

Original Research Paper

Effects of *Bradyrhizobium japonicum* on Some Chemical Properties of Ferralsols under Soybean (*Glycine max* (L.) Merr.) Cultivation

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Abstract: This study was conducted on acidic soils in two different agro-ecological zones in order to evaluate the influence of *Bradyrhizobium japonicum* on soil chemical properties in the Upper-Katanga (DR Congo). A split plot design with three replicates was installed in two sites. The main plots included three strains of *Bradyrhizobium japonicum* plus the untreated control and four soybean varieties in subplots. Seed inoculation was performed in the shade and sown on the same day. The results showed that *Bradyrhizobium* strains did not influence soil pH, Nitrogen, C: N ratio and organic matter neither at flowering nor at harvest. However, total and available phosphorus (P) were influenced by the different treatments at harvest in Kanyameshi site. The strain S1 induced the highest average of total and available P whereas, the strain S2 had the lowest value of total P, and S3 induced the lowest content in available P. By comparing the timing of soil sampling at flowering and harvesting, the Student test revealed significant differences in pH, total nitrogen, C: N ratio, organic matter and available P indicating that soil chemical properties was improved at harvest and are only partially influenced by applied *Bradyrhizobium* strains.

Keywords: Soybean, Inoculum, Soil Fertility, Haut-Katanga

Introduction

In sub-Saharan Africa, soils fertility is fundamentally low and exported nutrients are not adequately replaced (FAO, 2003; Mulaji, 2011) and nutrient depletion is a major aspect of soil degradation (Useni *et al.*, 2014; Mulaji, 2011). This situation leads in a drastic deficiency of mineral nutrients necessary for plant growth, which would be the basis of low crop yields (Houngnandan *et al.*, 2001; Javaheri and Baudoin, 2001). However, the sustainable management of soil fertility in humid tropical countries is still topical, especially since the majority of agricultural production in tropical soils is still based on the traditional farming systems.

Notably, agricultural production, which has leapt forward in recent decades, will have to grow even further in the future, as the world population has also increased (FAO, 2005). To meet future food needs, it will clearly be necessary to continue to rely on inorganic nitrogen

fertilizers while their consequences for the environment are well established (Kasongo and Banza, 2015).

Obviously, legumes play a significant role in soil improvement by their symbiotic association with soil bacteria of the rhizobium group and allow fixing up to 300 kg N ha⁻¹ year⁻¹ under good conditions (Smith and Hume, 1987). Therefore, the use of legumes in rotation and crop associations is of great important (Manyong *et al.*, 1996; Carsky *et al.*, 1997; Mako *et al.*, 213; Useni *et al.*, 2013), since it may reduce the costs and limit the damage caused by mineral fertilizer input (Kasongo and Banza, 2015). In this family of plants, soybean (*Glycine max* (L.) Merr.) is an important grain legume crop in the world in terms of total production and international trade (Kasongo and Banza, 2015; Baboy *et al.*, 2015). In the Democratic Republic of Congo, during the years 2003-2013, soybean yield varied from 4750 to 5476.19 Kg per hectare (www.fao.org). These yields are lower than that obtained in several countries including Kenya (3.76 t ha⁻¹, Verde *et al.* (2013), Serbia -

4.35 t ha⁻¹ - (Mrkovački *et al.*, 2008) and Tanzania where Phosphorus addition and inoculation allowed to reach 10.837 t ha⁻¹ (Tairo and Ndakidemi, 2013). A study conducted by Shahid *et al.* (2009) reported that soybean seed production could increase by 70-75% when soybeans are inoculated with appropriate bacterial rhizobium. As rhizobium can meet plant need in N, the use of additional inoculum could not only increase plant productivity but also N balance in the soil. Obviously, N is essential to the development of microorganisms in the rhizosphere, leading to the increased decomposition of soil organic matter and enhanced P availability because of mycorrhiza activities. However, the extent to which Bradyrhizobium strains could improve the fertility of ferralsols is not clear.

