corresponding to the plots of the trial (F1 A and F2 C). B and D: projection of the first and of the second factorial values on the map of the trial. (circles = positive factorial values; squares = negative factorial values).**



**Figure 6. Principal component analysis of the differences in the physico-chemical characteristics between the top and the deeper soil layer. A: factorial plan of the plots. The origin of the plan corresponds to the topsoil characteristics for each plot and the head of the arrow indicates the direction of the change, according to maps of the F1 variables (B) or to maps of the F2 variables (D). C and E: projection of the first and second factorial values on the map of the trial. (circles = positive factorial values; squares = negative factorial values)**

*Change in soil between the top and the deeper layer*

To study the changes between the top and the deeper layer, a PCA was performed on the differences between the two sets of data.

On the factorial plan, an arrow represented each plot (Figure 6A). The centre of the plan corresponds to the topsoil layer, and the head of the arrow indicates the direction of the change when moving to the deeper layer.

Most of the arrows were positioned in the quartile representing negative F1 and positive F2 values. This shows that, for the majority of the plots, the levels of the elements (correlated with the negative part of F1) increased in the deeper layer. These were mainly Al, Fe and Mn (Figure 6B). The contrary happened for soil characteristics correlated with the positive part of F1, mainly an increase in pH, Mg and Ca. The reverse was true for the few plots having arrows orientated in the positive part of F1, describing an unusual situation. When projected on the trial map, it appeared that this situation, represented by circles, was present in a few plots, already identified by the studies of the physico-chemical characteristics of the top and of the deeper layer (Figure 6C). For these particular plots, the pH increased when moving deeper in the soil and the Al decreased (Figure 3B). The second factor was not important and almost all plots were represented by positive factorial values (circles), indicating a relatively homogeneous change when moving to the deeper soil layer (Figure 6D and E).

### *Yields*

The average yield of the 23 plots corresponding to the area characterised by circles in the analysis of the 0-20 cm layer was significantly higher (71.7 t cane/ha) than the yield of the rest of the trial (55.1 t cane/ha) (Figure 2D and Table 1). The same was true when only the 10 plots with an unusual change between the top and the deeper layer were compared (78.5 t cane/ha versus 57 t cane/ha) (Figure 6C and Table 1). These patches possibly corresponded to ancient termite mounds, which would explain the better growth of the cane.

**Table 1. Comparison of sugarcane yields of the plots with positive F1 values (plots with circles located on top left of the trial) and of the plots on the rest of the trial (those with squares) (t test;  $P < 0.05$ ).**

Heterogeneity	Plots with positive F1 values			Other area		
	No. of plots	Average yield (t/ha)	SE	No. of plots	Average yield (t/ha)	SE
Topsoil	23	71.7	3.2	47	55.1	6.1
Bottom soil	10	78.5	8.9	60	57.0	2.9
		$P=0.012$			$P=0.008$	

When the different varieties and treatments were compared using the 70 plots, the nematicide treated plots of N16, N23 N12 and NCo376 gave the best yields (one-way ANOVA;  $P < 0.05$ ) (Table 2). When the 23 plots corresponding to the topsoil heterogeneity (circles) were removed from the analysis, and when the 10 plots corresponding to the change in soil with depth were removed, N17 treated with nematicide and untreated NCo376 shared the top of the table with the other four varieties (Table 2).



**Table 2. Comparison of the yields of the different varieties (treated or not treated with a nematicide). A: with all plots (one-way ANOVA), B: without the yields of 23 plots with a heterogeneous topsoil (positive F1 values ~ circle delimited area in Figure 2D) and C: without the 10 plots with contrasting 0-20 and 20-40 cm layers (Figure 6C). (C = untreated; T = treated with nematicide; NCo = NCo376)**

A (all the plots)				B (excl. 23 plots with positive F1 values for topsoil)				C (excl. 10 plots with contrasting layers)			
Variety	No. of plots	Yield (t cane/ha)	SE	Variety	No. of plots	Yield (t cane/ha)	SE	Variety	No. of plots	Yield (t cane/ha)	SE
N16T	6	92.1	10.6a	N12T	5	75.8	6.1a	N16T	4	78.9	10.4a
N23T	6	75.4	9.6ab	N16T	3	72.9	12.0ab	N12T	5	75.8	6.1a
N12T	6	73.0	5.8abc	N23T	5	71.1	10.4ab	N23T	6	75.4	9.6a
NCoT	6	69.9	4.5abcd	NCoT	4	70.8	5.7ab	NCoT	6	69.9	4.5a
N17T	6	67.9	3.8bcd	N17T	4	64.7	4.8abc	N17T	4	64.7	4.7ab
NCo	6	61.5	3.6bcde	NCo	4	61.7	4.9abcd	NCo	6	61.5	3.6ab
N24T	6	59.2	10.2bcde	N24T	4	53.0	5.1bcde	N23	6	51.2	9.1b
N12	6	53.9	7.6cde	N12	4	47.1	9.8cde	N12	5	50.5	8.4b
N23	6	51.3	9.1de	N23	4	47.0	12.5cde	N24T	4	48.3	6.2b
N17	6	49.8	7.0de	N17	3	40.1	4.9def	N16	6	46.3	5.9b
N16	6	46.3	5.9e	N16	4	37.8	2.1ef	N17	5	44.9	6.2b
N24	4	23.1	2.9f	N24	3	21.5	3.5f	N24	3	21.1	2.7c

### Conclusion

These statistical techniques are a very powerful way to describe soil heterogeneity and to study relationships between soil and yield, or soil and fauna, mainly because the PCA factors take into account not only one, but all soil elements together. This technique offers an unbiased method of eliminating replicates in a trials. However, reducing the number of replicates decreases the strength of the statistical analysis. A better approach would be to recalculate the yields according to the average content of the main soil components that induce the soil heterogeneity. This would not only reduce within-site variability, but would maintain the full complement of replicates and allow the use of the Fisher block ANOVA.

### REFERENCE

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