International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 42 No. 1 Mar. 2024, pp. 131-140

http://www.ijias.issr-journals.org/

Agro-pedological impacts of different crop rotations on a ferric acrisol in Burkina Faso

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ABSTRACT: Low crop yields are often explained by unfavourable rainfall conditions, the natural poverty of the soil in terms of nutrients and the low use of fertilisers. In order to find appropriate solutions for the sustainable management of soil fertility, a study was carried out on the Fertility Maintenance Trial (FTM), an experimental system established in 1960 in central western Burkina Faso, where organic and/or mineral fertilisation regimes combined with crop rotations have been tested. The approach of this study consisted of a synthesis of existing agronomic data from 2011-2019 on the three (03) crop rotations. Soil samples were taken from a depth of 0-20 cm for physico-chemical analysis. We also measured yields on the cotton and sorghum plots during the 2018 and 2019 seasons. The results show that yield variability can be attributed not only to fertilisation, but also to crop rotations and the annual rainfall recorded over the period. The sorghum-cotton and sorghum-cowpea rotations produced the highest average sorghum yields, at 547 $kg.ha^{-1}$ and 642 $kg.ha^{-1}$ respectively. Sorghum monoculture recorded the lowest sorghum production. Chemical analyses revealed higher phosphorus use in the sorghum-cowpea rotation compared with the other rotations. The study of cropping system efficiency also revealed the role of legumes in crop rotations in maintaining and preserving soil fertility. In addition, we recommend integrated soil fertility management (organic and mineral fertilisation, crop rotations, etc.) for sustainable management of productive capital on cotton farms in Burkina Faso.

KEYWORDS: Impacts, production, crop rotations, ferric acrisol, fertility, Burkina Faso.

1 INTRODUCTION

Strong demographic growth in recent years has led to high pressure on arable land resources. This high pressure on vegetation cover influences the capacity of soils to produce the biomass required to meet the needs of a growing population (Haddaway et al., 2014; FAO, 2018). By way of illustration, almost 60% of soil depletion has been attributed to various degrees of soil ecological processes, with agricultural practices becoming one of the main contributors (Affholder et al., 2013; Baize, 2017, Bolinder et al., 2020). Thus, research is exploring taking a significant position in the overall resilience of agricultural objectives by transforming scientific information about soils into real techniques that increase farmers' understanding of the sustainability of their farming activities (Han et al., 2016; Borchard et al., 2019; Krauss et al., 2020). One approach to sustainable agricultural management is to increase soil organic matter and reduce soil erosion through crop rotation (Laberge et al., 2011; Hopkins and Hansen, 2019; Krupnik et al., 2019; Rinot et al., 2019).

Crop rotation perturbs the reproduction of insects and pathogens, and therefore their life cycle. Plant nutrients are restored when certain plant species are included in the crop rotation, requiring less chemical fertiliser. Crop rotation is a useful technique in the practice of sustainable agriculture (Fontaine et al., 2011; Voisin et al., 2015; Guinet et al., 2019; FAO, 2018). Nevertheless, careful selection of a crop rotation pattern has the potential to reduce trade-offs between crop viability and environmental impacts, maintain long-term soil fertility and disrupt weed and disease cycling processes through intrinsic nutrient recycling (Bouthier and Trochard, 2015; Jeudy et al., 2016; Eva, 2019; Sierra et al., 2019).

The advantages of rotations including a legume in terms of food security and maintaining soil health are well established. After the rotation, the increasing heterogeneity of the agricultural production system will maintain or improve soil performance by increasing crop residues and various root systems, as well as increasing and developing activity (FAO, 2018; Bünemann et al., 2018; Eva, 2019; Chabert and Sarthou, 2020). To understand how soil fertility changes under crop rotations and to improve the sustainable management of soil fertility, an experiment was conducted at Saria, in the Centre-West region of Burkina Faso. Several research projects have been carried out on this experimental system, known as the Fertility Maintenance Trial (FTM). They focused on the impact of soil fertilisation options on carbon fractions, mineral balances, forms of phosphorus, soil organic matter and soil biology. Very little work has been done on the impact of the three (03) crop rotations on soil and crop productivity. This summary will make it possible to evaluate the evolution of sorghum and soil production under the influence of the different crop rotations tested since 1960.

