



**Full Length Article**

## Soil Acidification in Cocoa (*Theobroma cocoa*) Agrosystems in Côte d'Ivoire: II. Dolomite and Calcitic Lime Application Impact on Microbial Activities and Metals Availability in Soils

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### Abstract

Liming is a practice commonly used in agriculture to increase nutrient bioavailability and improve soil fertility, particularly in highly acidic, nutrient-poor soils. However, its impact on bacterial communities and the link to reduce metal mobility is still unknown in cocoa agrosystems. The aim of this study was therefore to investigate the interactions between lime application and bacterial activities in order to determine their effects on the availability of metals ( $Al^{3+}$  and  $Cd^{2+}$ ) in the cocoa rhizosphere. Three years after lime (agricultural quicklime and dolomite) application in experimental cocoa agrosystem plots, soils surface horizons (0–20 cm) were sampled in limed and unlimed plots at Béniankré, Yaou and Eholié sites in Aboisso region (south-east of Côte d'Ivoire) for chemical analysis (pH and metal content) and microbiological analysis (enzymatic activity, number of bacterial microflora). The results showed that application of lime significantly affected the acid-base status of the soil, with a net increase (+1 to 2 pH units) in pH in limed soils, reduce in the content of exchangeable and water-soluble  $Al^{3+}$  and  $Cd^{2+}$  (0 to 63%) content in soil, number of cultivable bacterial microflora (29 to 50%). This application did not affect significantly the total microbial activities in soil (fall ranging from 0 to 23%). These results could help in the sustainable management of cocoa agrosystem soils. However, they will need to be supplemented by additional studies to assess season impact on changes in the microbial community and their adaptation in these agroecosystems after liming. © 2023 Friends Science Publishers

**Keywords:** Liming; Metals; Microbial activity; Rhizosphere; Cocoa; Aboisso

### Introduction

Introduced to Côte d'Ivoire in 1888 in Aboisso region, cocoa farming has grown exponentially to occupy a central place in the Ivorian economy. With 2,400,000 tons of cocoa produced per year, Côte d'Ivoire ranks first in the world with 42% of global production (ICCO 2015). Covering around 2,500,000 ha, the expansion of cocoa farming in Côte d'Ivoire has been at the expense of forest areas, which have drastically declined more than 80% since the 1960s (Chatelain *et al.* 2004). Coupled with inappropriate agricultural practices, cocoa farming contributes to rapid soil degradation, in particular soil acidification, which is accelerated by natural acidification processes. In the Aboisso region, one of the first and pioneering cocoa-producing areas in the south-east of Côte d'Ivoire, soils are highly degraded and very acidic, with pH values varying between 3 and 5 (Kouakou *et al.* 2021). As a result, these first cocoa-producing areas are being abandoned and cocoa production area is migrating from the south-east and east to

the south-west and west of Côte d'Ivoire, with the direct consequence of irreversible loss of several hectares of primary forest (Verdeaux 1988).

In Côte d'Ivoire, soil acidification remains a major problem in cocoa-based agrosystems. It is known that soil acidification leads to the solubilization of metals such as aluminium (Al), manganese (Mn), iron (Fe), etc., in the soil solution (Koenig *et al.* 2011). These metals, in their free state in the soil solution, have various negative effects on the nutrition of plants, including cocoa (Alloway and Ayres 1997; Baligar and Fageria 2005). When released into the soil solution, the ions  $Al^{3+}$ ,  $Fe^{2+}$ ,  $Mn^{2+}$  are toxic to plants at a certain level and also fix phosphorus in acid soil, making it unavailable to the plant (Omenda *et al.* 2021). Furthermore, aluminium toxicity, along with other changes in soil chemistry and biology linked to pH decreasing, can have a major influence on the structure and function of the microbial community, thereby modifying the nutrient cycle, crop productivity and all ecosystem services. In acidic soil conditions, certain heavy metals such as copper

(Cu), iron (Fe), manganese (Mn), arsenic (As), cadmium (Cd) and zinc (Zn), when present in excess in the soil solution, can potentially be transferred and accumulate in the cocoa bean during biogeochemical processes (Ogunlade and Agbeniyi 2011; Gramlich *et al.* 2017; Anyimah-Ackah *et al.* 2019; Abt and Robin 2020; Gil *et al.* 2021; Vanderschueren *et al.* 2021). Soil acidification not only leads to a risk of loss of cocoa bean quality, but also to a fall in orchard yields, leading to their gradual abandonment or conversion to other monocultures i.e., rubber or oil palm (Balac 2002).

Sustainable cocoa production in Côte d'Ivoire is not assured, and research needs to be undertaken to restore soils degraded by acidification and prevent risks in new cocoa production areas. Liming appears to be a sustainable alternative to chemical inputs and other agricultural practices harmful to cocoa farming. It has beneficial effects on soil health, by reducing soil acidity so that beneficial organisms can thrive and continue to contribute to soil health and crop productivity (Fuentes *et al.* 2006; Mühlbachová and Tlustoš 2006). However, the benefits of carbonate soil improvers (increased pH, reduced metal concentration, etc.) on soil health are little known in tropical zones. It is in this context that lime was applied to three cocoa plots in the Aboisso region (south-east of Côte d'Ivoire) in order to investigate the impact of liming on bacterial activity and the dynamics of trace metal elements in the cocoa rhizosphere. A precise understanding of the response of beneficial soil microbial populations to liming in these agrosystems may provide valuable insights into sustainable approaches to soil acidification problems in the Aboisso region and current cocoa-growing expansion zones in Côte d'Ivoire. The study therefore verifies two hypotheses: (i) the lime might increase bacterial activity in the rhizosphere of the cocoa tree and (ii) this amendment might lead to a decrease in the content of free metals in the rhizosphere of the cocoa tree.