This study aims to study the effects of *Bradyrhizobium japonicum* on some soil chemical properties of ferralsols for evaluating the efficiency of biological fertilization.

Materials and Methods

Description of the Study Area

The experiments were conducted during the 2015-2016 cropping season in two sites. The first experiment was conducted at the experimental field of the Faculty of Agricultural Sciences of the University of Lubumbashi University (UNILU) and located at 1100 m altitude, 11°60'869"S and 027°47'692"E. The second experiment was conducted in the territory of Kipushi, precisely in Kanyameshi located at 11°45'25"S and 27°16'59"E and 1320 m altitude. The annual rainfall in these agro-ecological zones is around 1270 mm with a rainy season of 118 days, while the average annual temperature is around 20°C with great inter-annual stability. The rainy season runs from November to March, the dry season from May to September, while April and October are the transition months (Kasongo *et al.*, 2013). The area is characterized by ferralitic soil type with a pH-water oscillating around 5.2 (Kwey *et al.*, 2015).

Materials

Four soybean varieties and three strains of Bradyrhizobium were used as biological material. Among these four soybean varieties, two (TGX 1740-7F and 1880-3E) were obtained at the National Institute for Agricultural Research and Study (INERA), Kipopo Station. The other two varieties were obtained in Zambia (MUKANGA from ZAMSEED and PAN 1867 from PANNAR). Three commercial inoculum strains of the genus Bradyrhizobium were used. *Bradyrhizobium sp* strain of pasty formulation, Sojapak ® 50 liquid formulation strain and GraphEx™ powder formulation strain. The strain Bradyrhizobium sp was provided by the National Service of Fertilizer and Input (SENAFIC) and the other two strains (Sojapak ® 50 and GraphEx™) were obtained on the local market.

Conduct of the Experiment

Soil samples from the topsoil (0-20 cm) were collected before the setup of experiment, at flowering and at harvesting. The experiment was installed following a Split Plot device with three replicates. Three strains of Bradyrhizobium plus the untreated control and four soybean varieties were used. The combination of different strains and varieties resulted in 16 treatments. Planting was done at 0.40×0.20 m spacing at the rate of 2 seeds per pocket, i.e., a density of 250000 plants per hectare. The planting took place respectively in January 9 and 10, 2016 in Lubumbashi and Kipushi. Inoculation of soybean seeds was done at planting for concerned treatments. For symbiotic inoculation, a single procedure was used for all formulations to ensure that all seeds receive a thin layer of the inoculant. All inoculations were done in the shade just before planting in order to maintain the viability of the bacterial cells.

Laboratory Analysis and Statistics

All chemical analyses were performed on air-dried soil fractions (< 2 mm). Soil pH was measured potentiometrically in a 1:2.5 (W/V) suspension of H₂O. Organic carbon content (C_{org}, Walkley and Black) was determined as described by Van Ranst *et al.* (1999). The method is based on the oxidation of the organic matter by potassium dichromate (K₂Cr₂O₇) in a strongly acid medium (H₂SO₄). Total nitrogen and available phosphorus were determined by Kjeldahl and Bray II methods respectively, as described by Van Ranst *et al.* (1999).

One-way Anova was used to evaluate the effects of Bradyrhizobium strains on soil chemistry, with a post-hoc TUKEY test in case of differences between treatments. The Student test was applied to compare the sampling time (flowering and harvesting). All analyses were performed using Minitab 17 software considering P < 0.05 as the level of significance

Results

Effect of Rhizobial Strains on Soil pH at Flowering and Postharvest

Figure 1 illustrates the variation of soil pH at flowering and harvest based on rhizobia strains in the Kanyameshi and Kasapa sites. As a result, the different strains of rhizobium did not influence pH at flowering (p = 0.753) and harvest (p = 0.609). However, the pH varied in this site between 6.07 (S0) and 6.3 (S2) at flowering, 6.6 (S3) and 6.7 (S0, S1, S2) at harvest. The same trend is observed in the Kasapa site where the different strains of Bradyrhizobium did not influence pH neither at flowering (p = 0.339) nor at harvest (p = 0.57). However, the results showed highly significant differences after the student test between pH at flowering and after harvest compared to each other for both study sites (p = 0.00). The pH of the control treatment

increased slightly by 0.57 and 0.59 at the Kanyameshi

and Kasapa sites respectively at harvest.