2 MATERIALS AND METHODS

2.1 STUDY SITE

The study was conducted in the long-term trial plots at the research station of the Institute of Environment and Agricultural Research (INERA) in Saria, a village located at 2°16'N, 2°9'W at an altitude of 300 m in central western Burkina Faso. The average rainfall in 2015, 2017 and 2019 was 964, 898 and 992 mm, corresponding to 67, 65 and 74 days of rain respectively in the area. The vegetation type was an open woody savannah and the main species were Parkia biglobosa, Vitellaria paradoxa, and Tamarindus indica. The herbaceous component was dominated by Pennisetum pedicellatum, Andropogon sp., Loudetia togoensis, and Schoenfeldia gracilis.

2.2 METHODS

2.2.1 EXPERIMENTAL DESIGN: FERTILITY MAINTENANCE TRIAL (FTM)

The Fertility Maintenance Trial (FTM) was implemented in 1960 to study the fertility of a leached tropical ferruginous soil (ferric acrisol) under the influence of different cropping practices. These practices include mineral and organo-mineral fertilisation and three cropping systems: (i) sorghum monoculture *(Sorghum bicolor L.),* (ii) sorghum-cotton *(Gossipium hirsutum)* rotation and (iii) sorghum-leguminous rotation (groundnut until 1973, cowpea thereafter *cowpea (Vigna unguiculate L.))*.

Six identical treatments were applied to each of the three systems. The dose of mineral fertiliser depends on the nature of the crop and is expressed in kg of N, P_2O_5 and K_2O.

The main treatments were:

- **te**: control without any fertilizer addition,
- **fmr**: low mineral fertilization (37-23-14-6S-1B) + recycling of sorghum residues once every two years,
- **fmo:** low mineral fertilization (37-23-14-6S-1B) + low rate of cow manure (5 t MS ha $^{-1}$ 2ans^{-1}),
- **fm:** exclusive low mineral fertilization (37-23-14-6S-1B),
- **FMO:** high mineral fertilization (60-23-44-6S-1B) + high rate of cow manure (40 t MS ha $^{-1}$ 2ans^{-1}) + export of sorghum straw,
- **FM**: exclusive high mineral fertilization (60-23-44-6S-1B)

2.2.2 STUDY APPROACH

In this study, the available data on the three (03) rotations: sorghum-cotton, sorghum-cowpea and sorghum-sorghum from 1981 onwards, over a six-year period in the cotton-growing year (odd-numbered year) were used. Then, under this rotation systems based on sorghum, we assessed the agronomic and pedologic impact of the continuous application of mineral, organic and organic+mineral fertilisers. This will enable to assess the sorghum yield growth performance and soil chemical parameters evolution under the various soil fertilisation practices in sorghum-based rotations systems. In addition, to compare the agro-pedological efficiency of the three systems, we considered the years in which the trial was cultivated with sorghum (even-numbered years since 1960). In principle, this comparison evaluates the after-effects of sorghum, cotton and cowpea cultivation respectively in the sorghum-sorghum (a), sorghum-cotton (b) and sorghum-cowpea (c) rotation systems. These cropping systems were compared to a fallowed field since 1961. As a result, the impact of each crop cultivation in the rotation could be assessed. Soil carbon, soil phosphorus and pH as chemical parameters and the sensitivity to soil degradation as physical property were the concerning studied property. The study focused on years when all the plots were cultivated with sorghum.

The efficiency of the systems was studied by considering the individual and cumulative effects of the six fertilisers applied.

