OPEN ACCESS
Scholars Bulletin
Abbreviated Key Title: Sch Bull
ISSN 2412-9771 (Print) |ISSN 2412-897X (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Subject Category: Agriculture

Change in Soil Fertility Following the Addition of *Bradyrhizobium japonicum* in Soybean (*Glycine max* (L.) Merill) Cultivation in Southeastern DR Congo

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DOI: https://doi.org/10.36348/sb.2025.v11i10.003 | **Received:** 24.09.2025 | **Accepted:** 17.11.2025 | **Published:** 19.11.2025

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Abstract

The supply and bioavailability of mineral elements in heavily weathered soils are limited and continuously declining, leading to low agricultural production; hence the need to find sustainable alternatives to maintain the fertility of various soils. The objective of this study was to evaluate the change in the chemical properties of soils induced by the addition of *Bradyrhizobium japonicum* in soybean cultivation across three types of soil in the Haut-Katanga province. Two soil samplings were conducted to carry out this study. The first was done before the start of the trial; and the second during the experimental phase at the flowering stage of the soybeans. This experiment was conducted using a split-plot design, replicated three times. The collected soil samples were subjected to chemical analyzes. According to the results obtained, the changes in chemical properties were significantly different between the soil types. On one hand, a negative change was observed in the eutric Cambisol, namely a decrease in organic matter, total nitrogen, available and total phosphorus, as well as magnesium; on the other hand, a positive change was observed in the two Ferralsols (acric and xanthic), namely an increase in several chemical properties, except for ammoniacal nitrogen which decreased. Regardless of the soils, the addition of external strains led to a decrease in available phosphorus, while only the USDA 142 strain induced similar effects to the control on the increase of K as well as CEC. This has demonstrated the importance of soybean inoculation with Bradyrhizobium japonicum strains as an alternative option to improve crop productivity in acidic soils.

Keywords: *Bradyrhizobium japonicum*, Cambisols, Ferralsols, Soil fertility, *Glycine max* (L.) Merrill, Yield.

INTRODUCTION

Agriculture in sub-Saharan Africa is generally characterized by low productivity, mainly due to low soil fertility and inefficient soil management (Keino *et al.*, 2015; Raimi *et al.*, 2017). This low fertility is the result of a combination of factors, such as the nature of the parent rock, the degree of soil degradation, and the topography (Bado, 2002). Heavy rainfall leads to erosion and nutrient leaching, while soil acidity also contributes to this phenomenon (Raimi *et al.*, 2017; Verde *et al.*, 2013). Moreover, inappropriate agricultural practices, particularly the lack of adequate replenishment of nutrients taken from the soil by crops after harvests, exacerbate the situation (FAO, 2003; Mulaji, 2011). The soils in this region of Africa suffer from deficiencies and have a low capacity to provide essential nutrients to crops. Among them, nitrogen (N), phosphorus, and potassium are nutrients that limit yield in agricultural production (Hasan, 2018). Major inputs would therefore be needed to increase crop yields (Jaiswal *et al.*, 2021). Faced with the soaring prices of minerals necessary for agriculture and their impact on the environment, it becomes imperative, from a perspective of supporting sustainable agricultural practices, to consider fertilizing alternatives to limit the use of chemical fertilizers (Vericel *et al.,* Minette, 2010).

In humid tropical regions, particularly in the Democratic Republic of Congo, the majority of agricultural production is carried out in a traditional system. It is therefore imperative to implement sustainable soil fertility management in order to increase food crop yields (FIAB, 2011). It is therefore essential that smallholder farmers can reach their full potential by implementing a sustainable, low-cost, efficient, and integrated nutrient management system tailored to their socio-economic status (Raimi *et al.*, 2017). The use of microbial agents to improve agricultural production and soil and plant health is an ancestral practice (Gopalakrishnan *et al.*, 2015). This is a key technology in the integrated management of soil nutrients, based on the interaction between legumes and microorganisms present in the rhizosphere (Raimi *et al.*, 2017). On the one hand, these microorganisms are very useful due to the benefits they provide in terms of improving plant nutrition and yield, particularly thru biological nitrogen fixation, nutrient solubilization, etc. (Ahmed and El-Araby, 2012; Banayo *et al.*, 2012; Bhardwaj *et al.*, 2014). On the other hand, due to their ability to form a symbiotic association with soil bacteria from the Rhizobium group, the presence of legumes in the agroecosystem also helps to increase soil fertility and crop productivity (Smith and Hume, 1987).

Indeed, the symbiosis between legumes and Rhizobium can provide adequate nitrogen nutrition to plants, which allows farmers to save on the cost of chemical fertilizers (Faghire *et al.*, 2011; Tshibuyi, 2021). Various authors have highlighted the positive impact of rhizobia on the enrichment of soil chemical characteristics, particularly regarding nutrients such as nitrogen, phosphorus, and potassium, thereby contributing to the maintenance of soil nutritional balance (Adesemoye *et al.*, 2008; Egamberdiyeva, 2007; Guimarães *et al.,* 2016). A study conducted by Bambara and Ndakidemi (2010) in South Africa revealed a significant increase in soil pH and concentrations of calcium (Ca) and sodium (Na) after Rhizobium inoculation on Phaseolus vulgaris (Bambara & Ndakidemi, 2010). In their study, Tshibuyi and his colleagues (2019) suggested that the impact of Rhizobium strains on soil chemical characteristics could vary depending on the soil type. This observation corroborates that of Mukalay, who emphasizes the importance of not generalizing agricultural practices to all soil types in this region of the country (Mukalay, 2016).