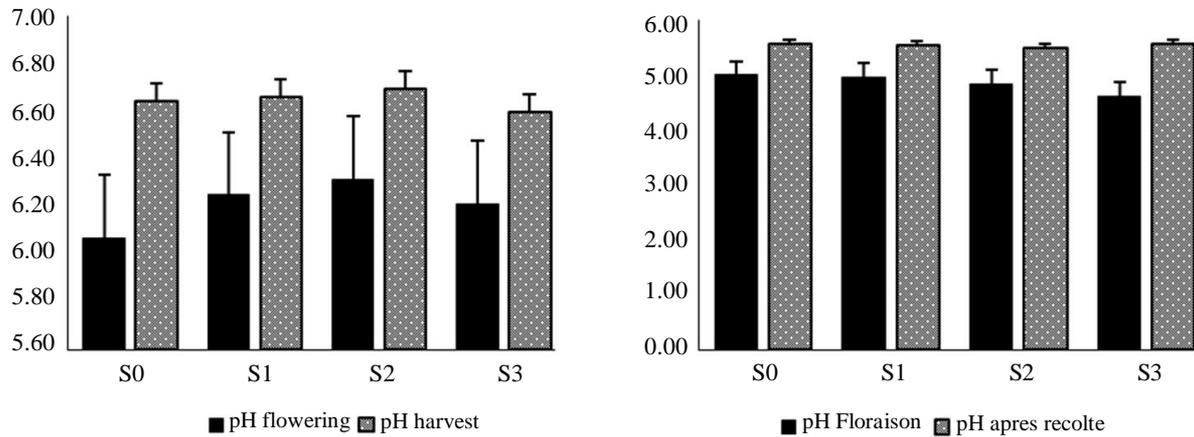


Fig. 1: Effect of rhizobial strains on soil pH change at flowering and harvest. Left for the Kanyameshi site and to the right of Kasapa

