World Journal of Agricultural Sciences 2 (3): 249-258, 2006
ISSN 1817-3047

Influence of Field Crop Plantations on Microbial Characteristics of Runoff Water: Effect on Plant Growth and Soil Functioning

[1]R. Duponnois, [2]C. Plenchette and [3]P. Cadet

[1]IRD. UMR 113 CIRAD/INRA/IRD/AGRO-M/UM2.
Laboratoire des symbioses tropicales et méditerranéennes (LSTM), Montpellier, France
[2]INRA. UMR BGA. 17 Rue Sully. 21000 Dijon, France
[3]IRD. Centre de Biologie et Génétique des populations (CBGP).
Campus International de Baillarguet, 34398 Montpellier cedex 5, France

Abstract: The present study was performed to investigate the microbial content of runoff water collected from different plots, cultivated (millet, groundnut) or not (fallow) and to determine their influence on the growth of millet or acacia (*Acacia seyal*) in a soil amended with crab shell chitin, an organic compound used to induce suppressiveness against plant parasitic nematodes. In this study, this soil organic amendment is required to evidence changes in microbial functional diversity among runoff water origins. The impact of runoff was studied using simulating rainfall in the Sudano-Sahelian area of Senegal, at the end of the dry season. The results showed that the origin of runoff water had a significant influence on their microbial composition. These modifications induced quantitative and qualitative differences in actinomycetes, fluorescent pseudomonas and mycorrhizal fungi after millet or *A. seyal* culture. For instance, after 3 month culture of millet in glasshouse conditions, the soil inoculated with runoff water collected from fallow plots, was highly colonized by fluorescent pseudomonas. Chitin amendment induced a higher development of actinomycetes which was depressed by runoff water collected from fallow plots. Plant growth was also significantly stimulated by runoff water inoculations. For instance, compared to the control, root biomass of *A. seyal* seedlings was increased (x 2) when the cultural soil was inoculated with the fallow runoff water. From a practical point of view, it could be important to manage plant cover in rainwater-harvesting agriculture to optimize runoff water in terms of plant beneficial micro organisms.

Key words: Actinomycetes · fluorescent pseudomonas · mycorrhizas · runoff · rain simulation

INTRODUCTION

The climatic conditions encountered in the Sudano-Sahelian zone of West Africa are one of the most rigorous of the world. They are characterized by low and highly variable rainfall, high temperatures and very poor soils, generally P and N-deficient soils. In Senegal, the dry season is very long, extending from November to June and followed by a wet season (3 to 4 months). During this short period, the rainfall episodes are scattered but violent and involve an important runoff causing dense erosion [1]. This soil erosion being a serious problem on agricultural land, it has been widely studied [2-5]. In Africa, different cultural practices have been described by Le Houérou [6] to reduce climatic risk. Among them, water storage (runoff farming) is used to minimize the interannual variations of crop production. It has been tested among the Mediterranean areas (e.g. Israel, Tunisia, Libya...) [7, 8] but also in the South of Sahara (Mali, Sudan, Burkina Faso[6, 9]. Runoff agriculture has a dual objective: (i) to collect and store large quantities of water using stone-walled terraces and (ii) to create new soils from finer, lighter-weight particles (clays, silts) carried off by runoff [10]. This stored water can also be used for supplementary irrigation. Whereas literature is voluminous on the extent and severity of erosion in Africa, there is a dearth of scientific data for erosion-induced effects on crop productivity. Although the crop yield and agronomic production are particularly influenced by biotic soil characteristics, information on the micro flora carried out by runoff water is lacking.

Corresponding Author: Dr. C. Plenchette, INRA. UMR BGA. 17 Rue Sully. 21000 Dijon, France

It has been previously demonstrated that arbuscular mycorrhizal fungi (AMF) are a major factor contributing to the maintenance of plant biodiversity [11]. These interactions are also influenced by plant species. Host plants belonging to the legume family are known to be highly mycotrophic [12]. Moreover, mycorrhizal symbiosis significantly changes the physiology and/or morphology of roots which affect the quality and quantity of root exudation and secretions [13] and cause new microbial equilibrium in the rhizosphere [14]. This mycorrhizal effect has been named "Mycorrhizosphere effect" [14]. Earlier studies showed that strong interactions existed between mycorrhizal fungal establishment and fluorescent pseudomonas [15] or actinomycetes [16].