## Materials and Methods

### Description of the study area

The study was carried out on cocoa plantations in the Aboisso region of south-eastern Côte d'Ivoire (Fig. 1). The area is characterised by a transitional equatorial tropical climate that is always humid. This climate is characterized by an average annual temperature of 27°C and an average annual rainfall of 1,500 mm. The vegetation consists of dense forests and hydromorphic formations. It was opened up at a very early stage to agriculture and is now heavily anthropized. The relief is very uneven, particularly in the north-east and east, with a geological substratum consisting of plutonic rocks (mainly granite), metamorphic rocks (Schist) and sands (Tertiary and Quaternary). The soils belong to the group of ferralsols that are highly leached at their base, very acidic and hydromorphic soils.

### Lime application and soil sampling

The soils of cocoa plantation experimental plots located at Béniankré, Yaou and Eholié in the Aboisso region are very acidic, with pH values varying between 4 and 5. A soil acidity correction treatment was therefore applied to experimental plots (1 ha) in the cocoa plantations. This correction consisted of a lime application to soil. Applied lime is composed of agricultural quicklime (94% CaO, Calbux® Granular 15) combined with dolomite (32.7% Ca, 19.5% Mg, LDC®). These products were applied by fractionation over three years at a rate of 3 t ha<sup>-1</sup>, i.e., 0.6 t ha<sup>-1</sup> year<sup>-1</sup> of quicklime and 0.4 t ha<sup>-1</sup> year<sup>-1</sup> of dolomite as a remedial and corrective amendment respectively. Three years after the first application of carbonates, a soil sampling campaign was carried out on the three plots. Soil samples were taken from each limed and control (unlimed) plot using an auger at a depth of 0–20 cm within a radius of 0.8 m containing the lateral roots around the cocoa trees. Five soil samples per tree were taken from around 10 cocoa trees per site. The five samples per tree were mixed on site to the same volume to form one composite sample per cocoa tree. In this way, 10 composite samples per treatment (limed or not) were taken at random from all 3 selected sites. For the microbiological analyses, soil samples were taken from each composite sample using a sterilized spatula and stored in hermetically sealed Nalgene tubes®. The remaining samples were sieved to 2 mm on site, packed in bags and transferred to the laboratory. In the laboratory, these samples were dried in ambient air until the weight stabilized prior to analyses.

### Microbiological and chemical laboratory analysis

After characterising the initial soil properties before the application of carbonates, microbiological and chemical analyses were carried out on sampled soil. Two types of microbiological analysis were carried out during this study. These were the total cultivable bacteria number and the total enzymatic activity of the soil as total microbial activities. The most probable number (MPN) technique was used to count the total bacteria microflora (Jarvis *et al.* 2010). One gram of fresh soil was mixed with 9 mL of sterile mineral water, then shaken for 1 h. The mixture was then centrifuged for 1 min at 500 rpm. One hundred (100) µL of the supernatant obtained was collected, and a series of daughter solutions was obtained by several dilutions (from 10<sup>-1</sup> to 10<sup>-6</sup>). Twenty (20) µL of each daughter solution was used to inoculate microplates containing 180 µL of NB (Nutriment Broth) culture medium. The microplates were incubated at 28°C for 48 h, after which the density was read at 620 nm using a spectrophotometer to count total microflora. The most probable number of microorganisms was then determined by calculating using the standard deviation of the log 10 MPN (SD log 10 MPN) and 95% confidence index.

The total enzymatic activity of the soil was determined by hydrolysis of fluorescein diacetate (FDA) (Green *et al.* 2006). Briefly, a mixture of 1 g of fresh soil, sodium phosphate and FDA was incubated after shaking at 28°C. The mixture was then centrifuged, and the optical density of the supernatant was measured at 490 nm using a spectrophotometer. Enzyme activity was then determined using a calibration curve based on a standard solution.

The chemical analyses focused on soil pH and metal content ( $\text{Al}^{3+}$  and  $\text{Cd}^{2+}$ ). The pH of soil was determined in the supernatant, after agitation and centrifugation of the soil-water mixture in a 1/5 (m/V) ratio. Heavy metal content was determined on the water extracts (water-soluble fraction) and using a neutral calcium chloride salt solution (exchangeable fraction). A 5 g sample of fine soil was mixed and then shaken in a tube with 50 mL of double-distilled water. After centrifugation, the supernatant is recovered and acidified for future assays. The pellet resulting from this extraction was placed in contact with 50 mL of a 0.1 M  $\text{CaCl}_2$  solution. Similarly, after shaking and centrifuging, the supernatant was collected for assay of heavy metals ( $\text{Al}^{3+}$  and  $\text{Cd}^{2+}$ ). Heavy metal content of the extracted solutions was determined by colorimetric methods using a spectrophotometer analysis kit on the HACH DR<sup>®</sup> spectrophotometer at wavelengths of 522 nm for  $\text{Al}^{3+}$  and 515 nm for  $\text{Cd}^{2+}$ .

### Statistical analysis of data

All univariate analyses were performed using JMP13, software. A repeated measures ANOVA was applied to identify the effect of treatments (limed or not). A one-way ANOVA followed by a comparison of Student-Newman-Keuls (SNK) test was used to identify significant differences between treatments for sites, with a significance level of 5% ( $P < 0.05$ ). We used ANOVA because data were sufficiently homogeneous within groups and with sufficient normality.