The soil degradation sensitivity (St) is determined by the method of Pieri (1989):

$$(St) = MO*100 / (A + Lf)$$

Where: *MO = organic matter, A = clay and Lf = fine silt.*

- St < 5, the soil is physically degraded and highly susceptible to erosion;
- 5< St<7, the soil is at high risk of physical degradation;
- St >7, the soil is not at risk of degradation.

2.2.3 Soil Sampling And Chemical Analysis

Analysis Soil samples were taken at the end of the cropping season of 2011. Three soil samples were taken per plot with an auger at 20 cm depth after harvest. The soil samples were then pooled and air dried and sieved at 2 mm. The soil organic carbon content was determined according to Walkley and Black (1934). The soil total N and P contents were determined after a wet digestion with H_2SO_4 solution, as described in Novozamsky et al. (1983) and a measurement using an automatic colorimeter (SKALAR SAN plus SYSTEM). Available phosphorus was extracted according to the Bray and Kurtz (1945) and then, its content determined with the automatic colorimeter. Soil pH was determined, using the electrometric method, in a soil solution with soil and water ratio of 1/2.5.

2.2.4 Statistical Analysis

Rstudio was used for the Principal Component Analysis and to produce the boxplots. Genstat 9th edition was used for the analysis of variance of the data and the Newman Keuls test for the separation of means at the 5% probability level.

3 Results

3.1 Sorghum Yield Evolution Over The Five Years Under The Various Fertilisation Practices And Sorghum Cropping Rotation Systems

The Table 1 shows a large variability in sorghum yields induced by different manures applied over time. The Newman Keuls test revealed a highly significant difference between the fertilisers, between years and the combined effects of fertilisers and years of cultivation. There was inter- and intra-annual variation between treatments.

Table 1. Average change in sorghum yield in cropping rotations under the impact of fertiliser options

Years	te	fmr	fm	fmo	FMO	FM
2007	350 ± 45	840 ± 58	598 ± 37	833 ± 35	912 ± 56	781 ± 41
2009	389 ± 18	617 ± 27	757± 37	946 ± 64	1074 ± 35	955 ± 16
2011	290 ± 85	619 ± 57	398 ± 21	829 ± 14	911± 30	634 ± 11
2013	319 ± 98	648 ± 92	427 ± 12	858 ± 12	940± 31	663± 12
2015	393 ± 40	782 ± 107	597 ± 39	804 ± 30	810 ± 17	731 ± 34
Newman Keuls test at the 5% level					**v.r.**	**p.r**
Treatements					**27,48**	**< 0,001**
Years					**13,52**	**< 0,001**
Treatements * Years					**2,27**	**< 0,001**
Rotations* Treatements					**3,82**	**< 0,001**
Rotations * Treatements *Years					**2,57**	**< 0,001**

***te**: control without any fertilizer addition, **fmr**: low mineral fertilization (37-23-14-6S-1B) + recycling of sorghum residues once every two years, **fmo:** low mineral fertilization (37-23-14-6S-1B) + low rate of cow manure (5 t MS ha $^{-1}$ 2ans^{-1}), **fm:** exclusive low mineral fertilization (37-23-14-6S-1B), **FMO:** high mineral fertilization (60-23-44-6S-1B) + high rate of cow manure (40 t MS ha $^{-1}$ 2ans^{-1}) + export of sorghum straw, **FM**: exclusive high mineral fertilization (60-23-44-6S-1B).*

Statistical analysis of average sorghum yields for the three crop rotations showed a highly significant difference between treatments (Table 1). The control had the lowest yields. It was followed respectively by the fm, fmr, FM, fmo and FMO manures. The organic+-mineral fertilisers had the highest annual yields. Annual increases of 31 to 56 % of sorghum yields with mineral fertilisers application are reached by sorghum residues annual returning to plots. However, the manure addition to mineral fertilised plots increases the sorghum yields by 25-39%, or at least double them. When the higher rates of manure are applied to plots fertilised with higher rates of mineral fertilisers, the sorghum yield increases range from 10 to 44%. The increase of sorghum yield in sorghum-cotton rotation was 19.4 % as compared to continuous sorghum-sorghum cropping system while in the case of sorghum-cowpea rotation, the yield increased by 40.2 %.