In our study, null hypotheses were (i) plant species community does not influence microbial content of runoff water and (ii) these runoff water collected from different plots, cultivated (millet, groundnut) or not (fallow) do not influence growth of millet or acacia (*A. seyal*) in a soil amended with crab shell chitin. Crab shell chitin, an organic compound usually used to induce suppressiveness against plant parasitic nematodes [17], is required to evidence possible changes in microbial functional diversity of runoff water. This experiment was conducted with a simulated rainfall in the Sudano-Sahelian region of Senegal.

MATERIALS AND METHODS

Field design : Three rectangular plots 5 x 10 m were marked out and protected from the surrounding area by a shallow, vertical metal sheet border. Their longest sides were parallel to the slope. Although, they were separated by 50 m from each other, they had the same slope (about 1%) and soil characteristics (Table 1) [18]. They were planted with millet (MP), or Groundnut (GP) which were harvested at the end of October the previous year. The third plot was uncultivated and considered as a fallow plot (FP). The study was carried out in April before the end of the dry season in Senegal.

The rainfall simulator: The basic unit of the simulator, designed by Esteves *et al*., [19] was constituted by a vertical galvanised standpipe (height: 6.58 m, diameter: 2.5 cm). At the top of the pipe was placed a HQ106 Spraying System nozzle which sprayed upwards. Control of the water pressure was ensured by a valve and an oil-immersed pressure gauge screwed at the bottom of each pipe. Each basic unit watered a square area of 8 x 8 m. The basic units were separated from each other by 5.5 m giving the best uniformity of rainfall. The simulation of the rainfall was performed in each experimental field plot (5 x 10 m) with 6 basic units divided on 2 lines.

Table 1: Physical soil characteristics of the three experimental plots (From Cadet *et al*., [18])

	(%)				
	Clay	Fine silt	Coarse silt	Fine sand	Coarse sand
GP[1]	6.7	11.0	20.0	43.6	18.6
FP	5.8	9.7	19.0	44.2	20.7
MP	5.7	9.2	17.9	44.9	21.8

[1]GP: Groundnut Plot; MP: Millet Plot; FP: Fallow Plot

Experimental design: Three simulated rains of 10 mm depth were performed during 2 weeks before the experiment. Their intensity did not induce runoff but moisten the top 10 mm of soil. They simulated the conditions at the beginning of the rainy season. Then three rains were applied on the plots on day 1, 3 and 5. The rainfall intensity was measured with collecting cans, placed one each square metre inside the 5 x 10 m field plots. It was 60 to 75 mm h^{-1}. All the rainfalls lasted 30 minutes.

Runoff harvesting: Runoff water coming from each plot was channelled into a single outlet point by a gutter bordering the lower side of the plot. Then they were collected from each plot in successive 15 l pails after each simulated rain. The suspensions were settled for at least 24 h and then, the top water was discarded to about one litre. The samples collected from each plot were mixed together and kept at 4°C in the dark. Bacterial cell counts were performed by acridine orange direct counts (AODC) as described by Richaume *et al*., [20].

Inoculation and plant culture:

Soil substrate: The soil used was collected (0-20 cm) at Sangalkam (about 50 km at the east of Dakar) in a stand of *Acacia holosericea*. It was sieved through 4 mm mesh screen and autoclaved (140°C, 40 min; twice one day apart). After autoclaving, its physico-chemical characteristics were as follows: pH (H_2O) 5.3; Clay 3.6%; Fine silt 0.0%; Coarse silt 0.8%; Fine sand 55.5%; Coarse sand 39.4%; Carbon 0.17%; Nitrogen 0.02%; Total P 39 ppm; Olsen P 4.8 ppm. Then the soil substrate was mixed with an autoclaved sand (140°C, 40 min) at the rate of 1:1 (v/v). This mixture was amended or not with 1% crab shell chitin (w/w).

