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Research Article

PRELIMINARY TRIAL OF EX SITU INOCULATION OF EARTHWORMS, HYPERIODRILUS AFRICANUS FOR THE RESTORATION OF DEGRADED SOILS IN THE KIKWIT REGION OF THE DEMOCRATIC REPUBLIC OF CONGO

Madikani, K.E., *Musibono, E. D., Koy, K. R., Milau, E.F. and Mafuka, M.P.

Laboratory of Ecotoxicology and Ecosystem Health ERGS, Department of Environmental Sciences,
Faculty of Sciences, University of Kinshasa, DR Congo

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Abstract

Soil macroinvertebrates, including earthworms, provide ecosystem services that are now considered key to the implementation of ecologically intensive agriculture. In order to improve the productivity of very poorly fertile and particularly acidic soils, the present study aims to inoculate earthworms for maize production under the edaphoclimatic conditions of the Kikwit region of the DRC. For this purpose, a trial was carried out in a completely randomized system, to better test the feasibility of introducing these engineers into the soil to improve the productivity of agrosystems. In soilless mesocosms, the inoculation of earthworms positively influenced the growth in height and diameter of maize plants on the 45th day of cultivation by 109, 37±3.7cm and 1.866±0.25cm respectively compared to the control with 82.07±4.92cm and 1.233±0.06cm respectively. The results still show that earthworms had a significant effect on all production and yield parameters. At harvest, the yield was 3±3tons.ha-1 of dry corn kernels, an increase of 46% over the control. The texture was on average 87.9% sand, 7% clay and 3.3% silt, so it was sandy-clay. In the presence of earthworms, we observed a relatively fine granulometry, from 50 to 250 µm. Regarding agronomic properties, the corn plants had a much better growth in height and diameter and the yield in dry corn grains is very high, up to 3 ton.ha^{-1} and thus exceeds 46.3% in the presence of earthworms. Physical properties such as soil aggregation, porosity and water use efficiency improved in the earthworm treatments. Soil ingested by earthworms has a slightly finer particle size and is rich in clay. Chemical properties, such as C and N stock, mineralization of nutrients such as assimilable P, Mg^{++}, Ca^{++}, K^{+}; pH and CEC, were very positively influenced by earthworms. All these chemical properties, their content is significantly higher in earthworm castings than in the surrounding soil. Biological properties, such as the stimulation of nitrifying bacteria for the degradation of organic matter, source of the maintenance of the high content of C and N, were very significantly influenced by the presence of earthworms. The inoculation of earthworms, therefore, in sandy soils that are particularly acidic and poor in biogenic elements, constitutes a serious alternative to the use of inorganic inputs that are not within the reach of small farmers. It allows to improve production, yield, carbon sequestration and erosion control in agrosystems.

Keywords: Earthworms, soil engineers, macroinvertebrates, inoculation, ecosystem services, ecological agriculture, Kikwit.