## Results

### Initial physical and chemical properties of soils

Table 1 gives an overview of selected initial physical and chemical properties of the soils. These results indicate that the soil textures were dominated by sandy types, with the exception of Yaou, where more than 40% of the granulometry was dominated by clay. The pH values of the soils enabled to distinguish two classes of acidity: the soils sampled at Béniankré had a  $\text{pH} < 5$  was soil with very acid reactions, and the soils at Yaou and Eholié had a  $\text{pH} < 5.5$ , thus belonging to the class of acid soils. According to the organic matter content, these soils are considered to be moderately poor in organic matter. These studied soils were also depleted in bases, with values below the minimum threshold limit of 11, 2.45 and 0.70  $\text{cmol}_c \text{kg}^{-1}$  respectively for  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$  (Snoeck *et al.* 2016). A fairly low fertility of these soils, suggested by the low values of

exchangeable bases, was confirmed by the CEC. The saturation rate clearly indicated a low proportion of base cations on the CEC. Al content of these soils was below the average content of the continental crust ( $158 \text{ g kg}^{-1}$ ), while Cd content was 4 to 5 times lower than the average composition of the continental crust ( $98 \mu\text{g kg}^{-1}$ ).

### Effect of calcomagnesium amendment on soil properties

Fig. 2 shows the variations in soil pH after amendment. The results showed an average increase of  $1.90 \pm 0.72$  pH units on all three sites, with variations ranging from 1.06 at Béniankré to 2.36 at Eholié. Fig. 3 showed the levels of metals ( $\text{Al}^{3+}$  and  $\text{Cd}^{2+}$ ) extracted from the soils using distilled water and a solution of  $\text{CaCl}_2$  from the various sites. Overall, Béniankré soils showed the highest levels of soluble metals, followed by the Eholié site with the highest levels of water-soluble Cd. The results also showed that, irrespective of the site, the metal content of limed soils was lower than that of unlimed soils. This reduction was greater when the extraction was carried out with water, particularly for Cd, whereas for aluminium, the difference between the two extracting solutions is not significant ( $P > 0.05$ ). For exchangeable and water-soluble Al (Fig. 3A–B), the content decreasing varied from 63% at Béniankré to 0% Eholié and from 56% at Béniankré to 0% Eholié compared with the unlimed soils for the salt and water extracts respectively. In the case of Cd (Fig. 3C–D), decreases in soil content ranged from 19% at Béniankré to 52% at Yaou and from 33% at Eholié to 59% at Yaou compared with unlimed soils for salt extracts and water, respectively.

### Bacterial activity in soils

Changes in total cultivable bacterial microflora in limed and unlimed soils at Béniankré, Yaou and Eholié sites are shown in Fig. 4A. Cultivable bacterial microflora in limed soils were systematically lower than in unlimed soils (Fig. 4A), by 49, 50 and 29% at Béniankré, Yaou and Eholié respectively. However, statistical analyses showed that the differences between the sites were not statistically significant ( $P < 0.05$ ), regardless of the treatment. This decline in cultivable soil bacterial microflora was also reflected in a slight fall in the total enzyme activity at the various sites (Fig. 4B), mainly at Eholié and Yaou. At all sites, enzyme activity was very low, ranging from 1.87 to  $3.30 \mu\text{g fluorescein g}^{-1} \text{ h}^{-1}$ . The decreases in enzyme activity induced by the lime application were on average 23, 7 and 0%, respectively in the Eholié, Yaou and Béniankré soils. However, these differences were not statistically significant ( $P > 0.05$ ) for Yaou and Eholié.

### Interaction between amendments, microbial activity and metal availability

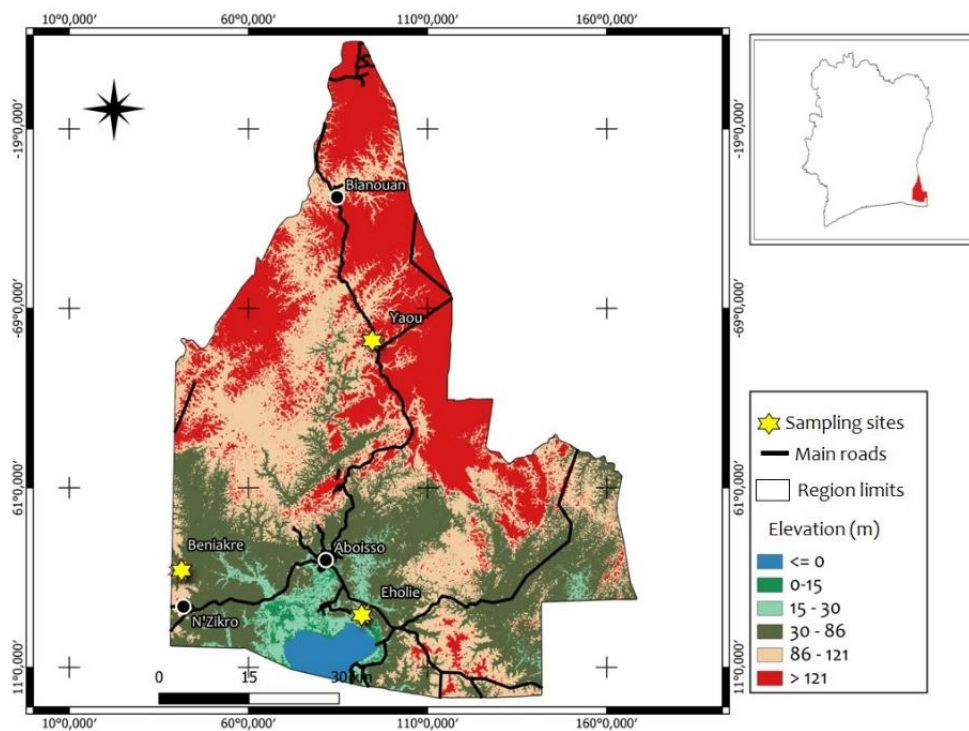
Tables 2 and 3 show the correlation matrices between the parameters analysed in this study for limed and control soils.