Greenhouse experiments: The study was divided into 2 sets of experiments carried out to simulate the plant

succession which could be affected by the biological characteristics of the run-off water. The first glasshouse experiment (Experiment 1) was performed with a millet plantation and the second (Experiment 2) with a sahelian *Acacia*, *A. seyal*.

The disinfected soil mixture amended or not with crab shell chitin was divided into 260 cm^3 plastic tubes (4.5 cm diameter, 17.5 cm high). Both experiments were conducted in a greenhouse under natural climatic conditions (temperatures ranged from 20°C to 35°C; about 15 h light per 24 h). The tubes were watered daily with tap water and no fertiliser was added.

Experiment 1: Seeds of millet (*Pennisetum typhoides* cv. IKMV) were surface sterilised in 0.1% Ca hypochloride for 5 min, rinsed with sterilized distilled water and germinated on 1% agar. Pre-germinated seeds were planted as one per plastic tubes. Then 5 mL of runoff water samples were injected with a syringe into each millet pot, one week after transplantation. Inoculation with sterilized runoff water, mixed together, acted as control. The experiment was set up as a 4 x 2 factorial design consisting of three run-off water origins with a non-inoculated control and the soil substrate amended or not with 1% crab shell chitin. The tubes were arranged in a randomized design with twenty five replicates per treatment combination.

Millet plants were harvested after 3 months culture. Roots were removed from the soil and washed under running tap water. The dry weight of shoots and roots (1 week at 60°C) was measured. Ground samples of leaves were ashed (500°C), digested in a nitric-perchloric acid mixture (2 mL HCL 6N and 10 mL HNO_3 N). They were analyzed by colorimetry for P [21] and by flame emission for K. Subsamples of plant tissues were digested in 15 mL H_2SO_4 36 N containing 50 g L^{-1} salicylic acid for N (Kjeldhal) determination.

The bacteria belonging to the group of fluorescent pseudomonas and actinomycetes were extracted (one gram of soil was sampled from each tube) by blending soil samples with 25 mL of sulphate magnesium 0.1 m sterile solution for 60 s in a Waring Blender. The soil suspensions were then serially diluted 10-fold in sterile $MgSO_4$ solution. Counts of fluorescent pseudomonas and actinomycetes were carried out by spreading 100 µL of each dilutions on selective agar media; fluorescent *Pseudomonas spp.* on King's B medium [22] and total actinomycetes on a medium which contained: starch, 10 g; casein, 0.3 g; KNO_3 2 g; K_2HPO_4, 2 g; NaCl 2 g; $MgSO_4.7H_2O$ 0.05 g; Na_2CO_3 0.02 g; $FeSO_4.7H_2O$ 0.01 g; agar 20 g; distilled water, 1 L) [23]. Three plates were inoculated per dilution. Bacterial colonies were counted after 4 days of incubation at 28°C in the dark.

Experiment 2: Millet culture substrates of each combination were carefully mixed and used to fill 260 cm^3 plastic tubes. Seeds of *A. seyal* from provenance Veluigara (Senegal) were surface sterilized for 30 min in 95% concentrated sulphuric acid, rinsed with sterilized distilled water and germinated on 1% agar. Pre-germinated seeds were planted as one per plastic tube. The tubes were arranged in a randomized design with eight replicates per treatment combination.

After 4 months culture, the plants were uprooted and the root systems gently washed. The soil from each tube was mixed and a 250 g subsample was taken from which the microbial analysis have been performed. These soil samples were kept at 4°C for 1 week before analysis.

Soil micro organisms: Microbial biomass was measured according to the fumigation extraction method [24]. The NH_4 and NO_3 contents were determined according to the method of Bremner [25]. Fluorescent pseudomonas and actinomycetes had been isolated and counted as described above. Spores of endomycorrhizal fungi were recovered from a 100 g sub sample from each pot, suspended in 1000 mL tap water, mixed, sieved at 50 µm and centrifuged in a density gradient to further extract the spores [26]. Then the mycorrhizal spores were counted with the aid of a stereomicroscope (x 40 magnification). For each combination, root nodules were counted.