INTRODUCTION

Slash-and-burn agriculture, the main agricultural practice in the tropics, has been overexploited for several years now, leading to soil degradation and reduced productivity and generating low yields per hectare. It leads to a significant loss of nutrients and is therefore not considered a sustainable agricultural practice (Brown *et al.,* 1994; Dupriez, 2007). The soils around Kikwit in Kwilu Province are acidic ferralitic soils, highly desaturated, poor in organic matter and in assimilable N and P. Indeed, the high content of iron and aluminum sesquioxides in these types of soils gives them a strong fixing power of available soil P (Mbala, 1990; Ratsiatosika, 2018). Thus, it is necessary to provide fertilizers to compensate for this low fertility. In this region, where the drop in soil fertility cannot be compensated for by massive fertilizer inputs, for obvious economic reasons, nor by long fallows, following the galloping demography, one of the solutions considered is to conserve, or even improve, soil fertility by manipulating biological processes within the framework of low-input farming systems. Soil fertility depends primarily on the quantity and quality of organic matter transformed by decomposer organisms. The efficiency of decomposers can be characterized by the rate of transformation of organic matter, which depends on environmental factors such as temperature, moisture, and characteristics of the material to be decomposed (Francis *et al.,* 2003). Several studies have shown that earthworms in the soil, play a primary role in the transformation of organic matter (Darwin, 1881; Lavelle *et al.,* 1998; Francis *et al.,* 2003; Blanchart *et al.,* 2008; Milau, 2016, Ratsiatosika, 2018). Earthworms as decomposers facilitate the release of mineral elements through the decomposition of organic matter. They not only affect the availability of nutrients to plants, but also influence the entire rhizosphere (Darwin, 1881; Scheu, 2003; Blanchart *et al.,* 2008). In the context of sustainable agriculture, maintaining soil fertility and thus improving agricultural production through the manipulation of earthworms is an avenue to be explored (Lavelle *et al.,* 1989; Lavelle *et al.,* 1998). Earthworms affect and improve crop production through five processes: (a) increase in the rate of mineralization of organic matter making nutrients available to the plant, (b) modification of soil structure, (c) stimulation of symbiont activities, (d) production of plant growth substances (hormones) through the stimulation of microbiological activities, and (e) control of pests and plant pests (; Brown *et al.,* 1994; Scheu, 2003; Puga Freitas, 2013). This mode of ecological and productive agriculture conserves and improves natural resources. It uses an ecosystem approach that leverages nature's contribution to plant growth and the increase of soil organic matter (Blanchart, 2006; Lavelle, 2012; Puga Freitas, 2013; Bouché, 2014). These organisms can, therefore, be considered as a natural resource of agronomic interest that can be used to increase agricultural

Corresponding Author:** ***Musibono, E. D.,
Laboratory of Ecotoxicology and Ecosystem Health ERGS, Department of Environmental Sciences, Faculty of Sciences, University of Kinshasa, DR Congo.

production in a sustainable manner by ensuring the maintenance of soil quality and health (Lee, 1985; Lavelle *et al.,* 1998). The manipulation of earthworms in farming environments has led to the development of a technology called Bio-Organic Fertilization (BOF) in tree plantations (Senapati *et al.,* 1999; 2002). BOF initially tested for 3 years in India resulted in an increase of more than 240% in tea production, an increase in farm profitability of up to 260%, and improvement in the physicochemical and biological properties of the soil (Shapitalo *et al.,* 2004; Sheehan *et al.,* 2006; Ratsiatosika, 2018). FBO has been transferred to other countries in the world such as China and Australia (Senapati *et al.,* 1999; Sheehan *et al.,* 2006). In the Democratic Republic of Congo in general and for the Kikwit area in Kwilu Province in particular, data on the impact of earthworms on agricultural productivity, unless we forget, are either very weak or almost non-existent (TRAN-VINH-AN, 1973, Milau, 2016). The present study will test that inoculation of earthworms into degraded soils in the Kikwit region not only improves the soil properties of these soils, but also increases agricultural productivity. The specific objective of this work is to inoculate earthworms as bio-organic fertilizers (BOF) for maize production under the soil and climatic conditions of the Kikwit region.

MATERIALS AND METHODS

The earthworm inoculation experiment was conducted in the northwestern part of the city of Kikwit, more precisely in the experimental field of the Faculty of Agricultural Sciences of the University of Kikwit. This site is located in Ndunga village, Kipuka sector, Bulungu Territory, Kwilu Province in the Democratic Republic of Congo (DRC). It is located 10 km from the center of Kikwit at 18°44'473" East longitude, 05°01'085" South latitude and at an altitude of 489 m. This site is represented on the Field Map below:

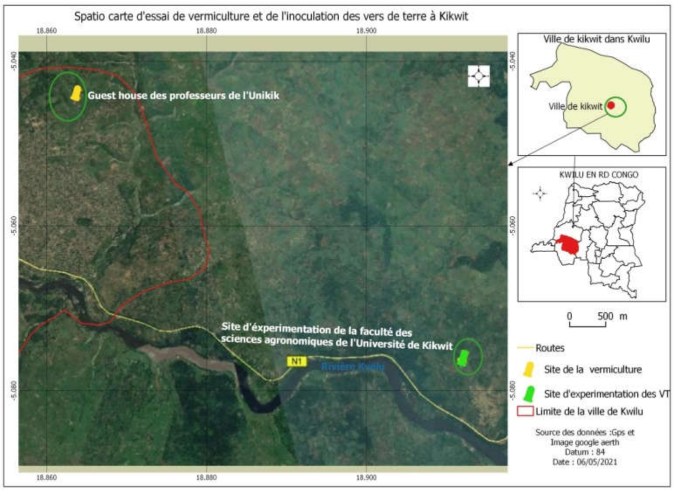

Figure 1. Map presentation of the study area and site

The Kikwit region enjoys a humid tropical climate with a sub-equatorial trend and a three-month dry season. This region is classified as an AW3 type climate according to the KOPPEN classification of 1936 (Nicolai, 1963; Fehr, 1990; Lubini and Kusehuluka, 1990; Masens, 1997). The average rainfall varies from 1200 to 1500mm per year. The average temperature is between 24 and 25°C and the relative humidity is 85% (Nicolai, 1963; Fehr, 1990; Masens, 1997). The soil in the Kikwit area is ferralitic, characterized by a high proportion of Kaolinite associated with iron and aluminum sesquioxides. The soil texture is sandy-clay with an average total sand content of about 80.6%. The low clay content (colloids) makes this soil vulnerable to erosion (Mbala, 1990). The original vegetation cover in the hinterland of the city of Kikwit has been completely modified and destroyed as a result of anthropogenic threats, such as the expansion of the city and the satisfaction of the needs of the population (Nicolai, 1963; Fehr, 1990; Lubini and Kusehuluka, 1990; Masens, 1997). The biological materials used in this study were earthworms and corn kernels. The earthworms were collected from the agroecosystems and the lawn located at the University of Kikwit next to the amphitheater. The earthworm species inoculated in this study was Hyperiodrilusafricanus (Beddard, 1891), as it is the large earthworm encountered in agro ecosystems under DRC conditions and is the most encountered under the lawn producing a large amount of castings (TRAN-VINH-AN, 1973; Milau, 2016). For the implementation of the trial, five types of substrates were used, including one containing the soil of the square (SP) taken under the population of Imperatacylindrica and serving as a control and four other fertilizers based on the soil of the square enriched with organic matter of animal origin consisting of living individuals of earthworms (VT) considered as bio organic fertilizers and chicken droppings (FP), of plant origin represented by the groundnut hulls (CA) and of mineral origin represented by the NPK. To collect earthworms in the field and soil samples, several materials were used, including formalin diluted to 40%, 20cc syringes, water, auger (probe), polyethylene bags, etc.

Many methods have been developed to optimize earthworm extraction, (Cluzeau *et al.,* 1999; Peres, 2003; Pelosi, 2008). The collection of earthworms in this study was performed using the method described by Bouché (1972a) and adapted by Cluzeau *et al.,* (1999) and used by Milau (2016), which combines the chemical method and manual sorting. Chemical extraction was performed using 20 ml of 0.40% formalin diluted in 10 liters of water spread over a 1m2 area delimited by a wooden quadra. Each time, three applications were made at the same place, spaced 15 minutes apart. The earthworms irritated by the formalin came out of their galleries and were then picked up by fine tweezers to keep them in the jars containing moist soil before they were transported to the inoculation site. Some individuals extracted by this method are intoxicated, become too weak and may die (Bouché and Gardner, 1986; Peres, 2003; Milau, 2016). Thus, the chemical method was supplemented by manual sorting applied to a soil monolith with a volume of 0.0125m3 (0.25m x 0.25m x 0.20m deep). Studies have emphasized the effectiveness of manual sorting, as it is satisfactory up to 93% for total abundance of individuals (Peres, 2003, Ratsiatosika, 2018). The presence of turricles had been used as an indicator for the choice of sites to be retained for the collection of earthworms, especially since we were targeting earthworms that could influence agroecosystems. The experiment was conducted in a completely randomized, block design with 3 blocks and 5 treatments, i.e. 15 elementary plots. The treatments studied consisted of the unfertilized soil of the site (SP), which served as a control. The organic, mineral and biological fertilizers used were: chicken droppings (FP), peanut shells (CA), NPK 17 and earthworms (VT). Polyethylene bags were used to carry the substrates above ground. A superficial ploughing of 15-20cm depth was carried out with the hoe to homogenize the soil. During plowing, the soil of the square was collected to fill half of the polyethylene bags that had been enriched with fertilizers, except for the pellets of the soil of the square and NPK. These organic substrates were mixed with the plaza soil in a 2:4 ratio. The NPK treatments were applied 2 weeks after sowing and at the time of disbudding, at a rate of 5 g per plant. For the earthworm treatments, the bags were filled halfway to allow the introduced worms not to escape. The other half of the soil was added gradually as the worms adapted. All pots were covered with a 5 cm layer of straw to maintain humidity. The sowing was done at 1x1m spacing and each elementary plot had 16 plants among which there were 4 central plants and the useful density consisted of 60 plants from which we took the data. To reduce the loss of the earthworm population, the inoculation was done in two phases: (i) during the first phase, inoculation took place 30 days before sowing to allow earthworms to adapt and begin bioturbation activities to make biogenic elements available to plants; (ii) the second phase had taken place 45 days after sowing.