Analyses of variance and probability on crop rotations revealed strong interactions between rotations and fertilisers on the one side, and their combined interaction with time on the other side. Thus, highly significant probabilities were recorded with these interactions.

3.2 Agronomic Efficiency Of Cropping Systems On Sorghum Yield

Figure 1 shows the efficiency of the preceding cropping systems on sorghum yield. The Newman Keuls test revealed highly significant differences between sorghum, cotton and cowpea. The sorghum-cotton and sorghum-cowpea rotations had the highest average yields, at 547 kg.ha-1 and 642 kg.ha-1 respectively. Sorghum monoculture had the lowest yield, at 458 kg.ha-1.

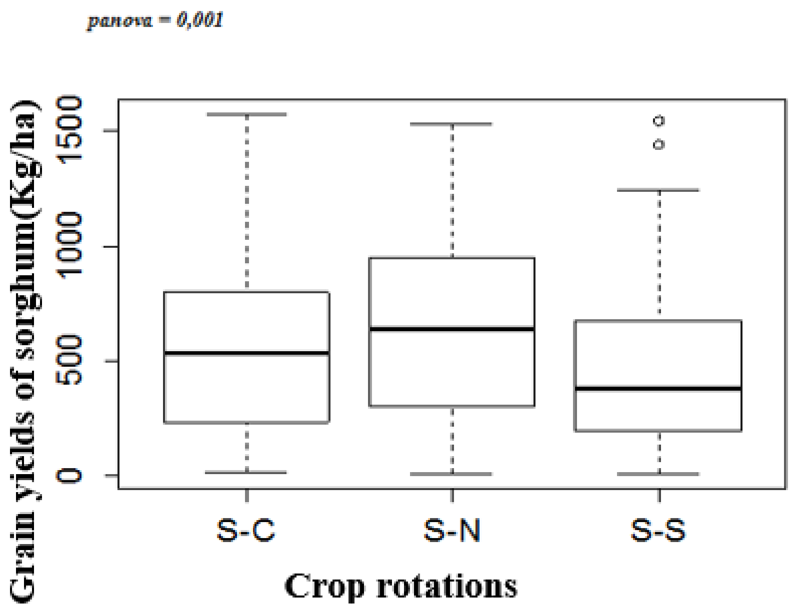

Fig. 1. Efficiency of the preceding cropping systems on sorghum yield

S-C: sorghum-cotton rotation; S-N: sorghum-cowpea rotation; S-S: pure sorghum.

3.3 Soil Fertility Impact Of The Various Fertilisation Practices In Sorghum Cropping Based Rotation Systems

3.3.1 Efficiency Of Crop Rotations On Soil Organic Matter, Assimilable Phosphorus And Soil Acidity

The figure 2 shows the impact of set-aside and crop rotations on soil organic matter. Soil organic matter content under set-aside and the sorghum-cowpea rotation is higher than under the other cropping rotations. The sorghum-cotton rotation had the lowest organic matter content.

A comparison of available phosphorus levels under the three crop rotations shows relatively higher levels under the sorghum-cotton and sorghum-sorghum rotations (Figure 2). The lowest levels were found on plots under set-aside and sorghum-cowpea rotations. The same observations were made regarding soil acidity (Figure 34) where pHwater 1: 2.5 S-C > pH water 1: 2.5 S-C > pH water 1: 2.5 S-C > pH water 1: 2.5 set-aside.