Mycorrhizal colonization of *A. seyal* plant root systems: The extent of endomycorrhizal colonization was estimated in terms of frequency and intensity (length of root fragments colonized / total length of root fragments) of mycorrhizal root pieces. The roots were clarified and stained according to the method of Phillips and Hayman [27]. They were cut into approximatively 1 cm pieces which were placed on a slide for microscopic observation (x 250 magnification) [28]. About fifty 1 cm root pieces were observed per plant.

Soil enzyme activities: The soil enzyme activities have been determined in each pot of each treatment. The β-glucosidase activity was measured as described by Hayano [29]. A soil sub-sample (100 mg) was incubated at 37°C for 2 h with 100 µL of paranitrophenyl β-D gluco-pyranoside 5 mM and 400 µL of a citrate phosphate buffer [30] at pH 5.8. The reaction was

Table 2: Effect of the runoff water on the growth of millet plants and the leaf mineral contents after 3 month culture in a soil amended or not with crab shell chitin (Experiment 1)

Origins of the runoff water	Chitin amendment	Shoot biomass (g dry weight)	Root biomass (g dry weight)	P (%)	N (%)	K (%)
Control	0	1.845a [1]	0.429a	0.048ab	0.667ab	1.988c
GP [2]	0	2.017a	0.824b	0.046a	0.637a	1.841c
MP	0	1.970a	0.689ab	0.051ab	0.703ab	1.655b
FP	0	1.814a	0.654ab	0.053b	0.736b	1.643b
Control	+	3.077cd	1.161c	0.062c	0.984c	1.197a
GP	+	3.284d	0.852bc	0.059c	1.036c	1.517b
MP	+	2.533b	0.796b	0.065c	1.332e	1.741bc
FP	+	2.811bc	0.803b	0.063c	1.194d	1.566b

[1]Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($p<0.05$)
[2]GP: Groundnut Plot; MP: Millet Plot; FP: Fallow Plot

stopped with 0.2% (w/v) of $CaCO_3$. The amount of Para nitro-phenol (PNP), final product of the reaction, was measured colorimetrically at 400 nm and expressed as μg PNP released g^{-1} h^{-1}. Chitinase activity was determined as described above with paranitrophenyl N-acetyl glucosaminide (5 mM) substrate solution. For the measurement of xylanase activity, 300 mg of soil subsample was incubated with 1 M (w : v) xylan and a citrate phosphate buffer at pH 5.8 [30]. Reducing sugar released during the incubation period (4 h) was boiled for 20 min after addition of 500 μL of Somogyi solution [31]. After cooling, 250 μL of Nelson solution [32] and 4 mL of distilled water were added. The xylanase activity was measured colorimetrically at 650 nm. Results were expressed as μg glucose g^{-1} h^{-1}. Protease activity was determined according the method developed by Ladd and Butler [33] and expressed as μg Tyr g^{-1} h^{-1}. Nitrate reductase activity was evaluated using KNO_3 as substrate and determined colorimetrically at 520 nm [34] and expressed as μg N g^{-1} h^{-1}. Urease activity was determined by incubating 100 mg of soil at 37°C with 50 μL of 720 mM urea solution (Sigma) and 400 μL of borate buffer (0.1 M) for 1 h. Released ammonium was extracted with 3 mL of KCl (2 M) solution and determined colorimetrically by a modified Berthelot reaction [35]. Urease activity was expressed as μg N g^{-1} h^{-1}. For the phosphatase activity, soil subsamples (100 mg) were incubated with 400 μL of citrate-phosphate buffer and 100 μL of a 5 mM di-sodium paranitrophenyl phosphate solution (SIGMA) at 37°C according to Tabatabai and Bremner [36]. The addition of 100 μL of $CaCl_2$ (0.5 M) and 400 μL of NaOH (0.5 M) stopped the reaction. It was necessary to add calcium chloride ($CaCl_2$) first to prevent any dissolution of humic substances and dispersion of clay minerals [37]. Soils were incubated for 1 h. Released phenols were estimated colorimetrically at 400 nm with a spectrophotometer (Ultrospec 3000, Pharmacia-Biotech). Two controls, i.e. soil without substrate, substrate without soil, were assayed under the same conditions as those for soil samples in order to check the absence of colored components extracted by the buffer or $CaCl_2$-NaOH treatment and of trace amounts of p-nitrophenol in p-nitrophenyl phosphate reagent, respectively.