The earthworms were inoculated in the evening in 5 cm deep trenches, previously moistened at a rate of 25g.m-2 or 10g per 0.40m2. A total of 1200g of earthworms were used to cover the 48 pits prepared to receive them (Tondoh and Konaté, 2005; Golli, 2013; Ratsiatosika, 2018). Soil samples were taken at a depth of 0 to 25cm using an auger and/or cylinders before the experiment to characterize the initial soil condition and after the trial to assess the effect of improving the physicochemical characteristics of the soil. The castings produced by the earthworms during the experiment were collected and analyzed in order to know the composition of the ambient soil and the one crossed by the earthworms. The composite soil samples and the castings were analyzed at the Pedology Laboratory of the Faculty of Agronomic Sciences of the University of Kinshasa. Measurements of agronomic properties consisted of evaluating growth and yield parameters of maize. Water use efficiency (WUE) was estimated using the formula of Ouédraogo *et al.,* (2006) in Golli, (2013) in order to know if the presence of earthworms favors a better water use by maize. This parameter was calculated from the following formula:

$$\text{WUE} = \frac{\text{Wasted biomass of treatment (kg/ha)} - \text{Bwasted biomass of control (kg/ha)}}{Total\ rainfall\ (mm)}$$

Statistical treatments were performed with the statistical software R (R Developpment Core Team, 2018) and Xlstat 2018, to perform the analysis of variance (ANOVA) and Student's Test (t) to compare the means of growth parameters and the evaluation of correlations and multivariate component analyses were also used to assess the correlation of soil parameters in relation to substrates.

RESULTS AND DISCUSSION

Agronomic parameters

Regarding the growth of maize plants, at day 30 and 45 (Table 1), ANOVA showed that the organic fertilizer treatments had the highest maize plant heights, with FP leading the way, followed by VT at 81.1±7.44 cm and 136.1 ± 6.1 cm, and 69.9± 2.44 and 109.3 ± 3.43 cm, respectively. The inorganic fertilizer (NPK) treatment showed an intermediate height 58.6± 4.46 cm on day 30, but it had experienced a slight increase of 6.36 cm over the VT treatment on day 45, 115.7± 5.32 cm. Nevertheless, there was not a significant difference between NPK and VT. The control still gave a low growth of maize at the last growth phase i.e. 53.0 ± 6.41 cm compared to CA which gave 82.0 ± 4.92 cm. Considering the crown diameter (Table 1), At day 45, according to ANOVA, chicken droppings had a significant effect on the crown diameter of maize plants with 3.1 ± 0.11 cm, followed by inorganic fertilizer, earthworms and peanut shells with 2.1 ± 0.12 cm; 1.9 ± 0.2 cm and 1.6 ± 0.12 cm respectively. But the soil of the square always gave a small diameter at the neck of the maize plants, 1.2 ± 0.06 cm. We observed that maize plants generally grew vigorously in height and thickness in the organic fertilizer treatments, including earthworms. This is due to the fact that these substrates sustainably enrich the soil with N and C, which are nutrients exploited by maize during the growth phase and especially that earthworms activate growth hormones, auxins in particular (Puga Freitas, 2013; Golli, 2013; Ratsiatosika, 2018). In relation to production and yield parameters, Analysis of variance reveals a significant difference between the average length of ears of different treatments in study at harvest ($p = 0.001$). Worms had significant effect with 14.4 ± 1.7 cm more than NPK and FP treatments which gave median lengths with 12.1 ± 1.8 and 11.8±1.3 cm respectively, while CA and SP treatments showed low lengths with 10.7 ± 1.6 and 9.3± 1 cm respectively (Table 1). The ANOVA shows that, the number of rows per corn cob is largely influenced in the presence of earthworms, followed by chicken droppings, with 13.8 ± 0.29 and 12.8 ± 0.29, respectively, compared to the others, including NPK, CA, and SP which did not show remarkable differences, i.e. 12.1 ± 1.04; 11.8 ± 1.2; and 11.8 ± 1.4, respectively. However, the number of rows per ear did not reveal significant differences between different treatments under study ($p = 0.002$) (Table 1). The highest 100-grain weight came from the earthworm treatment, 28.4 ± 1.75 g. The weight obtained in the NPK treatment is almost equal to that with the chicken droppings application, which had each 27.4 ± 0.72 and 27.2 ± 1.16 g. The peanut hulls produced an intermediate weight, 25.7 ± 0.55 g, while the square soil yielded a low weight, 22.4 ± 0.51 g.