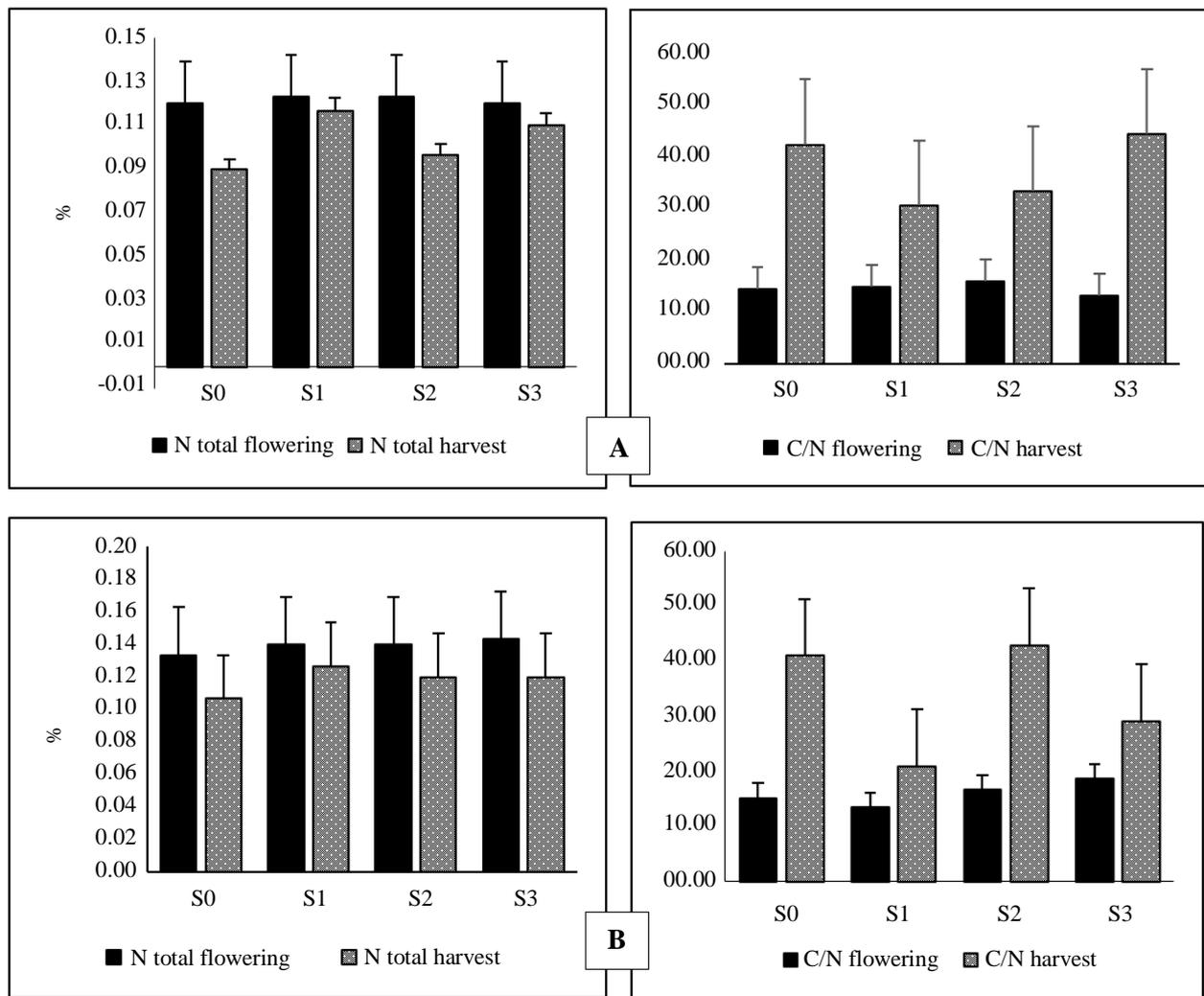


Fig. 2: Influence of Bradyrhizobium inoculation on total nitrogen (left) and soil C/N ratio (right) at flowering and harvest. (A): Kanyameshi site and (B): Kasapa site

Influence of Bradyrhizobium Strains on Soil Chemical Parameters

Figure 2 indicates that in Kanyameshi the total nitrogen and soil C/N ratio are not influenced by the different rhizobium strains at flowering and harvest. The same trend is observed in Kasapa site. Nevertheless, the Student test shows a significant difference in total nitrogen and C/N in Kanyameshi after comparing the situation in the soil at flowering and harvest. In Kasapa site, only the C/N ratio shows a highly significant difference ($p = 0.00$). The results show in both sites that soil nitrogen decreases after harvest compared to the flowering. An opposite trend is observed on the C/N ratio where in both sites the plots without strains (S0) presented after harvest near the triple of the situation at flowering.

The different rhizobium strain shows a significant effect on total and available phosphorus after soybean harvest in Kanyameshi soil (Fig. 3). Strain S1 had the highest mean total P and available P was 756.7 and 129.5 $\mu\text{g. g}^{-1}$ soil, respectively. Whereas, strain S2 recorded the lowest value of the total P and S3 for the available P. With regard to the flowering situation in the same site, the ANOVA result reveals similar effects between the strains.

For the Kasapa site, total and available soil phosphorus is not influenced by rhizobial strains applied regardless of the time of observation (i.e., flowering and harvest). Nevertheless, the Student test comparing the moment of observation between them shows a significant difference between the available phosphorus at flowering and harvest and not significant for the total P. The same trend is observed in the Kanyameshi site.