Statistical analysis: All data were subjected to analysis of variance and means were compared with the Newman-Keuls multiple range test ($p<0.05$). Mycorrhizal colonization data were transformed (arc sin$\sqrt{}$ x) before statistical analysis.

RESULTS

The number of bacteria counted in the runoff water collected from FP (Fallow Plot) was significantly higher ($2.4\ 10^2$ cfu.mL^{-1}) than those enumerated from GP (Groundnut Plot) and MP (Millet Plot) ($1.7\ 10^2$.mL^{-1} and $1.5\ 10^2$ mL^{-1}, respectively).

Experiment 1: After 3 month culture, shoot and root biomass of millet plants was significantly higher in the soil amended with chitin (Table 2). With chitin amendment, millet root growth was significantly inhibited with MP and FP inocula compared to the control (Table 2). Shoot biomass was significantly lower in the MP treatment than in the control (Table 2). Without chitin amendment, root biomass was significantly higher with GP treatment than in the control. No significant effect has been recorded for the shoot biomass. P and N leave content were significantly higher in the treatments with chitin (Table 2). Compared to the controls, no significant effects of the runoff water were recorded for P. In contrast, MP and FP runoff water have significantly enhanced N contents in the chitin amended soil compared to the control treatment (Table 2). In the soil not amended with chitin, K content was significantly lower in MP and FP treatments. With chitin, all the treatments have significantly increased leaf K content compared to the control (Table 2).

Table 3: Counts of Actinomycetes and fluorescent pseudomonads populations in the millet soil after 3 month culture in glasshouse conditions

Origins of the runoff water	Chitin amendment	Fluorescent pseudomonads (10^3 cfu.g^{-1} of soil)	Actinomycetes (10^3 cfu.g^{-1} of soil)
Control	0	0.4ab [1]	18.6a
GP [2]	0	1.5b	13.4a
MP	0	1.2ab	11.0a
FP	0	3.1c	4.8a
Control	+	0.7ab	82.0c
GP	+	0.1a	104.1d
MP	+	0.1a	102.6d
FP	+	0.1a	48.4b

Table 4: Effect of the runoff water on the growth *of A. seyal* seedlings, rhizobial symbiosis and the leaf mineral contents after 4 month culture in a soil amended or not with crabshell chitin (Experiment 2)

Origins of the runoff water	Chitin amendment	Height (cm)	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	No. of nodules per root systems	P (%)	N (%)	K (%)
Control	0	21.7b[1]	315.0b	186.7a	0.51ab	0.127bc	1.780a-d	0.984cd
GP (2)	0	25.0b	461.7cd	360.0d	2.17bc	0.117b	2.013c-e	0.986cd
MP	0	25.7b	518.3d	351.7cd	2.00bc	0.107b	1.510a	0.837b
FP	0	25.5b	500.0d	378.6d	3.14c	0.128bc	1.667a-c	1.074d
Control	+	12.3a	164.3a	194.3ab	0.00a	0.050a	1.610ab	0.693a
GP	+	14.4a	361.4bc	310.0b-d	0.14a	0.145cd	2.060de	0.871bc
MP	+	25.0b	425.0b-d	283.3a-d	0.66ab	0.156d	1.967b-e	1.060d
FP	+	25.1b	385.7bc	231.4a-c	2.28bc	0.199e	2.220e	1.097d