**Table 1:** Selected characteristics of initial soil properties in Aboisso region

Characteristics	Unit	Béniankré	Yaou	Eholié
Clay	%	14.30 ± 3.45	49.60 ± 2.75	18.00 ± 1.53
Silt	%	4.40 ± 3.09	24.30 ± 2.42	7.90 ± 1.82
Sand	%	81.20 ± 3.72	26.10 ± 3.8	74.20 ± 2.86
pH	-	4.50 ± 0.35	5.10 ± 0.30	5.40 ± 0.4
OM	(%)	2.10 ± 0.19	3.60 ± 0.13	1.90 ± 0.21
CEC	-	4.40 ± 1.94	4.60 ± 1.14	4.40 ± 1.13
Ca <sup>2+</sup>	cmol <sub>c</sub> kg <sup>-1</sup>	0.10 ± 0.04	1.50 ± 0.19	0.30 ± 0.20
Mg <sup>2+</sup>	cmol <sub>c</sub> kg <sup>-1</sup>	0.10 ± 0.06	0.20 ± 0.03	0.10 ± 0.04
K <sup>+</sup>	cmol <sub>c</sub> kg <sup>-1</sup>	0.03 ± 0.01	0.06 ± 0.03	0.03 ± 0.02
Na <sup>+</sup>	cmol <sub>c</sub> kg <sup>-1</sup>	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.02
S/T	%	3.90 ± 1.34	14.10 ± 2.70	10.50 ± 2.10
Al <sub>2</sub> O <sub>3</sub>	g kg <sup>-1</sup>	129.60 ± 3.00	90.47 ± 4.7	90.20 ± 2.50
Pseudo-total Cd	mg kg <sup>-1</sup>	0.17 ± 0.02	0.10 ± 0.02	0.20 ± 0.03

**Table 2:** The correlation matrices between the parameters analysed in this study for unlimed soil

Variables	Al <sub>CaCl2</sub>	Al <sub>H2O</sub>	Cd <sub>CaCl2</sub>	Cd <sub>H2O</sub>	FDA	MPN
pH	0.36	0.47	0.63	-0.34	0.36	0.64
Al <sub>CaCl2</sub>		0.97	0.90	0.15	0.55	-0.19
Al <sub>H2O</sub>			0.96	0.03	0.55	-0.11
Cd <sub>CaCl2</sub>				-0.09	0.52	0.06
Cd <sub>H2O</sub>					0.20	-0.25
FDA						0.11



**Fig. 1:** Location of study site

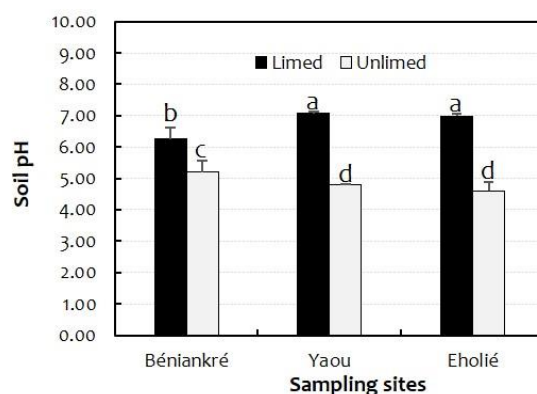
In the control soils (Table 2), exchangeable Al<sup>3+</sup> and Cd<sup>2+</sup> had a positive impact on the total enzymatic activity of the soil, with the exception of water-soluble Cd<sup>2+</sup>. On the other hand, the total cultivable bacterial microflora was weakly, even independent of the presence of these cations, but seemed to be controlled by the soil pH. In contrast under

these conditions, it is very surprising to find a positive interaction between these cations and soil pH, with the exception of water-soluble Cd<sup>2+</sup>. For limed soil (Table 3), water-soluble and exchangeable metals and the total enzymatic activity of the soil are inversely affected by the

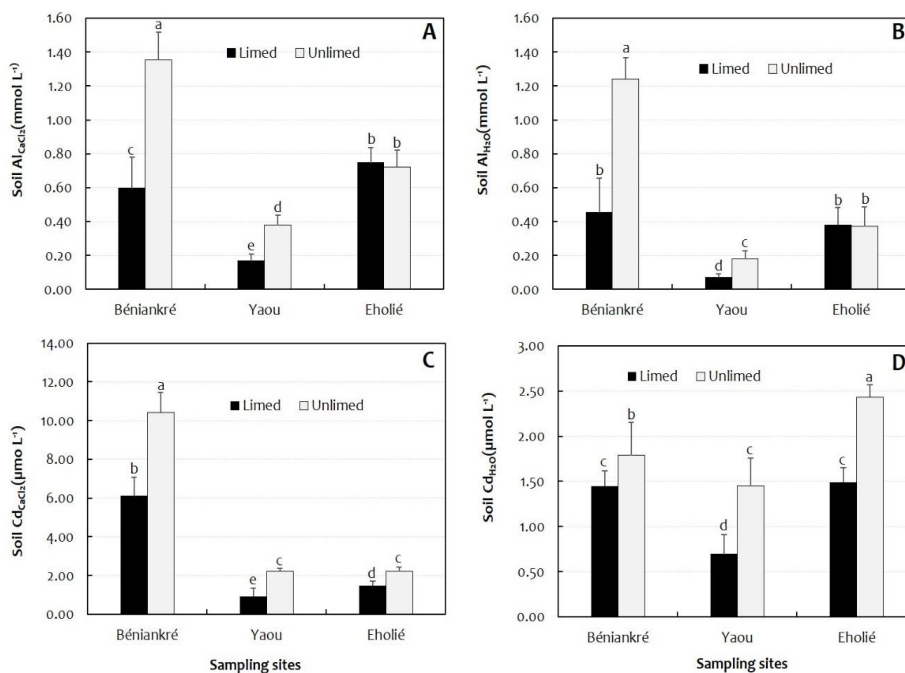


**Table 3:** The correlation matrices between the parameters analysed in this study for limed soil

Variables	Al <sub>C<sub>a</sub>Cl<sub>2</sub></sub>	Al <sub>H<sub>2</sub>O</sub>	Cd <sub>C<sub>a</sub>Cl<sub>2</sub></sub>	Cd <sub>H<sub>2</sub>O</sub>	FDA	MPN
pH	-0.48	-0.78	-0.93	-0.55	-0.80	0.00
Al <sub>C<sub>a</sub>Cl<sub>2</sub></sub>		0.90	0.40	0.92	0.10	0.41
Al <sub>H<sub>2</sub>O</sub>			0.66	0.86	0.42	0.35
Cd <sub>C<sub>a</sub>Cl<sub>2</sub></sub>				0.56	0.86	-0.07
Cd <sub>H<sub>2</sub>O</sub>					0.33	0.37
FDA						-0.02