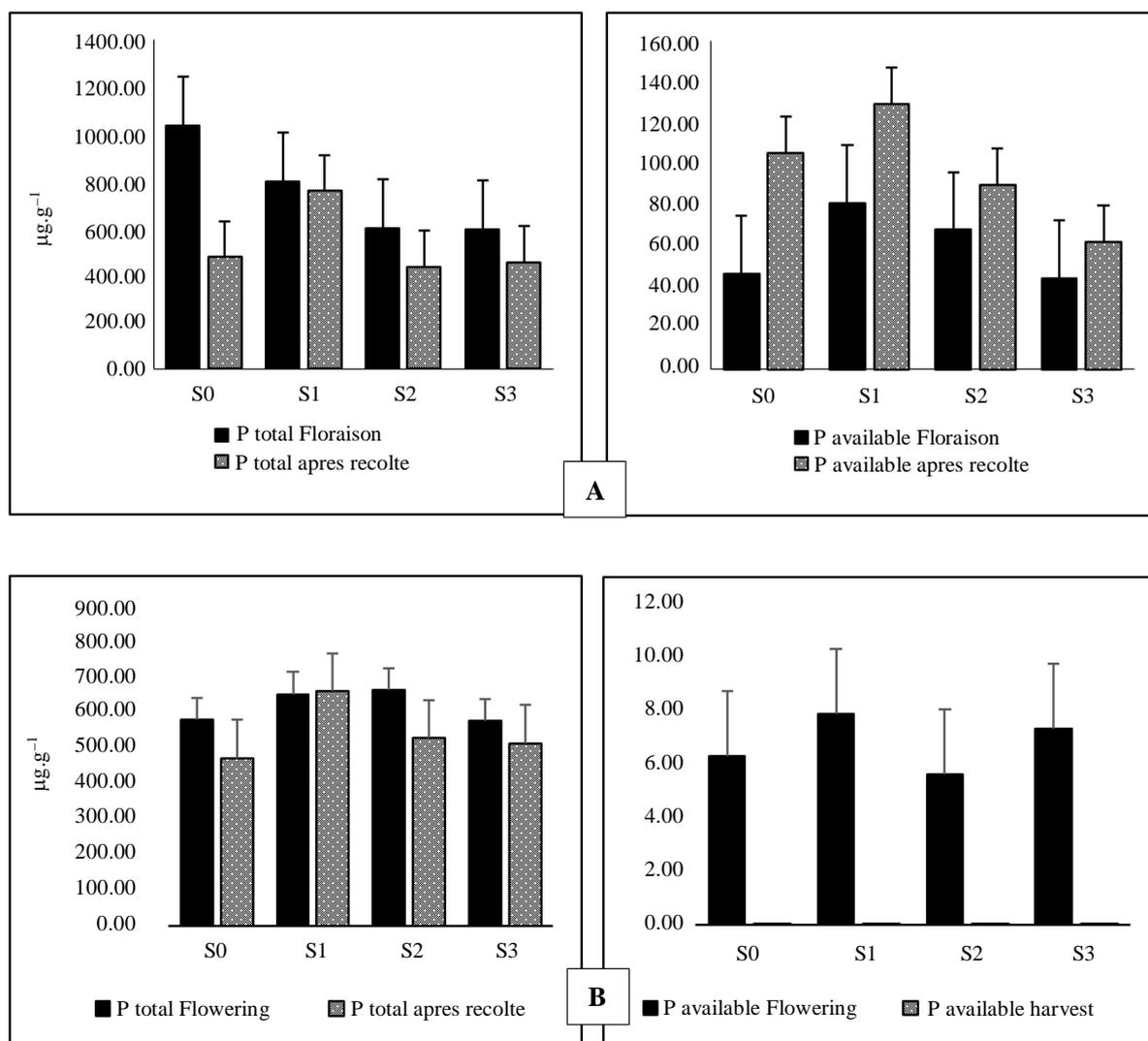


Fig. 3: Influence of Bradyrhizobium inoculation on total phosphorus (left) and available soil phosphorus (right). A: Kanyameshi site and B: Kasapa site