According to Kyei-Boahen *et al.*, (2023), data regarding the performance of rhizobium strains in tropical soils are very limited. Thus, a few previous studies have examined the ability of soybean rhizobium strains to influence the nutritional status of soils (Nguyen *et al.*, 2020). While information is available on the effects of Rhizobium and soybean varieties on nitrogen availability in soils, few studies have explored their impact on the availability of other nutrients in the legume rhizosphere, across different soil categories. However, the application of *B. japonicum*-based biofertilizer to soybeans could have variable effects on chemical parameters depending on the soil type. This research examines the influence of *B. japonicum*, various soybean varieties, and different soil types on the levels of nutrients available to plants in the soybean rhizosphere. The objective of this study is therefore to analyze the impact of interactions between Bradyrhizobium and soybeans on the chemical characteristics of soils.

MATERIALS AND METHODS

Description of Study Sites

This study was conducted in the Kipushi region, in the Haut-Katanga province (figure 1.), during the 2020-2021 growing season. Three sites were chosen to represent three distinct soil types: the Eutric Cambisol, the Acric Ferralsol, and the Xanthic Ferralsol (IUSS Working Group WRB, 2015). Two Ferralsols were selected due to their predominance in the region and designated as the most representative soil group of the area and the most degraded (Kasongo, 2008; Mukalay, 2016). Conversely, a Cambisol, characterized by low weathering and less differentiated profiles, was chosen to contrast with the Ferralsols, which are significantly more weathered.

The eutric Cambisols are represented by the Kanyameshi site, a research station of the National Institute for Agricultural Studies and Research (INERA KIPOPO), located on the Kipushi road; the acric and xanthic Ferralsols are respectively represented by the Eliora farm site, located on the Kasenga road, and by the Katandula farm site, located on the Kasumbalesa road.

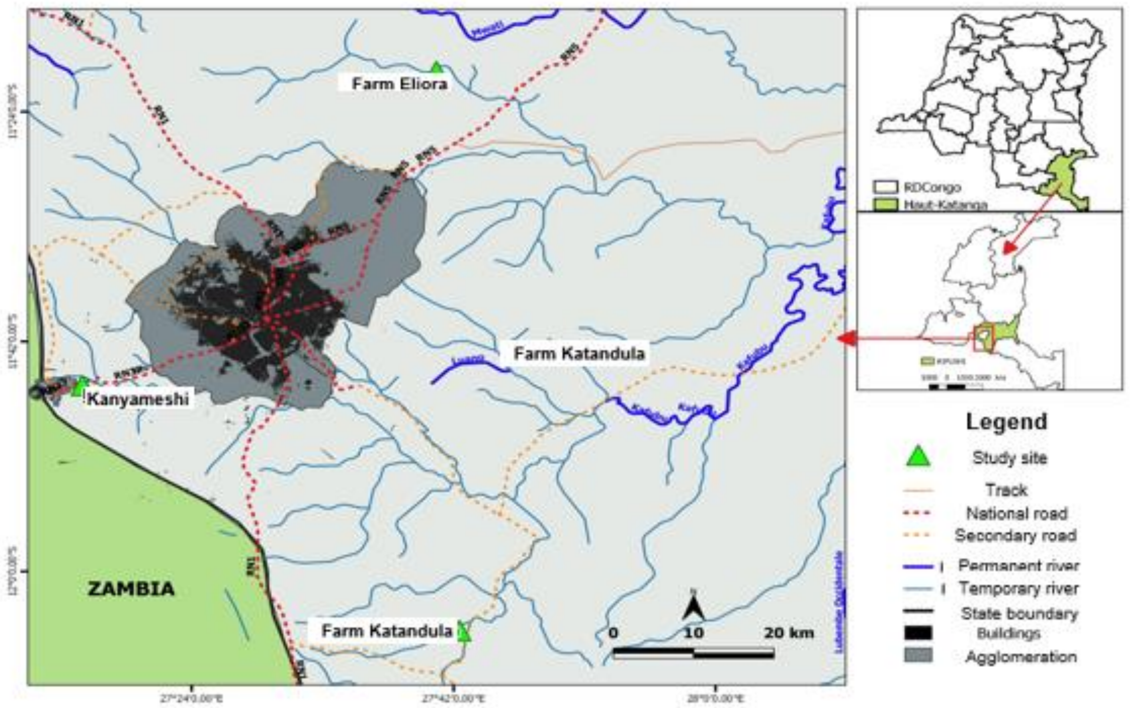

Figure 1: Location of experimental study sites

The study area experiences a CW6 climate according to the Köppen classification, characterized by a rainy season, a dry season, and two transition months (Figure 2.). The rainy season extends from November to March, while the dry season runs from May to September. The months of April and September are considered transition months (Kasongo *et al.*, 2013). The average annual rainfall is 1,270 mm, with extreme values of 717 and 1,770 mm. The average annual temperature is around 20°C (Kidinda *et al.*, 2015; Tshibuyi *et al.*, 2019).