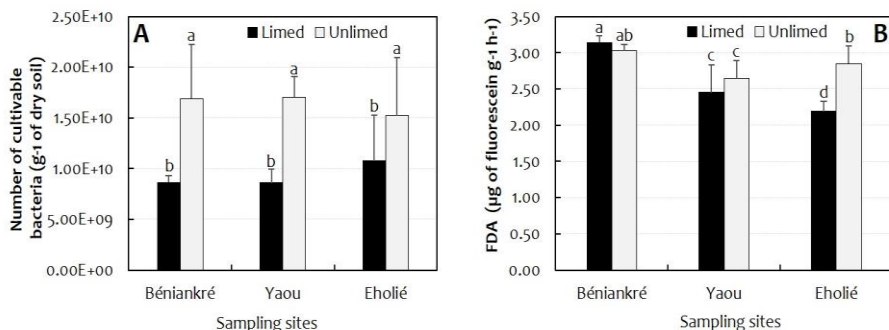
**Fig. 2:** Soil pH variation after soil amendment. Bars indicate the standard deviation (N=10) and the same letters in each figure indicate that the difference is not statistically significant at  $P > 0.05$



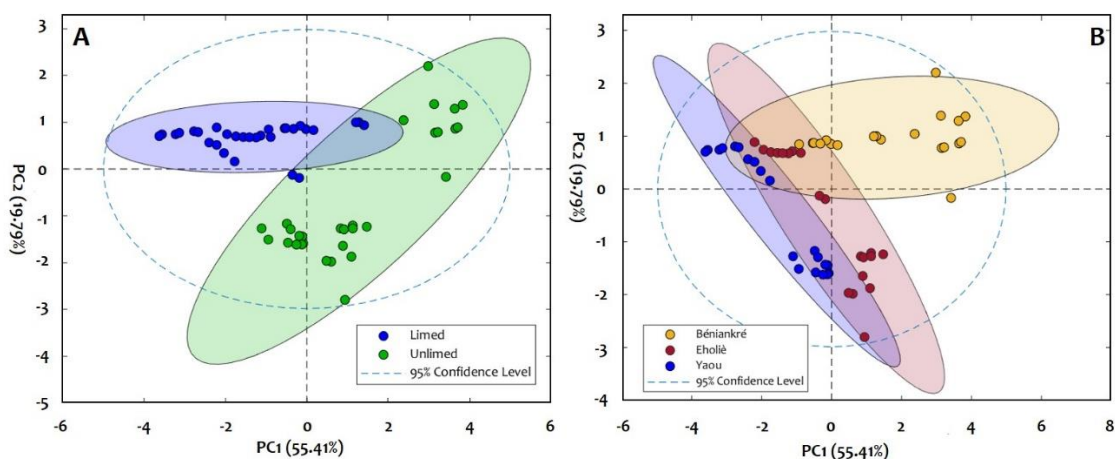
**Fig. 3:** Exchangeable and labile metal contents of Al ( $\text{mmol L}^{-1}$ ) (A and B) and Cd ( $\mu\text{mol L}^{-1}$ ) (C and D) in soil. Bars indicate the standard deviation (N=10) and the same letters in each figure indicate that the difference is not statistically significant at  $P > 0.05$

decrease of soil pH, unlike the total cultivable bacterial microflora. There was also an effect of  $\text{Al}^{3+}$  (exchangeable and water-soluble) and water-soluble  $\text{Cd}^{2+}$  on total cultivable bacterial microflora. Under these conditions, soil

acid-base status of the soil would be one of the factors regulating microbial activity and cation levels ( $\text{Al}^{3+}$  and  $\text{Cd}^{2+}$ ) in the soil (Fig. 4), and the second factor would be the nature of the site (Fig. 5).



**Fig. 4:** Number of cultivable bacteria ( $\text{g}^{-1}$  of dry soil) (A) and total microbial activity using fluorescein diacetate (FDA) ( $\mu\text{g}$  of fluorescein  $\text{g}^{-1} \text{h}^{-1}$ ) (B) in soil. Bars indicate the standard deviation ( $N=10$ ) and the same letters in each figure indicate that the difference is not statistically significant at  $P > 0.05$



**Fig. 5:** Principal component analysis (PCA) of the soil chemical parameters, soil cultivable bacteria and total microbial activity in control and limed soil groups (A) or based on studied sites (B)

## Discussion

The effect of liming on microbial activity showed a general decrease in the number of cultivable bacterial microflora. This lower proliferation of bacteria in the presence of the lime could be due to the change in environmental conditions. Various authors have revealed that lime application to soil induces a change in the acid-base status, with a tendency towards alkalinisation (Johnson *et al.* 2005). This variation in soil pH could therefore lead to a decline in total microflora, both bacteria and fungi. However, some results underline that lime application effect on the bacterial community depends on the quantity of applied lime (Ding *et al.* 2023). For small quantities, carbonate soil improver will tend to improve the structure of the bacterial community and increase the relative abundance of species in the soil bacterial community, whereas the application of large quantities could inhibit the growth and activity of microorganisms, leading to a reduction in soil microbial biomass and diversity (Johnson *et al.* 2005; Ding *et al.* 2023).