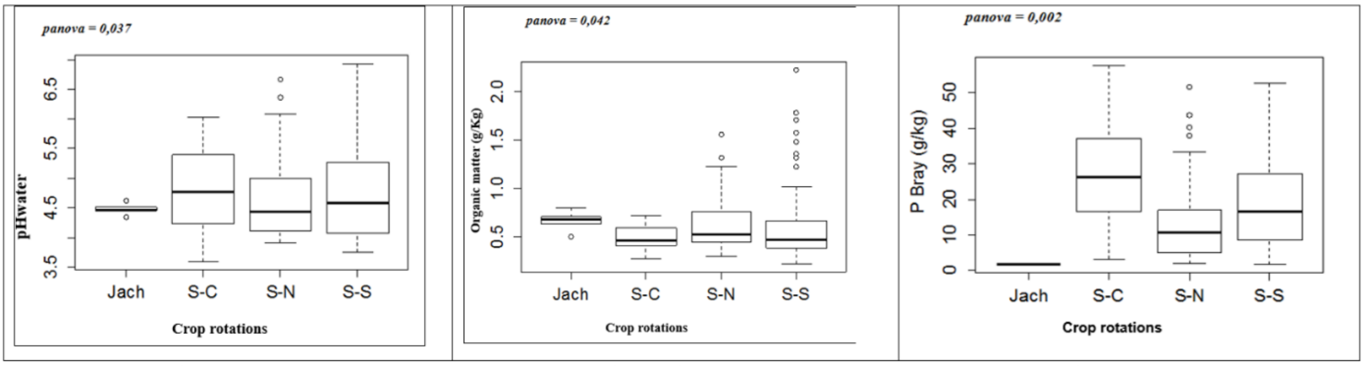

Figure 2a) Figure 2b) Figure 2c)

Fig. 2. Impact of cropping systems on soil organic matter, available phosphorus and pH$_{water\ 1:\ 2.5}$

Jach: set-aside; S-C: sorghum-cotton rotation; S-N: sorghum-cowpea rotation; S-S: pure sorghum. The horizontal line inside the box plot represents the median.

3.3.2 Efficiency Of Set-Aside And Crop Rotations On Soil Granulometric Fractions

The crop rotations led to a change in soil granulometric composition comparatively to the **set-aside** (Figure 3). The clay content is higher on plots under sorghum-cowpea, sorghum-sorghum and sorghum-cotton rotations. The silt fraction is in increasing order on plots in sorghum-sorghum, sorghum-cowpea and sorghum-cotton rotations. The plots under sorghum-cotton and sorghum-sorghum rotations are very sandy. In most cases, these soils are predominantly silty-sandy. The clay fraction is low.

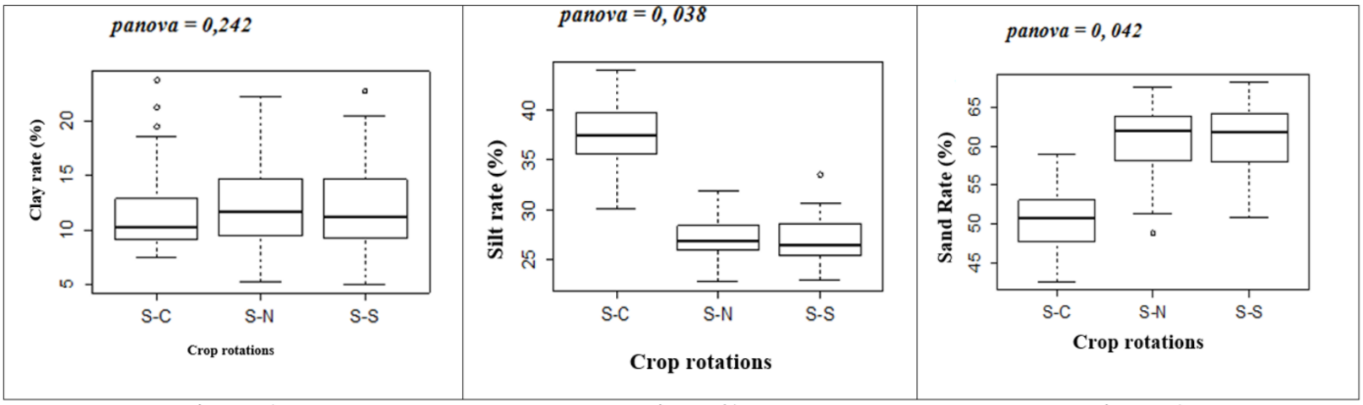

Figure 3a) Figure 3b) Figure 3c)