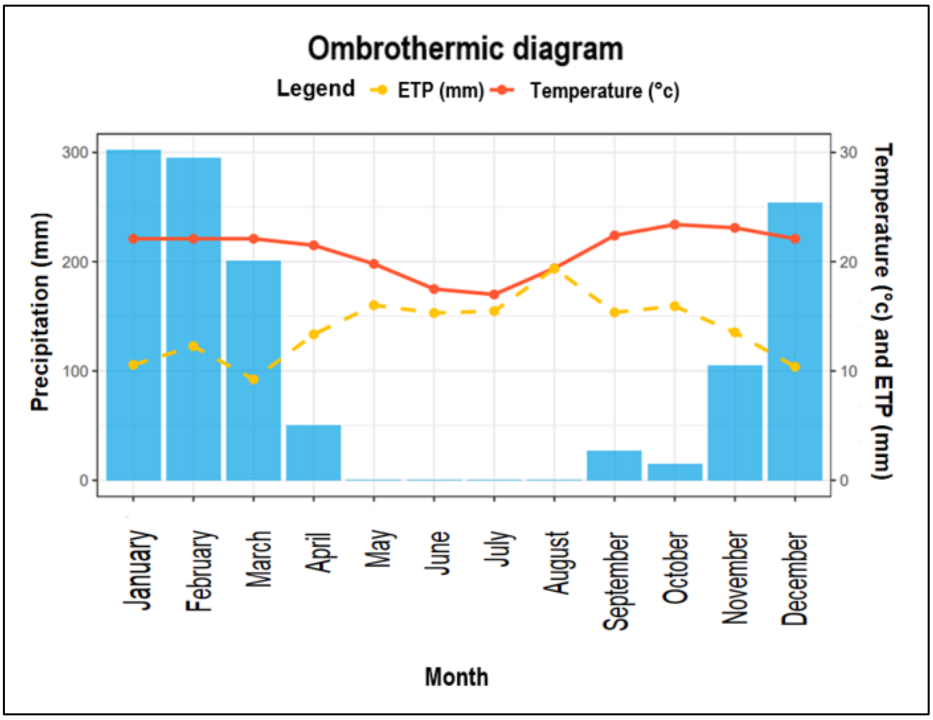

Figure 2: Climatic diagram of the study area (Period 2000-2020) (source: several stations)

Experimental setup and experimental procedure

In each experimental site, a trial was set up on a fallow field of about five years, following a split-plot design with three strains of *Bradyrhizobium japonicum* and five soybean varieties, repeated three times. The three strains used were USDA 110 (S1), USDA 136 (S2), and USDA 142 (S3), all from the Agricultural Research Service (NRRL) culture collection. They were chosen based on their ease of acquisition and their use in other regions of Africa. These strains are genetically different, which allows them to adapt differently to an environment and a variety of soybeans. Four soybean varieties were introduced in the Lubumbashi region: Pka 06 (V1) and Imperial (V5) from INERA Mulungu (Bukavu), Safari MX (V3) and Kafue (V4) from SEED CO (Zambia), as well as TGX1893-10F. The fifth variety is a local variety obtained at INERA Kipopo, in Lubumbashi. Control plots bearing the varieties in question, but without the addition of B. japonicum, were also integrated into the experimental setup, bringing the number of experimental units to 20 (figure 2.).

The soybean sowing was carried out at a distance of 0.40 m x 0.20 m, with three seeds per hill, for a density of 375,000 plants per hectare. For symbiotic inoculation, a single procedure was used for all formulations to ensure that all seeds received a thin layer of inoculant to enhance biological nitrogen fixation (Gemell *et al.,* 2005). All inoculations were carried out in the shade, just before sowing, at a rate of 500 g of inoculant per 100 kg of soybeans per hectare.

Sampling before the experiment

Before the trial was set up at the various selected sites, soil samples were taken for subsequent laboratory analyzes. These samples were taken from the area designated for the installation of the experimental plot, using a manual auger, in the surface layer (from 0 to 20 cm deep), according to the methodology described in Tropical Soil Biology (Huising *et al.,* 2012). In short, the area reserved for the experimental plot was subdivided into three sub-plots (replicates) within which 36 soil samples were taken using an auger along transects crossing the sub-plots.

In total, twelve elementary samples were systematically taken from each sub-plot, then mixed to obtain a single composite sample representative of the plot. Thus, three samples were obtained for each site, corresponding to one composite sample per block.

Soil sampling during the experiment

During the experiment, soil samples were taken at the time of plant flowering in each of the experimental units. More specifically, the soil from the soybean rhizosphere was collected when 50% of the plants had flowered. The soil samples were taken from three planting pockets in the middle rows of each plot, excluding the border plants. The soil was carefully collected at a depth of 10 to 20 cm around the targeted soybean plants, which were uprooted for this purpose. The rhizosphere soil adhering to the plant roots was shaken into labeled bags, air-dried, and then sieved thru a 2 mm mesh before being shipped to the laboratory.

Laboratory analysis

The soil analyzes in the laboratory were conducted on two sets of samples (taken before and during the experiment) to identify possible changes induced by the different combinations of soybean-Rhizobium. These analyzes were conducted at the Mt Makulu research station in the soil fertility chemical laboratory in Lusaka, Zambia.

The soil pH was determined by the potentiometric method (with a pH meter) in a 1:2.5 (W/V) suspension, in a 0.01 M CaCl2 solution for pH (CaCl2) (Van Ranst *et al.,* 1999). The Walkley and Black (1934) wet digestion method was used to determine the soil carbon content, and the percentage of soil organic matter was obtained by multiplying the percentage of soil organic matter by a factor of 1.724. The cation exchange capacity (CEC) and exchangeable bases (Ca, Mg, K, and Na) were determined after extracting the soil samples with ammonium acetate (1 N NH4OAc) at pH 7.0. The exchangeable Ca and Mg in the extracts were analyzed using an atomic absorption spectrophotometer, while Na and K were analyzed by flame photometer (Black and EVAN, 1965). The cation exchange capacity was then estimated by titration thru distillation of ammonium, which was replaced by sodium from a NaCl solution (Chapman, 1965). The available phosphorus, which was determined by the Bray 2 method (Van Ranst *et al.,* 1999), while the total phosphorus was determined by the colorimetric method based on vanadomolybdate (Black and EVAN, 1965). Total nitrogen was determined using the Kjeldahl method (Bremner, 1960). Nitrate as well as ammonium were determined by the colorimetric method.