Table 1. Average variation of the growth and yield parameters of corn as a function of time

Parameters Time / Treatments	Growth parameters: Parameters Average plant height cm)			Average plant neck diameter (cm)			Average parameters of production and yield of corn at harvest					
	15th D	30th D	45th D	J15th D	J30th D	45th D	LEP (cm)	NR	PCG (gr)	PTGS (gr)	RTHa (t/ha)	WUE (kg/mm)
VT	45,7±3,43	69,9±2,44	109,3±3,43	1,3±0,06	1,8±0,25	1,9±0,2	14,4±1,7	13,8±0,29	28,4±1,75	301,8±35	3,0±17	46,6±0,3
CA	36,96±4,92	50,4±3,29	82±4,92	0,9±0,15	1,4±0,15	1,6±0,12	10,7±1,6	11,8±1,2	25,7±0,55	143,2±8,72	1,4±10,44	12,6±1,51
NPK	50,5±5,32	58,6±4,46	115,7±5,32	1,2±0,06	1,5±0,31	2,1±0,12	12,1±1,8	12,1±1,04	27,4±0,72	247,8±26	2,4±17,2	28,2±0,32
SP	46,23±6,41	33,3±6,14	53±6,41	0,3±0	0,9±0,29	1,2±0,06	9,3±1	11,8±1,4	22,4±0,51	116,4±12	1,1±5	31,7±0,57
FP	19,26±6,1	81,1±7,44	136,1±6,1	1,3±0,06	2,1±0,12	3,1±0,11	11,8±1,3	12,8±0,29	27,2±1,16	180,3±9	1,8±14,19	36,6±0,7

Legend: **VT**: earthworm, **CA**: peanut shells, **NPK**: mineral fertilizer composed of nitrogen, phosphorus and potassium, **SP**: field soil (control), **FP**: chicken droppings, **LEP**: ear length, **NR**: number of rows, **PCG**: hundred gram weight, **PTGS**: total dry weight of grain, **RTHa**: yield in tons per hectare, **WUE**: water use efficiency.