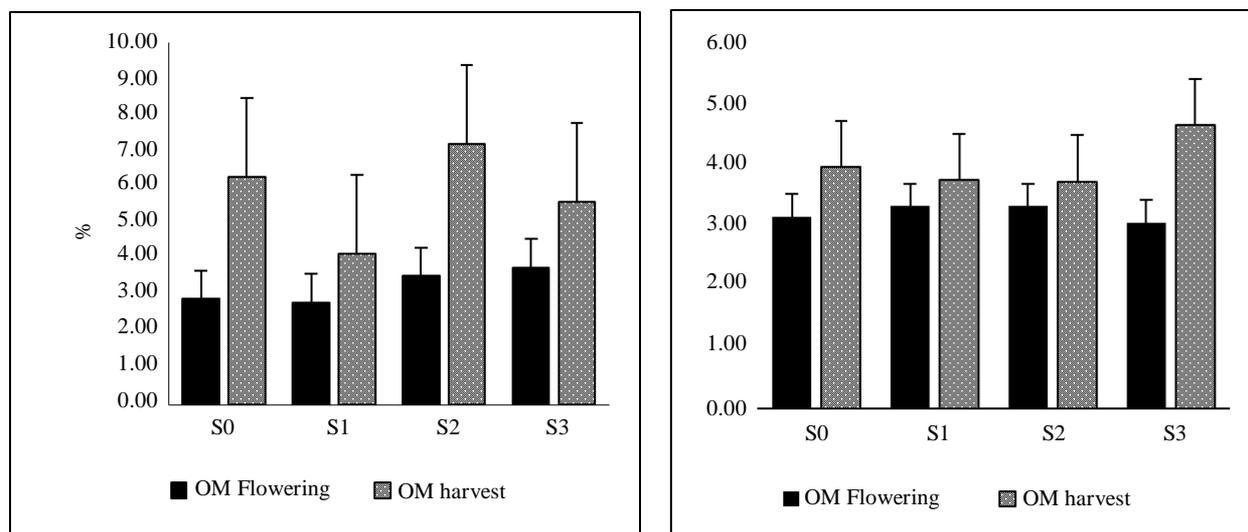


Fig. 4: Influence of inoculation by Bradyrhizobium on organic matter. On the left the site Kanyameshi and on the right the site Kasapa

Figure 4 shows that Bradyrhizobium strains did not produce a significant effect on soil organic matter in both Kanyameshi and Kasapa study sites at flowering and harvest. By comparing the time of observation (i.e., flowering and harvest) between them, the results reveal a highly significant difference for both study sites. These results show that the level of organic matter in the soil has improved after harvest. Thus, the control plots (S0) in the Kanyameshi site show that soil organic matter doubled at harvest.

Discussion

The results revealed similar effects between different rhizobial strains on pH, Nitrogen, C/N ratio and soil organic matter at flowering and harvest in both study sites ($p > 0.05$). Several studies have shown that the success of inoculation depends not only on the strain itself, but also on its ability to withstand poor environmental conditions (Smil, 2002; Thies *et al.*, 1991; Nazih *et al.*, 1993; Tshibuyi *et al.*, 2019) on the one hand and, on the other hand, to survive in the soil as saprophytic microorganisms beyond the first month following the inoculation (Gibson and Harper, 1985). The pH under the inoculated treatments remains close to neutral compared to the control inoculated in Kanyameshi site at harvest. Total and available phosphorus, only in the Kanyameshi site was influenced by the different strains of Bradyrhizobium at harvest. Strain S1 had the highest average in total and available P whereas; strain S2 recorded the lowest value of the total P and S3 for the available P. The diversity of rhizobia, like that of all other microorganisms, is extremely high (Amarger *et al.*,