Statistical analyzes

The soil analysis data were subjected to various statistical analyzes to evaluate the effect induced by the treatments. Exploratory multivariate principal component analyzes (PCA) were used, consisting of reducing the entire dataset into a few principal components to highlight the change in physicochemical parameters in a comprehensive manner. A three-factor ANOVA was used to evaluate the effects of soil types, Bradyrhizobium strains, and soybean varieties on the change in soil physicochemical properties between the soil before the experiment and at the flowering of about 50% of the soybeans. Next, the results were analyzed separately for each soil type using a two-factor ANOVA (the effects of strains and varieties) to evaluate the change in soil chemical parameters. Fisher's least significant difference (LSD) was used to compare treatment means at a significance level of 5%. All these analyzes were performed using R 4.1.1 software.

RESULTS

I suggest first presenting the results in the form of tables so that we know the levels of concentrations observed before moving on to the correlations (PCA).

Initial chemical composition of soils

The initial chemical composition is significantly different between the soil types (table 1). It emerges from these results that the eutric Cambisol is a soil with a significantly higher chemical composition (pH($CaCl_2$), available phosphorus, total phosphorus, exchangeable bases (Ca, Mg, K, and Na), CEC, and base saturation) compared to the two Ferralsols. Between the two Ferralsols, the xanthic Ferralsol exhibited a pH($CaCl_2$), although acidic, with significantly higher Mg and base saturation than in the acric Ferralsol. It was also observed, similarities between the soils in the composition of total nitrogen, NH_4-N, NO_3-N, and Na.

Table 1: Average chemical composition of each soil type before the experiment. The Means followed by similar letters in a column are not significantly different from each other at the 0.05 threshold

Sites	Acric Ferralsol	Xanthic Ferralsol	Eutric Cambisol	*P-value*
pHKCl	3,63±0,03^{a}	4,6±0,06^{b}	5,5±0,1^{c}	*0,00*
Exchangeable acidity	2,45±0,32^{b}	0,54±0,06^{a}	0,23±0,05^{a}	*0,00*
OM %	1,96±0,24	1,88±0,2	2,5±0,29	*0,22*
Total-N %	0,16±0,02	0,18±0,01	0,19±0,01	*0,17*
NH_4-N ppm	4,21±0,35	4,65±0,53	5,98±0,57	*0,55*
NO_3-N ppm	13,75±1,51	14,92±1,07	17,14±1,96	*0,36*
Available-P ppm	2,33±0,33^{a}	2±0,58^{a}	46±3,51^{b}	*0,00*
Total-P ppm	130±10^{a}	193,33±6,67^{a}	510±55,08^{b}	*0,00*
Ca cmol/kg	0,61±0,05^{a}	2,34±0,29^{a}	5,94±0,65^{b}	*0,00*
Mg cmol/kg	0,25±0,02^{c}	0,79±0,09^{b}	2,12±0,16^{c}	*0,00*
K cmol/kg	0,22±0,01^{a}	0,20±0,02^{a}	1,64±0,15^{b}	*0,00*
Na cmol/kg	0,12±0,01	0,09±0,01	0,12±0,01	*0,13*
CEC cmol/kg	3,56±0,33^{a}	3,73±0,37^{a}	9,43±0,52^{b}	*0,00*
Base saturation %	34±1^{a}	91±2^{b}	104±1^{c}	*0,00*

The principal component analysis (Figure 3) shows notable differences between the properties of the three types of soils involved in this study, both before the installation of the trial and at the time of flowering. Before the installation of the trial (Figure 3a), the acric Ferralsol is distinguished by high levels of exchangeable acidity as well as these two components (Al^{3+} and H^+); whereas the eutric Cambisol is characterized by high levels of several chemical parameters. Finally, the xanthic Ferralsol is seen as an intermediate soil between the first two. At flowering (figure 3b), notable differences between the properties of these different soils maintained the same initial trends, although strong variabilities in the parameters were observed in the different soils.

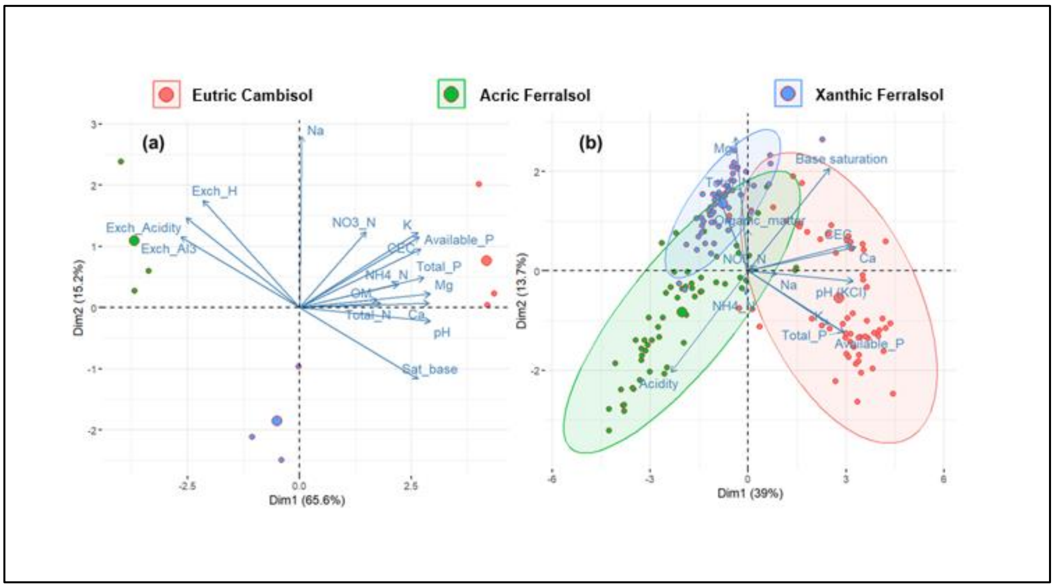

Figure 3: Chemical properties of soils: before the installation of the trial (a) and at flowering (b) in the three sites

Change in chemical parameters based on soil types

In light of the results in Table 2 presenting the analysis of variance, no three-level interaction was observed between soil types, *B. japonicum* strains, and soybean varieties on the change in soil chemical parameters. Nevertheless, a two-level interaction (soil*strain) was only reported on the change of 3 chemical soil parameters, including total nitrogen content, available phosphorus, as well as potassium (K). Overall, an influence of soil type was observed on the majority of chemical parameters, except for NO3-N which was similar across soil types. The strain effect was also reported on the change in available phosphorus, potassium, and CEC.