[1]Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($p<0.05$)
[2]GP: Groundnut Plot; MP: Millet Plot; FP: Fallow Plot

The chitin amendment has involved a better development of actinomycetes (Table 3). The number of actinomycetes was significantly higher in the GP and MP treatments compared to the control. In contrast, this microbial population was significantly lower in the FP treatment (Table 3). No significant effects have been recorded with the fluorescent pseudomonas in the treatments with or without chitin, excepted for FP without chitin where the number of fluorescent pseudomonas was significantly higher than in the control (Table 3). The number of actinomycetes was negatively correlated to the number of fluorescent pseudomonas ($R^2=0.236$, $p<0.003$).

Experiment 2: The height of *A. seyal* seedlings was significantly lower in control + chitin and GP + chitin treatments after 4 months culture as compared to the treatments with runoff water from MP or FP chitin treatments (Table 4). A positive effect of the runoff water inoculation has been recorded on the shoot growth in the soils with or without chitin. For instance, the best stimulations have been found in the MP treatments, + 64.4% in the soil without chitin and + 159.1% in the soil with chitin compared with the corresponding controls. This positive effect of the runoff water has only been recorded in the soil without chitin for the root biomass (Table 4). FP treatment has significantly increased the number of root nodules per plant in both soils compared with the corresponding controls (Table 4). In the soil without chitin, no effect of the runoff water inoculation has been recorded for P and N leave contents. An opposite effect has been observed with the chitin amended soil. The P content has been significantly increased in all the treatments compared to the control whereas N content was higher in GP and FP treatments (Table 4). In the soil amended with chitin the K content was significantly enhanced in MP and FP compared to the control and GP treatments but was not higher than in treatments with the non amended soil (Table 4).

Microbial biomass has not been significantly affected by runoff water in the soil without chitin (Table 5). In contrast, it was significantly decreased in the GP treatment with the chitin amended soil (Table 5). The number of actinomycetes was significantly higher in the control with chitin than that without chitin. An opposite effect of the chitin amendment was observed for fluorescent pseudomonas (Table 5). The runoff water inoculation has significantly increased the number of actinomycetes in all the treatments with or without chitin amendments whereas no significant effect was found for the group of fluorescent pseudomonas excepted for FP treatment in the soil without chitin where a significant decrease was observed (Table 5). No significant difference was observed on the number of mycorrhizal spores and mycorrhizal colonization

Table 5: Influence of the origin of the runoff water on the microbial composition of the *A. seyal* cultural substrate after 4 month culture

Origins of the runoff water	Chitin amendment	Microbial biomass (µg C g^{-1} of soil)	Fluorescent pseudomonads (10^3 cfu.g^{-1} of soil)	Actinomycetes (10^3 cfu.g^{-1} of soil)	No. of mycorrhizal spores per 100 g of soil	Mycorrhizal rate (%)
Control	0	116.0a-c [1]	32.0c	6.0a	0.7a	0.1a
GP [2]	0	63.3a	22.8bc	49.3c	14.3ab	5.3ab
MP	0	109.0ab	16.0a-c	25.6b	31.2b	15.3b
FP	0	84.0a	12.1ab	33.1bc	30.7b	14.6b
Control	+	213.7d	5.3a	73.7d	1.9a	3.2ab
GP	+	145.3bc	3.0a	119.0f	14.7ab	4.5ab
MP	+	170.0cd	7.5ab	118.8f	2.7a	0.8a
FP	+	170.0cd	5.4a	93.8e	12.6ab	3.8ab

Table 6: Influence of the origin of the runoff water on the NO_3^- and NH_4^+ contents of the *A. seyal* cultural substrate after 4 month culture

Origins of the runoff water	Chitin amendment	NH_4^+ (µg N g^{-1} of soil)	NO_3^- (µg N g^{-1} of soil)
Control	0	0.16a [1]	1.9a
GP [2]	0	0.36ab	10.2ab
MP	0	0.21ab	3.9ab
FP	0	0.30ab	6.8ab
Control	+	0.14a	17.2b
GP	+	0.42b	8.2ab
MP	+	0.22ab	3.9ab
FP	+	0.24ab	10.1ab