1996), which would influence their effectiveness. On the other hand, in the Kasapa site, the different strains did not induce significant effects. Phosphorus deficiency has already been shown to affect the multiplication of rhizobia in the rhizosphere, resulting in reduced infection, reduced nodule growth and inhibited plant activity (Adebayo, 1992). Similarly, phosphorus has already been shown to be one of the many factors affecting yields and symbiotic nitrogen fixation in legumes (Mafongoya *et al.*, 2004). Pereira and Bliss (1989) showed that the amount of nitrogen fixed by the bean is severely limited by phosphorus soil deficiency. This situation could explain the results obtained in this study.

Several authors have reported that the tolerance to environmental stress of Rhizobium strain is a very important factor for good efficiency (Danso *et al.*, 1988). Thus, high temperatures ($> 40^{\circ}\text{C}$) inhibit nodulation and reduce N_2 fixation activity (Kichou and Sahraoui, 2001). The response to temperature likely vary considerably depending on the strains involved. When soil temperatures are high at the surface, nodulation tends to be localized in deeper horizons (Dommergues *et al.*, 1999). Similarly, under conditions of water deficit, the growth and survival of rhizobia are affected (Hungria and Vargas, 2000), nodulation is reduced and N_2 fixation is decreased (Brockwell *et al.*, 1995).

By comparing pH at flowering and harvest, the results showed highly significant differences in all sites. For this result, the pH increased from flowering to harvest at all sites. For total nitrogen and C/N, the Student test shows a significant difference on total nitrogen and C/N at Kanyameshi whereas in Kasapa only C/N showed a significant difference after comparing the situation in the soil at flowering and harvest. However, nitrogen content

in the soil decreased at harvest and in the case of C/N, the opposite situation is observed. Nutritional constraints likely limited the symbiotic nitrogen fixation by affecting rhizobial survival and multiplication, initiation, development and nodule functioning and growth of the host plant (Delgado *et al.*, 1994). In both Kanyameshi and Kasapa, the untreated control (S0) showed a post-harvest C/N ratio that was almost triple the flowering situation. This situation may be the beneficial effect of legumes in improving the soil chemical status through Nitrogen input and resultant decomposition of residues (Diop *et al.*, 2013; N'gbesso *et al.*, 2013).

Conclusion

The results showed that the different strains of rhizobium did not influence pH, nitrogen, carbon-nitrogen ratio and soil organic matter neither at flowering nor at harvest in both Kanyameshi and Kasapa. However, total and available P were influenced by different strains of rhizobium in Kanyameshi at harvest. The strain S1 managed to induce the highest total P value and, strain S2 induced the highest available P. Significant differences were obtained after comparing the situation of soil at flowering and harvesting on pH, total nitrogen, the carbon-nitrogen ratio, organic matter and available P soil. However, untreated controls (S0) showed after harvest nearly the triple of the situation observed at flowering for C/N ratio in Kanyameshi and Kasapa. This increased C/N ratio compared to inoculated treatment would suggest that there exists in the rhizosphere a competition between applied and native strains leading to limited N₂ uptake and soil fertility improvement.

This study showed that the direct effect of Bradyrhizobium on soil chemical properties is limited at certain strains and environmental conditions.

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Author's Contributions

Ben Tshibuyi Kasu-Bandi and Laurent Kidinda Kidinda: Contributed in paper preparation, development and publication.

Germain Nyembo Kasendue, Kasongo Lenge Emery, Antoine Kanyenga Lubobo, John Banza Mukalay, Mick Assani Bin Lukangila, Meschac Ilunga Tshibingu: Contributed in paper preparation and development.

Louis Baboy Longanza: Contributed in paper development.

Ethics

Authors declare that there not any conflict of interest or any other ethical issues that may arise after the publication of this manuscript. The data are original and from experiments conducted by the Authors of this manuscript.

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