Table 2: F values and Results of the 3-factor Analysis of Variance between soils, *B. japonicum* strains, and soybean varieties on the change in soil chemical properties *: *significance at <0.05; **: significance at 0.01; *: significance at 0.001.***

Parameters	Soils	Strains	Variety	Soils*Strains	Soils*Variety	Strains*Variety	Soil*Strainété
pH ($CaCl_2$)	*18,9 ****	*1,7 ns*	*0,2 ns*	*1,1 ns*	*0,8 ns*	*0,3 ns*	*0,4 ns*
Exchangeable acidity	*143,6 ****	*0,6 ns*	*0,9 ns*	*1,2 ns*	*1,2 ns*	*0,2 ns*	*0,1 ns*
OM %	*19,4****	*0,3 ns*	*0,4 ns*	*0,4 ns*	*0,8 ns*	*0,5 ns*	*0,8 ns*
Total nitrogen	*22,7 ****	*1,5 ns*	*1,1 ns*	*2,4 **	*0,4 ns*	*0,3 ns*	*0,3 ns*
NH_4-N ppm	*12,2 ****	*0,7 ns*	*0,8 ns*	*0,5 ns*	*0,4 ns*	*0,5 ns*	*0,9 ns*
NO_3-N ppm	*2,1 ns*	*0,7 ns*	*1,1 ns*	*1,9 ns*	*0,4 ns*	*0,9 ns*	*0,4 ns*
P-available ppm	*19,2****	*5,2 ***	*0,1 ns*	*5,4****	*0,5 ns*	*0,2 ns*	*0,2 ns*
P-total pmm	*4,4 **	*1,1 ns*	*0,2 ns*	*1,4 ns*	*0,1 ns*	*0,1 ns*	*0,2 ns*
Ca cmol/kg	*3,3 **	*2,4 ns*	*0,7 ns*	*1,1 ns*	*1,2 ns*	*0,3 ns*	*0,6 ns*
Mg cmol/kg	*139,4 ****	*0,4 ns*	*0,5 ns*	*0,5 ns*	*0,6 ns*	*0,7 ns*	*0,3 ns*
K cmol/kg	*50,9 ****	*3,7 **	*0,1 ns*	*2,5 **	*0,4 ns*	*0,3 ns*	*0,3 ns*
Na cmol/kg	*18,4 ****	*0,1 ns*	*0,2 ns*	*1,2 ns*	*0,4 ns*	*0,3 ns*	*0,2 ns*
CEC	*41,2 ****	*3,6 **	*1,3 ns*	*1,1 ns*	*1,1 ns*	*0,3 ns*	*0,5 ns*
Base Saturation %	*149,8 ****	*0,7ns*	*1,2 ns*	*1,2 ns*	*1,9 ns*	*0,2 ns*	*0,1 ns*

Looking at the results in Table 3, two trends emerge in the change of soil chemical parameters; the first trending toward an increase in chemical parameters and the second toward a decrease. In general, the two Ferralsols showed a positive and significant change in several parameters compared to the Eutric Cambisol, which showed a negative change in most parameters. For the parameters of total nitrogen, available phosphorus, base saturation, magnesium (Mg), and potassium (K); the change was significantly high in the acric Ferralsol followed by the xanthic Ferralsol and finally a decrease in the eutric Cambisol. The pH ($CaCl_2$) significantly increased in the acric Ferralsol followed by the eutric Cambisol, in contrast to a decrease in the xanthic Ferralsol.

Table 3: Change in soil chemical parameters between the initial situation and soybean flowering according to different soil types. Negative values indicate a decrease in the observed parameter between the two periods, OM: organic matter, pH: hydrogen potential, N: nitrogen, P: phosphorus, CEC: cation exchange capacity, Ca: calcium, Na: sodium, K: potassium, Mg: magnesium; the different letters (a, b, c) are to be compared between the columns. The Means followed by similar letters in a row are not significantly different from each other at the 0.05 threshold

Parameter	Acric Ferralsol	Eutric Cambisol	Xanthic Ferralsol
pH ($CaCl_2$)	0,41±0,05 ^{c}	0,16±0,06 ^{a}	0,19±0,05 ^{b}
Exchangeable acidity	-1,53±0,09 ^{a}	-0,03±0,01 ^{c}	-0,29±0,03 ^{b}
OM %	0,43±0,13 ^{b}	-0,16±0,1 ^{a}	0,68±0,08 ^{b}
Total -N %	0,04±0,01 ^{c}	-0,02±0,01 ^{a}	0,02±0 ^{b}
N-NH4 ppm	-1,84±0,25 ^{a}	0,59±0,29 ^{c}	-0,85±0,14 ^{b}
N-NO3 ppm	8,87±0,85	8,45±0,89	6,57±0,67
P-available ppm	5,53±0,42 ^{c}	-13,07±2,26 ^{a}	2,03±0,34 ^{b}
Total - Pppm	88,01±5,23 ^{b}	-34,67±26,53 ^{a}	9,83±3,84 ^{a}
CEC cmol/kg	1,71±0,21 ^{b}	0±0,22 ^{a}	2,1±0,19 ^{b}
Base Saturation %	50,8±2 ^{c}	1,7±0,2 ^{a}	11,7±0,5 ^{b}
Ca cmol/kg	1,96±0,18 ^{ab}	1,66±0,19 ^{a}	2,32±0,17 ^{b}
Mg cmol/kg	0,9±0,04 ^{c}	-1,19±0,14 ^{a}	0,74±0,05 ^{b}
K cmol/kg	0,53±0,07 ^{b}	0,02±0,01 ^{a}	0,06±0,01 ^{a}
Na cmol/kg	0,028±0 ^{a}	0,043±0 ^{b}	0,034±0 ^{ab}