Fig. 3. Impact of crop rotations on soil granulometric fractions

Jach: set-aside; S-C: sorghum-cotton rotation; S-N: sorghum-cowpea rotation; S-S: pure sorghum. The horizontal line inside the box plot represents the median.

3.4 Soil Fertility Management Impacts On The Sensitivity To Soil Degradation

The results on sensitivity to soil degradation (Table 2) show that most of the plots cultivated after 52 years are vulnerable to physical degradation. All the treatments are sensitive to soil degradation, except for the high rates application of organic + mineral fertilisers. (40 t.ha-1.2y^{-1}). The recommended dose of manure (5 t.ha-1.2y-1), show a weak sensibility of soil degradation after 52 years of farming. This sensitivity to soil degradation is more pronounced with the continuous application of low and high mineral fertilisers and in mining agriculture practice (control).

From another point of view, the soil degradation sensitivity induced by the Control, the low mineral fertiliser + sorghum residues and the low mineral fertiliser + low manure rate treatments under the three cropping rotations shows that the sorghum-cotton rotation has the lowest levels. That result to a relatively high sensitivity to degradation. As to sorghum-Cowpea rotation, the low values of soil degradation sensitivity were assessed with treatments high rate of mineral fertiliser and with high rates of mineral + organic fertilisers application. A comparison between crop rotations shows that plots in the sorghum-cotton rotation are more exposed to degradation.

Table 2. Sensitivity to soil degradation under different fertilization and cropping rotations

Treatements	Sorghum-Sorghum	Sorghum-Cotton	Sorghum-Cowpea	Cumulative effects of fertilisation
Te	2.33 ± 0.63 a	2.26 ± 0.79a	2.63 ± 0.36 a	2.48 ± 0.9 a
Fmr	2.47 ± 0.02 a	2.13 ± 0.91a	3.45 ± 0.8 a	3.04 ± 0.34 ab
Fmo	4.88 ± 0.39 a	3.44 ± 0.60a	4.42 ± 0.16 a	4.65 ± 0.28 ab
Fm	2.37 ± 0.12 a	3.65 ± 0.36a	3.70 ± 0.56 a	2.96 ± 0.41 ab
FMO	11.83 ±3.84 b	8.60 ± 3.55b	8.53 ± 2.27 b	10.18 ± 2.81 c
FM	3.08 ±0.42 a	3.40 ±0.65a	2.81 ± 0.45 a	2.94 ± 0.43 a
Probability	***< 0,001***	***< 0,005***	***< 0,001***	***< 0,001***
Newman keulhs	***S***	***S***	***S***	***S***
Crop rotations	4,49 ± 0,54	4,05 ± 0,25	4,26 ± 0,29	……
Probability		***0, 124***		……

*te: control without any fertilizer addition, **fmr**: low mineral fertilization (37-23-14-6S-1B) + recycling of sorghum residues once every two years, **fmo:** low mineral fertilization (37-23-14-6S-1B) + low rate of cow manure (5 t MS ha $^{-1}$ 2ans^{-1}), **fm:** exclusive low mineral fertilization (37-23-14-6S-1B), **FMO:** high mineral fertilization (60-23-44-6S-1B) + high rate of cow manure (40 t MS ha $^{-1}$ 2ans^{-1}) + export of sorghum straw, **FM**: exclusive high mineral fertilization (60-23-44-6S-1B).*

4 DISCUSSION

4.1 AGRONOMIC AND SOIL EFFICIENCY OF CROPPING SYSTEMS (SORGHUM-SORGHUM, SORGHUM-COTTON, SORGHUM-NIEBE, FALLOW)

By studying the agronomic and soil efficiency of cropping systems, it is possible to assess their potential for sustainable fertility management. This efficiency depends on the practices intrinsic to each system.