In this study, results indicating variability in the decline in the number of cultivable bacteria following the site could be explained by initial soil chemical parameters such as organic matter and soil pH, as suggested by several studies (e.g., Andersson and Nilsson 2001; Kemmitt *et al.* 2005; Schroeder *et al.* 2018). These studies found an increase in microflora, probably due to the presence of nutrients readily available to microorganisms. Balland-Bolou-Bi and Poszwa (2012), linked this to the heterogeneity of the communities of microorganisms present at each site and their specific function. According to Bongoua-Devisme *et al.* (2012), an organic amendment to saline soils induces a proliferation in the number of bacteria and a change in soil salinity after the organic amendment has been applied. These results therefore suggested that fall in the number of cultivable bacteria could be linked to the presence of strict acidophilic microflora whose numbers and activities are affected by the alkalinisation of the soil (+1 to 2 pH units), resulting in drop-in total enzymatic activities. Soil enzymes are mainly due to microbial origin (Ladd 1978). They are linked to the activity and abundance of

microorganisms (Dick *et al.* 1996). Measurements of the enzymatic activity of the microflora in studied soils generally revealed a higher level of enzymatic activity in unlimed than limed soils, irrespectively of the site. However, statistical differences were not significant ( $P > 0.05$ ) between the three sites. Recent research has shown that microbial abundance decreases in acidic soils while it increases in neutral soils (Shangguan *et al.* 2019). Thus, the link between the fall in the number of bacterial microflora and the total enzymatic activities could reflect a change in the bacterial and fungal community as reported by numerous studies (Cassman *et al.* 2016; Wan *et al.* 2019). According to Ahmad *et al.* (2013) and Yin *et al.* (2021), long-term liming not only reduces soil acidity, but also promotes the growth of neutrophilic bacteria by reducing the carbon available for bacterial growth.

Exchangeable metals have higher concentrations on average than water-soluble metals. This efficiency of the saline solution in extracting more metals may be due to the fact that it allows the extraction of potentially bioavailable fraction, which includes the exchangeable and free fractions (Gupta and Sinha 2007; Sutherland *et al.* 2011), unlike water extraction, which only allows the water-soluble fraction to be assessed (Peijnenburg and Jager 2003). The results showed an increase of the content of water-soluble and exchangeable  $Al^{3+}$  and  $Cd^{2+}$  from unlimed to limed soils. This suggested a beneficial effect of calcium-magnesian amendment in adjusting pH to reduce the relative solubility of metals in soils (Brallier *et al.* 1996). This beneficial effect is reflected in a decrease of the content of water-soluble and exchangeable  $Al^{3+}$  and  $Cd^{2+}$  in limed soils. This fall in metal content can be explained by the replacement of  $Al^{3+}$  and  $Cd^{2+}$  ions bound to soil colloidal complexes by  $Ca^{2+}$  and  $Mg^{2+}$  cations (Rahajaharitombo 2004). In fact, rapid decrease in  $Al^{3+}$  content and  $Cd^{2+}$  in soils could correspond to the neutralization and fixation of these cations and protons in unavailable forms by the  $OH^-$  ions added by the soil improver limes, leading to an increase in pH (Chuan *et al.* 1996). This would mean that the exchange reactions of  $Al^{3+}$  and  $Cd^{2+}$  ions at the level of colloidal complexes take place before the neutralization of protons (Djondo 1995). The difference between sites in terms of metal depletion could be linked to the initial soil content.

Under this study conditions, the number of cultivable bacteria appeared to be independent of the metal content, whose affinity with enzymatic activity could have resulted from a change in the community, as discussed above. The data therefore indicated a generally significant positive relationship between microbial activity (enzymatic activity) and the levels of exchangeable and water-soluble metals ( $Al^{3+}$  and  $Cd^{2+}$ ). In other words, microbial activity would be enhanced by the presence of metals (Jaworska and Lemanowicz 2019; Bartkowiak *et al.* 2020), both before and after soil liming. On the contrary, significant negative correlations were observed between heavy metals and phosphatase activity in certain soils (Madejón *et al.* 2001).

This negative correlation was probably due to a reduction in enzyme activity in soils fertilized with soluble phosphate and therefore with high levels of available P (Madejón *et al.* 2001). In this study, a positive effect of  $Al^{3+}$  (exchangeable and water-soluble fractions and  $Cd^{2+}$  (exchangeable fraction) on total enzymatic activities could be due to the development of new strategies by the bacteria remaining in the cocoa rhizosphere (Certini *et al.* 2004; Carson *et al.* 2007), after liming which led to a change in the chemical conditions of soil. This would result in a variation in the density and activities of the microflora, probably due to specificity of the current bacterial communities present in soil (Balland-Bolou-Bi and Poszwa (2012). This suggested that solubilization of metals was due to an increase in organic acids in the soil by bacteria adapted to the new environmental conditions. Santelli *et al.* (2001) found, for example, that bacterial abundance was positively correlated with the degree of mineral weathering. The results of these previous studies indicate that minerals are colonized by bacterial communities that are specific to the environmental conditions, resulting in extensive alteration of these minerals, as suggested by the correlations between enzymatic activity and metal content. The solubilization of metals induced by the presence of acidophilic bacteria in the soil would be offset by the neutralization of these metals by the massive addition of calcomagnesium amendments. This neutralization would be more important for  $Al^{3+}$  than  $Cd^{2+}$  in these soils.

## Conclusion

The application of lime (agricultural quicklime and dolomite) significantly affected the acid-base status of the soil, with a net increase in pH in limed soils compared with the control soils. This alkalization of the soil resulted in a reduction in the content of exchangeable and water-soluble  $Al^{3+}$  and  $Cd^{2+}$  in soil, which may be due to the displacement of elements from more labile forms to more stable form and therefore less extractable forms. Increase in soil pH led to a decrease in the total number of cultivable bacteria, while having a positive impact on total enzymatic activity in the soil. The new specific microbial communities would have developed different and effective strategies for adapting, probably by releasing organic acid, which was highly complexing and led to a drop-in the availability of  $Al^{3+}$  and  $Cd^{2+}$  in the soils following the changes in chemical conditions induced by the liming. In order to better understand the changes induced by soil liming in the cocoa agrosystems, it is advisable to carry out polymerase chain reaction analyses of the bacteria and fungi present in the cocoa rhizosphere in order to identify and characterise the types of microorganisms functionally involved in the mobilization of metals. In addition, it would be appropriate to assess the impact of the season on changes in the microbial community and their adaptation in these agroecosystems after liming.