In relation to the strain effect (table 4), available phosphorus significantly decreased with the addition of *B. japonicum* strains, unlike the control which showed a high positive change of about 5.3ppm. It was observed for potassium (K) and CEC positive changes, the most significantly high of which were observed in the control compared to strains S1 and S2 for potassium and S1 only for CEC. Overall, Strain S3 led to changes in potassium and CEC in a similar manner to the control.

Table 4: Effect of B. japonicum strains on the change of chemical parameters. K: potassium, CEC: cation exchange capacity, P: phosphorus. The means followed by similar letters in a column are not significantly different from each other at the 0.05 threshold

Strains	P-available ppm	K cmol/kg	CEC cmol/kg
S0 (Control)	5,31±1,24*b*	0,28±0,11*b*	1,98±0,29*b*
S1 (USDA 110)	-0,11±1,91*a*	0,01±0,07*a*	1,6±0,29*a*
S2 (USDA 136)	-3,07±2,02*a*	0,01±0,08*a*	0,86±0,31*ab*
S3 (USDA 142)	-0,38±1,3*a*	0,08±0,05*ab*	1,48±0,24*ab*

In Table 2, interactions between soil types and *B. japonicum* strains were reported. Thus, from the results in Table 5, it appears that the strains of *B. japonicum* acted differently from one soil to another on available phosphorus, potassium (K), and total nitrogen. A significant increase in available phosphorus was obtained in the Cambisol without the addition of *B. japonicum* strains (control), a similar change with strain S2 in the acidic Ferralsol. In all three soils, the addition of *B. japonicum* strains led to decreases in available phosphorus, except in the eutric Cambisol. Regarding total nitrogen (N), significantly high increases were observed in the xanthic Ferralsol regardless of the strain, a similar situation in the acric Ferralsol with the addition of strains S2 and S3. Particularly in the eutric Cambisol, any external addition of *B. japonicum* strains led to a decrease in total nitrogen in the soil. Regarding potassium (K), a significantly high increase was observed in the acric Ferralsol without the addition of strains (S0), followed in the same soil by the addition of strain S2 (USDA 136). In the Eutric Cambisol, a decrease in potassium was generally observed in the soybean rhizosphere. In the xanthic Ferralsol, however, an intermediate increase in potassium was observed between the acric Ferralsol and the eutric Cambisol.

Table 5: Interactive effect between soils and B. japonicum strains on the change of chemical parameters in the rhizosphere of soybeans. The means followed by similar letters in a column are not significantly different from each other at the 0.05 threshold

Soils	Strains	P-available ppm	N-total %	K cmol/kg
Eutric Cambisol	S0	8,33±3,5*d*	0,01±0,01*ab*	-0,24±0,13*abc*
	S1	-8,73±4,83*ab*	-0,02±0,02*ab*	-0,32±0,14*ab*
	S2	-17±3,97*a*	-0,05±0,02*a*	-0,51±0,12*a*
	S3	-6,87±3,28*abc*	-0,06±0,02*a*	-0,16±0,06*abc*
Xanthic Ferralsol	S0	2,67±0,63*bcd*	0,06±0,01*b*	0,14±0,02*bcd*
	S1	3,4±0,99*bcd*	0,03±0,01*b*	0,07±0,02*bcd*
	S2	1,73±0,56*bcd*	0,02±0,01*b*	0,07±0,0*bcd*
	S3	1,8±0,3*bcd*	0,04±0,01*b*	0,09±0,02*bcd*
Acric Ferralsol	S0	4,94±0,79*cd*	0,02±0,01*ab*	0,93±0,2*ef*
	S1	5,38±0,77*cd*	0,02±0,01*ab*	0,29±0,07*cd*
	S2	6,07±1,03*d*	0,04±0,01*b*	0,46±0,12*def*
	S3	3,94±0,69*cd*	0,05±0,02*b*	0,31±0,09*cd*

DISCUSSION

The productivity of soils in Africa depends on their nutritional status and fertility (Anup & Ghimire, 2019). This is partly related to the availability of chemical components, particularly the primary and secondary macronutrients essential for plant growth, development, and production (Nyoki & Ndakidemi, 2014). The progressive decrease of these macronutrients (nitrogen, phosphorus, potassium, calcium, magnesium, sodium, etc.) is caused by various factors (Raimi *et al.*, 2017 ; Steiner *et al.*, 2008), leading to a decline in yield. In order to achieve better quality sustainable compromises, the present study was undertaken to evaluate the impact of different soil types, *B. japonicum* strains, and soybean varieties on the availability of chemical elements in the rhizosphere.