4.1.1 AGRONOMIC EFFICIENCY OF THE THREE (3) CROP ROTATIONS

The average yields obtained over time with sorghum cropping rotation systems comparatively to pure cropping sorghum system show a higher performance The sorghum-cowpea and sorghum-cotton rotations largely contributed to improving sorghum yields. Similar performance of cereal yields are found in rotations and associations cropping systems that include cowpea (Zingore et al., 2008; Tittonell et al., 2013; Ripoche et al., 2015). Effect and residual effect of N symbiotic fixation by the leguminous crops in the rotation or association (cowpea and groundnut) are advanced to explain the cereal yields increase. Bado (2002) and Bado et al. (2006) in Burkina Faso has shown that groundnut and cowpea are able to cover 27-34% and 52-56% of their N requirements respectively through symbiotic nitrogen fixation process. This fixed nitrogen makes a greater contribution to plant growth than nitrogen fertilisers application (Bado et al., 2006; Carroué et al., 2012; Guinet et al., 2019). Leguminous crop residues are richer in nitrogen and help to enrich the soil in this element (Asimi, 2009; Haddaway et al., 2014; Jeudy et al., 2016).

Sorghum following cowpea and cotton can benefit indirectly from nutrients through the residues left by the two crops or residual fertilisers applied (Beillouin et al., 2015; Voisin et al., 2015; Sierra and Tournebize, 2019). These effects identified as "rotational effects" are explained by the improvement of soil chemical and biological properties by the leguminous crops which later can enable the physical one. Crop rotation also has other advantages, such as reducing pest attacks (Constantin et al., 2012; Ouandaogo et al., 2016; Guinet et al., 2019). However, crop rotation is not practised systematically by farmers, no doubt because of the limited available land. Combining cereal crops with leguminous plants is the most common practice. In Burkina Faso, more than 80% of sorghum and cowpea are grown in association.

4.1.2 SOIL EFFICIENCY OF CROPPING SYSTEMS

The Set-aside and the sorghum-cowpea rotation contributed to maintaining the organic status of the soil compared to the pure sorghum cultivation and sorghum-cotton rotation. Improving soil organic status in set-aside could be explain by the presence of plant biomass, which turned to increase the soil organic C content. In the case of the sorghum-cowpea rotation, the improved soil organic status is the result of cowpea biomass residual stock and the increase of soil N content due to symbiotic fixation. Otherwise, soil N content is always linked to that of C. The contribution of fallow and crop rotations can be attributed both to carbon sequestration through the roots and to various crop exports. Somé et al (2007), in the case of improved fallows, recorded a significant increase in soil organic matter stock of over 40% due to litter. They asserted that, given that the spiked biomass of set-aside is mown and exported at the end of each season, its contribution to observed variations in soil chemical elements is low. The subsurface litter parts of the grasses largely contribute to the increase in fallow soil C content (Craheix et al., 2012; Krupnik et al., 2019; Krauss et al., 2020). Subsurface litter parts (root) can vary between 2 and 7 t C ha^{-1} $year^{-1}$ (Garay et al., 2000; Douxchamps et al., 2010; Fontaine et al., 2011). That probably explains the difference in organic matter content between the set-aside and the three rotations. The difference can be attributed to the difference in root biomass ploughed in by each cropping system. In addition, the different manures influence crop nutrient uptake at different levels. This explains the differences in nutrient reserves within each fertilisation regime. Added to this are the biological processes under the impact of these fertilisation practices, on which depend the degree of mineralisation and the capacity of each system to store carbon in the soil.