The production of dry maize grains was largely influenced by the inoculation of earthworms, followed by inorganic fertilizer, chicken droppings and peanut shells, with 301.8 ± 35 g; 247.8 ± 26 g; 180.3 ± 9 g and 143.2 ± 8.72 g respectively. The soil of the square, on the other hand, yielded a low total dry kernel weight of 116.47± 12. Maize yield was largely influenced by the presence of earthworms, followed by mineral fertilizer, chicken droppings and peanut shells, with 3 ± 3 T/ha, 2.4 ± 2.1 T/ha, 1.7 ± 0.5 T/ha and 1.3 ± 0.4 T/ha respectively. The lowest yield was observed on the plaza soil (control) with 1.1 ± 0.2 T/ha. The analysis of variance reveals significant differences between the yield of different treatments under study (p-value = 0.001). This better performance of dry corn grain yield based on earthworms increased compared to that obtained mainly with the control (unenriched soil) by 46.1%, inorganic fertilizer (NPK) by 11.1% and other organic fertilizers, such as chicken droppings by 27.7%. These results show that the introduction of soil engineers, including earthworms and the triggering of their positive activity is involved in the restoration of fertility of degraded and acidic agroecosystems and increase their productivity (Senapati *et al.,* 1999 and 2002; Golli, 2013; Gilot, 1994; Ratsiatosika, 2018). It boils down to say that in addition to the improvement of soil porosity and aggregation by earthworms (Darwin, 1881; Blanchart *et al.,* 1999; Golli, 2013; Ratsiatosika, 2018), the improvement in maize production would be explained by better utilization of soil mineral nutrients in the presence of earthworms (Lavelle *et al.,* 1998; Brown *et al.,* 1994 and Scheu, 2003). The ANOVA shows that the highest EUE was observed in earthworms, followed by chicken droppings and the control, presenting 46.687 ± 0.3 kg/mm, 36.613 ± 0.7 kg/mm and 31.760 ± 0.57 kg/mm respectively. Mineral fertilizer and peanut shells gave lower values than the control, 28.217±0.32 kg/mm and 12.657±1.51 kg/mm respectively. This is explained by the fact that earthworms improved the physical properties of the soil, including structure through the creation of a network of galleries and bioturbation movements. The soil structure is one of the main components of soil fertility by modulating the water regime, infiltration, drainage, aeration, retention and spatial distribution of nutrients, and facilitates roots to progress (Blanchart, 2008, Golli, 2013, Ratsiatosika, 2018).

Pedological properties

Regarding the granulometry, the texture of the soil of our study site consists on average of 87.9% sand, 7% clay and 3.3% silt, this soil is sandy-clay. These results are similar to those obtained by (Mbala, 1990). The analysis of the eight-fraction particle size shows that the soil under study is characterized by two predominant fractions, including the 250 µm and 125 µm fractions (Figure 2). The 250 µm fractions are positively and strongly correlated with the SIAE and FP treatments and with respect to axis 2, the 100 µm fractions are positively correlated with the earthworm treatment. This can be explained by the fact that the initial soil not reworked before the experiment and the one receiving an organic fertilizer, rich in nitrogen, maintained the initial granulometric composition, i.e. 250 µm observed naturally in the soil. The results obtained in this study clearly show that the soil has a sandy-clay texture. The work done by Nicolaï, 1963; Mbala, 1990; Lubini and Kusehuluka, 1990, Masens, 1997, confirms the texture of this soil. However, small grains were observed, 100 µm, in the presence of earthworms because there is evidence that less solid mineral particles can be ground through the gizzard and intestine of earthworms into finer particles (Meyer, 1943, in TRAN-VINH-AN, 1973, Charpentier, 1996). The 250 µm fraction is negatively correlated with the treatment consisting of the castings could be explained by the fact that the excreta maintain the textural fraction acquired by the earthworms, as the ability to grind mineral particles no longer exists (TRAN-VINH-AN, 1973; Golli, 2013; Ratsiatosika, 2018).