Characterizations of the different soils under study

According to the data illustrated in figure 3a and in table 2, the acric Ferralsol is considered less favorable for agriculture, being surpassed by the xanthic Ferralsol, which in turn is surpassed by the eutric Cambisol due to its advantageous chemical composition. According to Mukalay (2016), the Ferralsols present in this study area are presented as altered soils with lower fertility compared to Cambisols, which are young and fertile soils. In the two Ferralsol soils studied, the concentration of available phosphorus was below the critical threshold, while the eutric Cambisol showed levels above 20 ppm, required to ensure sustainable production in tropical areas (Tetteh *et al.*, 2017). The total nitrogen (N) level in the three soils was average, as described in the literature (Mthimunye *et al.*, 2023). The pH measured with $CaCl_2$ was strongly acidic in the acric Ferralsol, followed by a moderately acidic pH in the xanthic Ferralsol, and finally a slightly acidic pH in the eutric Cambisol (Belinda, 2000).

Change in chemical parameters in different types of soils

The results of this study demonstrated that the cultivation of all varieties was favorable, even in the absence of Bradyrhizobium inoculant, which led to changes in soil composition. Indeed, no significant differences were observed in the chemical parameters of the soil among the different varieties, whether individually or in interaction with other factors. In this regard, various studies have highlighted similar conclusions regarding various species of legumes (Mthimunye *et al.*, 2023). The effects of the strains were manifested only on three soil parameters, thus demonstrating the adaptation of the strains to various soil types for several chemical soil parameters.

During this study, it was observed that all chemical parameters, except for NO_3-N, underwent significant variations depending on the soil types. Indeed, most mineral elements experienced a notable increase in Ferralsols (acric and xanthic), while decreases were recorded in eutric Cambisols. The increase in the concentration of macro and micronutrients in the rhizosphere soils of soybeans in the two Ferralsols compared to the Cambisol can be attributed to various factors.

First of all, the increase in pH ($CaCl_2$) and the reduction of exchangeable soil acidity favored the availability of most plant nutrients (Bagayoko *et al.*, 2000; Condron *et al.*, 1993). This study revealed a decrease in exchangeable soil acidity, as well as an increase in soil pH in the rhizosphere of the three types of soils examined. However, this increase was more pronounced in the two Ferralsols than in the eutric Cambisol, which could explain the increase in macronutrients and micronutrients in the rhizosphere soils of soybeans grown in Ferralsols. The reduction in exchangeable acidity may also result from the increasing substitution of Al by Ca in the exchange sites and the subsequent precipitation of Al in the form of $Al(OH)_3$, due to the calcifying effect observed in several soil strains (Chimdi *et al.*, 2022). What was observed in our study thru a significantly high increase in Ca and Mg in both Ferralsols compared to the Eutric Cambisol.

Secondly, the microorganisms present in the rhizosphere carry out the mineralization process, which has the ability to mobilize mineral elements such as phosphorus, thus making them available in the soil (Nyoki &Ndakidemi, 2018). Which would have led to an increase in available phosphorus in our Ferralsols. Similar conclusions were mentioned by Dakora *et al.*, (Dakora *et al.*, 2008). It is well established that rhizobacteria have the ability to directly modulate phosphorus availability by producing organic anions or enzymes (Plassard *et al.*, 2015; Richardson *et al.*, 2009). Additionally, it has been observed that under conditions such as those present in our Ferralsols, there is an increase in the secretion of organic acids by plants of the Fabaceae family, which generally colonize infertile soils. These organic acids play a role in the mineralization process of soil organic phosphorus (Lazali *et al.*, 2020).

Gilbert *et al.*, (1999) as well as Richardson *et al.*, (2001) further emphasize that the transformation of unavailable organic phosphorus into inorganic phosphorus available to plants can result from root mineralization or the production of microbial phosphatases (Hansen *et al.*, 2017). Thirdly, the decomposing cells of microorganisms release nutrients, thereby making them available in the rhizosphere soil (McCully, 2001; Nyoki & Ndakidemi, 2018). Fourthly, mineral elements can be released into the rhizosphere soil in the form of exudates from plant roots (Ae *et al.*, 1990; Nyoki & Ndakidemi, 2018).

Fifthly, the decomposition of plant biomass leads to an increase in nutrients present in the soil. It has been reported that agricultural systems that integrate legumes help improve soil fertility and health, notably by increasing the availability of organic matter in the soil and enriching nitrogen and phosphorus concentrations (Jensen *et al.*, 2020; Thapa *et al.*, 2021). Tshibuyi *et al.*, observed in 2019 an increase in pH and C/N ratio of acidic soils in Lubumbashi, from the flowering stage to the harvest stage of soybean plants (Tshibuyi *et al.*, 2019). This research also highlighted the positive impact of legumes on the enrichment of the chemical quality of acidic soil, due to their nitrogen contribution and the subsequent decomposition of residues (Diop *et al.*, 2013; N'gbesso *et al.*, 2013).

In this study, another observation was significantly higher increases in exchangeable cations in both Ferralsols compared to the Cambisol. The increase in pH ($CaCl_2$) and the decrease in exchangeable acidity recorded in the soils can explain the high content of exchangeable bases in the soil, which remains within an acceptable range for the availability of nutrients such as Ca, Mg, and Na (Belinda, 2000). Moreover, the use of a strain adapted to acidic and infertile soil can lead to an increase in the concentrations of basic cations in the soil solution (Chimdi *et al.*, 2022), indicating that the soybean rhizobacteria were well-suited to the cultivation and various soils with respect to certain exchangeable cations, except for K, which was significantly high in the control but similar with the application of the USD 142 strain (S3). Furthermore, the notable increase in exchangeable cations could also result from rhizosphere deposits, thereby promoting nutrient availability for plant assimilation (Mthimunye *et al.*, 2023; Plotegher & Ribeiro, 2016).

Despite the significant improvement in most chemical parameters, the levels of nitrogen and its various forms did not show significant differences between the soils, whether before the start of the experiment or at the flowering stage. It has been shown that in an acidic soil with high nitrogen availability, this can result in a decrease, or even inhibition, of the symbiotic efficiency of introduced rhizobium strains (Akley *et al.*, 2022; Kasper *et al.*, 2019), which would potentially reduce the biological nitrogen fixation activity in the various soils.