As regards available phosphorus under these cropping systems, the biennial sorghum-cotton rotation and continuous sorghum-cultivation provide a better supply of available phosphorus. This is due to the yields obtained in these cropping systems, which imply relatively low Phosphorus or nutrients? exports compared with those obtained in the sorghum-cowpea rotation. It should be noted that cowpea allows good use of P by the subsequent cereal crop. In Burkina Faso, Bado (2002) showed that after groundnut and cowpea precedents, sorghum took up 2 to 3 times more nitrogen and twice as much P to enhance the yields increases by about 60 to 300% respectively. This explains the lower P content and higher yields of sorghum in this rotation, compared with the continuous sorghum cropping and sorghum-cotton rotation systems.

Crop rotations influenced the soil granulometric composition. The fine fractions [(0-50 µm), (50-200 µm)] shows that the plots under sorghum-cowpea and sorghum-cotton rotations were more clayey and silty respectively.

With total sand content (200-2000 µm), we have the same trend as that on soil organic matter content (Sorghum-Cotton Sands < Sorghum-Sorghum Sands < Sorghum-Cowpea Sands). This confirms once again the close relationship between the coarse fraction and soil organic matter content. This difference can be justified by the soil protection function provided by each crop in the rotation. In the sorghum-cowpea rotation, the cowpea, as it develops, acts as a biological cover, and thus protects the soil against erosion factors (wind, run-off). It also acts as an anti-erosion agent by retaining fine fractions. When we compare the biomass of continuous sorghum cropping system and sorghum-cotton rotation over one rotation cycle, we obtain a higher production under the continuous sorghum cropping system. Sorghum often has a branched surface structure, which reduces runoff and hence the loss of the fine fraction.

4.2 Sensitivity To Soil Degradation

The study on soil sensitivity to degradation argues in favour of integrated soil fertility management strategies. The mining agriculture and the continuous application of mineral fertilisers are not recognised for sustainable soil use. In the long term, these agricultural practices expose soils to degradation (Luo et al., 2010; Kiba, 2012; Pouya et al., 2013b). Only the high-rate input of organic and mineral fertilisers has been able to conserve the soil better. Low rate of organic and-mineral fertilisers reduced the soil degradation. Our results confirm the benefits of crop rotations for soil fertility management. A rational crop rotation balances in nutrients exports and is an excellent farm management plan (Ly et al., 2020; Young et al., 2021).

5 Conclusion

After 50 years cultivation, the lixisol in the West-central part of Burkina Faso lead to a general fluctuation of crop yields linked not only to rainfall patterns but above all to different fertilisation practices. The risks of the mining agriculture on the decreasing cotton yields and on soil fertility depletion have been demonstrated Fertilisation practices that combine organic matter and mineral fertilisers are recognised to sustainable production. We recommend applying manure or compost at the rate of 6 $t.ha^{-1}.2y^{-1}$ plus an annual input of 100Kg.ha-1 of NPK and 50 $Kg.ha^{-1}$ of urea to the cotton plant. The dose of 5t. $ha^{-1}.2y^{-1}$ of organic matter seems unsuitable to maintain the soil fertility. Crop diversification through rotations, especially by the incorporating of leguminous (cowpea or groundnut), offers definite agronomic and soil-related advantages over the continuous sorghum cultivation. We therefore recommend a triennial sorghum-cotton-cowpea rotation for cotton cultivation. In this case, an annual input of $3t.ha^{-1}$ of compost or manure is suitable. In addition, the cotton residues can be transformed into compost by using an appropriate and proven technic to, thereby helping to diversify the sources of organic soil improvers.

Acknowledgments

The authors are grateful to the “Laboratoire Sol-Eau-Plante” of the “Institute of the Environment and Agricultural Research (INERA)”, Burkina Faso, for supporting this research. We are grateful to the field technician Sanon Martin for their assistance during the fieldwork, and to Moyenga Momini, Ramdé Martin and Kaboré Jean-Paul for the lab analysis.

Competing interest

The authors declare that they have no competing interest concerning this article.

Authors’ contributions

All authors contributed to the realization of the work and to the manuscript preparation.

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