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## Author Contributions

BBBE – Conceptualization, methodology, visualization and writing original draft and funding acquisition; KAH – Methodology, Investigation and formal analysis; EDJB – Methodology, investigation and visualization and supervision. All the authors read and approved the final manuscript.

## Conflicts of Interest

The authors declare that they have no conflict of interest.

## Data Availability

Data presented in this study will be available on a fair request to the corresponding.

## Ethics Approval

Not applicable to this paper.

## References

- Abt E, LP Robin (2020). Perspective on cadmium and lead in cocoa and chocolate. *J Agric Food Chem* 68:13008–13015
- Ahmad W, B Singh, FA Dijkstra, RC Dalal (2013). Inorganic and organic carbon dynamics in a limed acid soil are mediated by plants. *Soil Biol Biochem* 57:549–555
- Alloway BJ, DC Ayres (1997). *Chemical Principles of Environmental Pollution*, 2<sup>nd</sup> edn. Blackie Academic & Professional (Chapman & Hall), London
- Andersson S, SI Nilsson (2001). Influence of pH and temperature on microbial activity, substrate availability of soil-solution bacteria and leaching of dissolved organic carbon in a mor humus. *Soil Biol Biochem* 33:1181–1191
- Anyimah-Ackah E, IW Ofori, HE Lutterodt, G Darko (2019). Exposures and risks of arsenic, cadmium, lead, and mercury in cocoa beans and cocoa-based foods: A systematic review. *Food Qual Saf* 3:1–8
- Balac R (2002). Dynamiques migratoires et économie de plantation. In: *La Côte d'Ivoire à L'aube du 21<sup>ème</sup> Siècle: Défis Démographiques et Développement Durable*, pp:195–231. Tapinos G, P Hugon, P Vimard (Eds.). Karthala, Paris, France
- Baligar VC, NK Fageria (2005). Soil aluminum effects on growth and nutrition of cacao. *Soil Sci Plant Nutr* 51:709–713
- Balland-Bolou-Bi C, A Poszwa (2012). Effect of calco-magnesian amendment on the mineral weathering abilities of bacterial communities in acidic and silicate-rich soils. *Soil Biol Biochem* 50:108–117
- Bartkowiak A, J Lemanowicz, R Lamparski (2020). Assessment of selected heavy metals and enzyme activity in soils within the zone of influence of various tree species. *Sci Rep* 10:14077
- Bongoua-Devisme AJ, C Mustin, J Berthelin (2012). Responses of iron-reducing bacteria to salinity and organic matter amendment in paddy soils of Thailand. *Pedosphere* 22:375–393
- Brallier S, RB Harrison, CL Henry, X Dongsen (1996). Liming effects on availability of Cd, Cu, Ni and Zn in a soil amended with sewage sludge 16 years previously. *Water Air Soil Pollut* 86:195–206
- Carson JK, D Rooney, DB Gleeson, N Clipson (2007). Altering the mineral composition of soil causes a shift in microbial community structure. *FEMS Microbiol Ecol* 61:414–423
- Cassman NA, MF Leite, Y Pan, M de Hollander, JA van Veen, EE Kuramae (2016). Plant and soil fungal but not soil bacterial communities are linked in long-term fertilized grassland. *Sci Rep* 6:23680
- Certini G, CD Campbell, AC Edwards (2004). Rock fragments in soil support a different microbial community from the fine earth. *Soil Biol Biochem* 39:1119–1128
- Chatelain C, H Dao, L Gautier, R Spichiger (2004). Forest cover changes in Côte d'Ivoire and Upper Guinea. In: *Biodiversity of West African Forests: An Ecological Atlas of Woody Plant Species*, pp:45–82. Bongers F, P Loorens, FN Kouamé, WD Hawthorne (Eds.). CABI Publishing, Wallingford, UK
- Chuan M, G Shu, J Liu (1996). Solubility of heavy metals in a contaminated soil: Effects of redox potential and pH. *Water Air Soil Pollut* 90:543–556
- Dick RP, DP Breakwell, RF Turco (1996). Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. *Meth Assess Soil Qual* 49:247–271
- Ding Z, B Ren, Y Chen, Q Yang, M Zhang (2023). Chemical and biological response of four soil types to lime application: An incubation study. *Agronomy* 13:504
- Djondo MY (1995). Propriétés d'échange ionique des sols ferrallitiques argileux de la vallée du Niari et sableux du Plateau de Mbe-Batéké au Congo: Application à la correction de leur acidité. *Thèse de Doctorat*. ORSTOM Centre de Montpellier, France
- Fuentes J, D Bezdicek, M Flury, S Albrecht, J Smith (2006). Microbial activity affected by lime in a long-term no-till soil. *Soil Till Res* 88:123–131
- Gil JP, S López-Zuleta, RY Quiroga-Mateus, D Bravo (2021). Cadmium distribution in soils soil litter and cacao beans: a case study from Colombia. *Intl J Environ Sci Technol* 19:2455–2476
- Gramlich A, S Tandy, C Andres, JC Paniagua, L Armengot, M Schneider, R Schulin (2017). Cadmium uptake by cocoa trees in agroforestry and monoculture systems under conventional and organic management. *Sci Total Environ* 15:677–686
- Green VS, DE Stott, M Diack (2006). Assay for fluorescein diacetate hydrolytic activity: Optimization for soil samples. *Soil Biol Biochem* 38:693–701
- Gupta AK, S Sinha (2007). Assessment of single extraction methods for the prediction of bioavailability of metals to *Brassica juncea* L (var Vaibhav) grown on tannery waste contaminated soil. *J Hazard Mater* 98:1788–1794
- ICCO (2015). Quarterly bulletin of cocoa statistics, Vol XLI No 4 Cocoa year 2014/15. *International Cocoa Organization (ICCO)*. Westgate House London, UK. Available at: <http://www.icco.org/about-us/internationalcocoa-agreements/catview/30-relateddocuments/46-statistics-production.html> (Accessed 15 March 2023)
- Jarvis B, C Wilrich, PT Wilrich (2010). Reconsideration of the derivation of Most Probable Numbers their standard deviations, confidence bounds and rarity values. *J Appl Microbiol* 109:1660–1667
- Jaworska H, J Lemanowicz (2019). Heavy metal contents and enzymatic activity in soils exposed to the impact of road traffic. *Sci Rep* 9:19981
- Johnson D, JR Leake, DJ Read (2005). Liming and nitrogen fertilization affects phosphatase activities microbial biomass and mycorrhizal colonization in upland grassland. *Plant Soil* 271:157–164
- Kemmitt SJ, D Wright, D Jones (2005). Soil acidification used as a management strategy to reduce nitrate losses from agricultural land. *Soil Biol Biochem* 37:867–874
- Koenig R, K Schroeder, A Carter, M Pumphrey, T Paulitz, K Campbell, D Huggins (2011). *Soil Acidity and Aluminum Toxicity in the Palouse Region of the Pacific Northwest*. Washington State University Extension Fact Sheet N° FS050E, Washington DC, USA
- Kouakou YKN, TJ Gala-Bi, A Yao-Kouamé (2021). Physico-chemical quality of soils under cocoa farm in Koffikro-Affema (Aboisso) in South-East of Côte d'Ivoire. *Eur J Agric Food Sci* 3:47–54