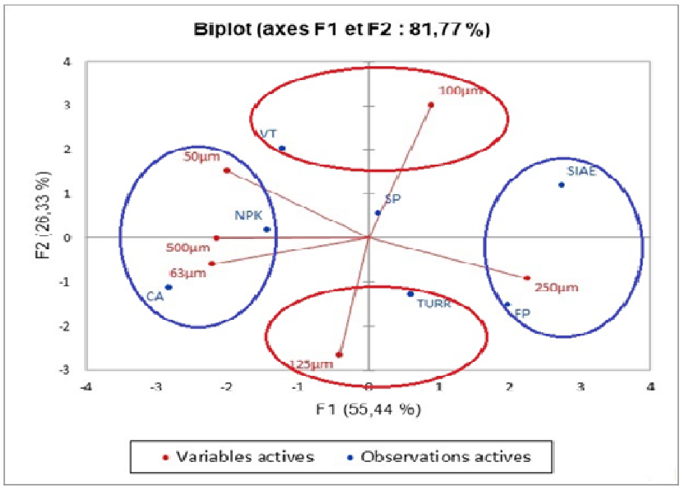

Figure 2. Correlation of the granulometric fractions to eight fractions

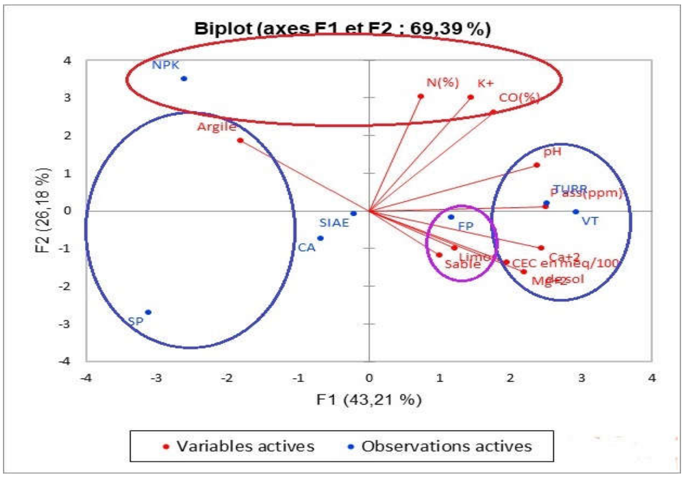

Figure 3. Correlation of chemical parameters to different substrate

The analysis of correlation circles between chemical parameters and observations in the plane defined by the three principal components (Figure 2), shows that pH, Pass, Ca^{++}, Mg^{++} , CEC, N, and K^{+} are highly positively correlated with VT and Turr treatments. This could be explained by the fact that earthworms through the production of stable calcium-rich biostructures increase pH, which in turn improves the cation exchange capacity and availability of nutrients in the soil and their uptake by plants (TRAN-VINH-AN, 1973, Charpentier, 1996). The phenomenon of organic carbon (OC) stock is explained by the fact that, on the one hand, earthworms stimulate the activity of microorganisms in their digestive tract and in their fresh turricles promoting the mineralization of C in the short term (Lavelle *et al.,* 1998; Brown *et al.,* 1999; Ratsiatosika, 2018). On the other hand, they produce biostructures (stable aggregates) capable of physically protecting C, thus increasing the long-term C stock (Bouché and Garner, 1986; Milau, 2016; Ratsiatosika, 2018). The improvement in corn grain yield observed in the earthworm treatments up to 3tonnes.ha-1 is confirmed, as the earthworms were able to reduce the pH from 5.2 to 5.5 -5.6. It is well documented that corn and other crops perform well in this slightly acidic pH range (Anonymous, 1994). The work of Jeanson, 1971, in TRAN-VINH-AN, 1973, has remarkably highlighted the existence of a clay-humus complex in the earthworm castings, which allows the exchange of cations with the soil solution.

Conclusion

The present study has provided a very important advance on the influence of soilless earthworm inoculation on the growth and yield of the maize crop. The study shows that it was the "earthworm" treatment that was able to give satisfactory results at the end of the experiment. Regarding the agronomic properties, the maize plants had a much better growth in height and diameter and the yield of dry maize grains was very high, up to 3ton.ha^{-1} and thus exceeded 46.3% in the presence of earthworms. The performance of the maize crop was stimulated by the improvement of the soil properties of the soil. Physical properties such as soil aggregation, porosity and water use efficiency improved in the earthworm treatments. Soil ingested by earthworms has a slightly finer particle size and is rich in clay. Chemical properties, such as C and N stock, mineralization of nutrients such as assimilable P, Mg^{++}, Ca^{++}, K^{+}; pH and CEC, were very positively influenced by earthworms. All these chemical properties, their content is significantly higher in earthworm castings than in the surrounding soil. Biological properties, such as the stimulation of nitrifying bacteria for the degradation of organic matter, source of the maintenance of the high content of C and N, were very significantly influenced by the presence of earthworms. Considering all these results, the inoculation of earthworms in agroecosystems allows to improve not only the production and yield of the corn crop, but also other ecosystem services, such as C sequestration and erosion control. From this study, it can be deduced that earthworm inoculation thus represents a serious alternative to the use of inorganic inputs that are not within the reach of small-scale farmers, and that a sandy soil, considered one of the poorest, can be improved to some extent in terms of fertility through earthworm inoculation. However, further study on the economic evaluation of this technology seems inescapable before any promotion.

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