The regression of the chemical state of Eutric Cambisols could be attributed to the fact that, in fertile soil, symbiotic mechanisms and nutrient mobilization processes are not activated, as plants and rhizobia can already meet most of their needs by drawing from soil elements, leading to a decrease in nutrients in this soil. Some researchers have mentioned that the levels of carbon, phosphorus, and calcium in the soil are essential nutrients that impact the microbial population (Navarro-Noya *et al.*, 2013) as well as their activities in the soil (Lori *et al.*, 2017). Moreover, previous studies have indicated a negative correlation between the ability of bacteria to solubilize phosphorus and C/N ratios, as well as the concentrations of nitrogen, phosphorus, carbon, and magnesium in soils (Alemneh *et al.*, 2022; Argaw, 2014). Some authors emphasize that in a context of nutrient-rich soils (Denison & Kiers, 2004; Graham, 2008), the partnership between legumes and rhizobia loses its importance for the concerned organisms, particularly the legumes which play a predominant role in this mutualistic relationship, which helps the plants conserve their energy resources (Ferguson *et al.*, 2019; Nguyen *et al.*, 2020; Nishida & Suzaki, 2018; Reid *et al.*, 2011). The impact of rhizobium inoculation was only observed on the levels of available phosphorus, potassium, and CEC in the soybean rhizosphere (Table 4). Interactions were observed in the rhizosphere soils of soybeans between the soil and the strains of *B. japonicum*, regarding available phosphorus, potassium, and total nitrogen.

In general, the strains of *B. japonicum* in this study led to a reduction in the levels of available phosphorus in the soybean rhizosphere, as opposed to the control group where an increase was observed. This highlights the commonly encountered issue when using external strains of *B. japonicum*, where the success of inoculation is conditioned by various factors, such as the presence or absence of native rhizobia (Kyei-Boahen *et al.*, 2023; Sanz-Sáez *et al.*, 2015) and the quality of the inoculum used (Rodríguez-Navarro *et al.*, 2011). According to the literature, the strains introduced in our study were able to compete with the native strains, which are often very competitive and adapted to the environment (Grönemeyer *et al.*, 2014). This competition would represent one of the major constraints for the introduction of more efficient rhizobium strains (Vieira *et al.*, 2010). Inefficient native rhizobium strains, adapted to the local environment, tend to compete effectively with high-performing introduced strains, which can lead to poor nodule colonization by the inoculant (Kyei-Boahen *et al.*, 2023; Thilakarathna and Raizada, 2017). Thus, Thies *et al.*, revealed in 1991 that when native rhizobium populations are at > 100 cells g/soil, there was little or no response to external inoculation (Thies *et al.*, 1991). Regarding the other two parameters (K and CEC) for which a strain effect was observed, strain S3 (USDA 142) showed comparable results to the control, although slightly lower numerically. These results suggest that this strain might be better adapted to local conditions than the other strains. It has been reported that inoculant strains exhibit variations in their ability to effectively colonize the rhizosphere and compete with native rhizobia for nodule occupancy (Mendoza-Suárez *et al.*, 2021).

According to our results, it is observed that soybean cultivation, whether inoculated or not, has a positive impact on the physicochemical properties of Ferralsols. This improvement is more significant for acric Ferralsols than for xanthic Ferralsols, while it has a negative effect on eutric Cambisols. Therefore, an increase in nutrient availability was observed from sowing to flowering of soybeans grown in Ferralsols, thus creating favorable conditions for the growth of subsequent plants. Within the framework of a sustainable production approach aimed at restoring soil fertility, it is essential to consider the use of inoculated or non-inoculated soybeans in a crop rotation or intercropping system. This corroborates the conclusions of Manyong *et al.*, (1996), Carsky *et al.*, (1997), Mako *et al.*, (2013), and Useni *et al.*, (2013), who highlight the importance of using legumes in crop rotation and intercropping. According to these authors, it is recommended to introduce legumes at the end of the crop rotation on nutrient-rich and less altered soils, while on nutrient-poor soils, legumes should be placed at the beginning of the rotation. Which turned out to be consistent with our results. This study leads us to advocate for the integration of soybeans into Ferralsols, not only because of its positive impact on soybean crop yields (as observed by Tshibuyi *et al.*,2019), but also for its role in improving the fertility of these soils. This could promote the improvement of the nutritional status and fertility of Ferralsols, highly weathered soils in the study area, as indicated by Schwab *et al.*, (2015). Moreover, regarding Cambisol, it is recommended to promote soybean cultivation without the addition of allochthonous rhizobium strains, preferably within a crop rotation system where legumes are placed last in the rotation.

CONCLUSION

This study was initiated to evaluate the effects of soil types, soybean varieties, as well as different strains of *B. japonicum* on the change in the chemical composition of soils.

The results obtained showed a significant change in most chemical properties among the different soil types. Chemical properties such as CEC, base saturation, macronutrients, and micronutrients significantly increased in Ferralsols (acric and xanthic) followed by a decrease in Eutric Cambisol. A strain effect was observed only on a few chemical properties such as available phosphorus, the level of which decreased with the addition of external strains, demonstrating the presence of indigenous rhizobacteria in the soil that would have competed with the introduced strains. Regarding the level of K as well as the CEC, the USDA 142 strain led to similar increases with the control, suggesting an adaptation of this strain in the study area.

From a sustainable development perspective, the cultivation of inoculated soybeans should be integrated into a crop rotation system where, for soils less altered in nutrients, soybeans would be placed at the end of the rotation, while in highly altered soils, they would be at the beginning of the rotation.

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