- Ladd JN (1978). Origin and range of enzymes in soil. In: *Soil Enzymes*, pp:51–96. Bums RG (Ed.). Academic Press London
- Madejón EP, BR López, F Cabrera (2001). Soil enzymatic response to addition of heavy metals with organic residues. *Biol Fert Soils* 34:144–150
- Mühlbachová G, P Tlustoš (2006). Effects of liming on the microbial biomass and its activities in soils long-term contaminated by toxic elements. *Plant Soil Environ* 52:345–352
- Ogunlade MO, SO Agbeniyi (2011). Impact of pesticides use on heavy metals pollution in cocoa soils of Cross-River State Nigeria. *Afr J Agric Res* 6:3725–3728
- Omenda JA, KF Ngetich, MN Kiboi, MW Mucheru-Muna, DN Mugendi (2021). Phosphorus availability and exchangeable aluminum response to phosphate rock and organic inputs in the Central Highlands of Kenya. *Heliyon* 7:e06371
- Peijnenburg WJGM, T Jager (2003). Monitoring approaches to assess bioaccessibility and bioavailability of metals: Matrix issues. *Ecotoxicol Environ Saf* 56:63–77
- Rahajaharintompo RL (2004). Gestion de la fertilité et de la fertilisation phosphatée des sols ferrallitiques des hautes terres de Madagascar. *Thèse de Doctorat*. Université d'Antananarivo, Madagascar
- Santelli CM, SA Welch, HR Westrich, JF Banfield (2001). The effect of Fe-oxidizing bacteria on Fe-silicate mineral dissolution. *Chem Geol* 70:99–115
- Schroeder KL, DC Schlatter, TC Paulitz (2018). Location-dependent impacts of liming and crop rotation on bacterial communities in acid soils of the Pacific Northwest. *Appl Soil Ecol* 130:59–68
- Shangguan YX, Y Qin, H Yu, K Chen, Y Wei, X Zeng, Z Zhou, S Guo, S He (2019). Lime application affects soil cadmium availability and microbial community composition in different soils. *Clean-Soil Air Water* 47:1800416
- Snoeck D, L Koko, J Joffre, P Bastide, P Jagoret (2016). Cacao nutrition and fertilization. In: *Sustainable Agriculture Reviews*, pp:155–202. Lichtfouse E (Ed.). Springer, Cham, Switzerland
- Sutherland WJ, S Bardsley, L Bennun, M Clout, IM Côté, MH Depledge, LV Dicks, AP Dobson, L Fellman, E Fleishman, DW Gibbons, AJ Impey, JH Lawton, F Lickorish, DB Lindemayer, TE Lovejoy, R MacNally, J Madgwick, LS Peck, J Pretty, SV Prior, KH Redford, JPW Scharlemann, M Spalding, AR Watkinson (2011). Horizon scan of global conservation issues for 2011. *Trends Ecol Evol* 26:10–16
- Vanderschueren R, D Arguello, H Blommaert, D Montalvo, F Barraza, L Maurice, E Schreck, R Schulin, C Lewis, J Vázquez, De Castro, P Umaharan, E Chavez, G Sarret, E Smolders (2021). Mitigating the level of cadmium in cacao products: Reviewing the transfer of cadmium from soil to chocolate bar. *Sci Total Environ* 781:146779
- Verdeaux F (1988). Paradoxes et rationalités de la "déforestation" en Côte d'Ivoire. *Nat Sci Soc* 6:26–35
- Wan S, Z Liu, Y Chen, J Zhao, Q Ying, J Liu (2019). Effects of lime application and understory removal on soil microbial communities in subtropical *Eucalyptus* L 'Hér Plantations. *Forests* 10:338
- Yin C, DC Schlatter, DR Kroese, TC Paulitz, CH Hagerty (2021). Impacts of lime application on soil bacterial microbiome in dryland wheat soil in the Pacific Northwest. *Appl Soil Ecol* 168:104113