[1]Data in the same column followed by the same letter are not significantly different according to the one-way analysis of variance ($p<0.05$)
[2]GP: Groundnut Plot; MP: Millet Plot; FP: Fallow Plot

Table 7: Influence of the origin of the runoff water on the soil enzymatic activities of the *A. seyal* cultural substrate after 4 month culture

	Origins of the runoff water							
	Control		Groundnut Plot		Millet Plot		Fallow Plot	
Soil enzymatic activities	- Chitin	+ Chitin	- Chitin	+ Chitin	- Chitin	+ Chitin	- Chitin	+ Chitin
β-Glucosidase (µg Glucose g^{-1} h^{-1})	0.00a [1]	5.60a	2.20a	17.80b	0.00a	2.60a	0.00a	41.90c
Phosphatase (µg PNP g^{-1} h^{-1})	5.90a	5.50a	4.70a	21.60ab	0.60a	10.00a	2.70a	86.10b
Urease (µg N g^{-1} h^{-1})	0.14 a	0.00a	0.00a	0.00a	0.34a	0.00a	1.30a	0.40a
Nitrate reductase (µg N g^{-1} h^{-1})	0.00a	101.30c	2.30a	299.60d	0.00a	0.00a	48.40b	228.90d
Xylanase (µg Glucose g^{-1} h^{-1})	1.89b	1.67b	0.34a	0.14a	0.35a	0.60 a	0.58a	0.00a
Chitinase (µg PNP g^{-1} h^{-1})	1.20a	51.70b	20.50ab	49.50b	0.00a	13.90a	7.70a	1.20a
Protease (µg Tyr g^{-1} h^{-1})	0.30a	3.50bc	3.80c	1.10a	0.00a	3.20bc	2.60b	0.70a

[1]Data in the same line followed by the same letter are not significantly different according to the one-way analysis of variance ($p<0.05$)

between the treatments in the soil amended with chitin. In contrast, MP and FP treatments significantly increased the mycorrhizal establishment in soils without chitin (Table 5).

Soil NO_3^- content was also modified by chitin as its concentration was significantly higher in the control with chitin than in the control without chitin (Table 6). Runoff water inoculations did not involve significant differences excepted for GP treatment + chitin in which NH_4^+ content was significantly higher than that measured in the control + chitin (Table 6).

The effects of the origin of runoff water on the soil enzymatic activities are shown in Table 7. Some enzymatic activities measured in the soil without chitin have been significantly modified by the runoff water. Nitrate reductase and protease activities have been significantly stimulated by FP runoff water whereas xylanase activity was significantly reduced by all the runoff water inoculations (Table 7). In the soil with chitin, β-glucosidase and nitrate reductase activities in GP and FP treatments and phosphatase activity in FP treatment were increased (Table 7). In contrast, chitinase

and protease activities were significantly reduced in the MP and FP, GP and FP treatments, respectively (Table 7). As in the soil without chitin, xylanase activity was significantly inhibited in all the runoff water inoculations treatments (Table 7).

DISCUSSION

Soil erosion is a large problem on agricultural land in several regions of Africa [38-40]. Large amounts of soil carried by runoff water are the principal causes of soil loss in the world [41]. It has been previously demonstrated that nematodes which are localized in the top 20 cm soil layer [42] could be carried over large distances [18, 43]. In the same way, the data recorded in this experiment showed that this runoff might be used to distribute micro organisms which could improve plant growth and soil fertility such as fluorescent pseudomonas, actinomycetes and mycorrhizal fungi. It is well known that bacteria belonging to the fluorescent pseudomonas group can suppress various soil borne diseases [44, 45] have PGPR (Plant Growth Promoting Rhizobacteria) effects [46] or can improve mycorrhizal establishment [15]. Actinomycetes are involved in soil functioning especially through carbon metabolism [47]. Mycorrhizal fungi increased plant nutrient uptake [48, 49], enhanced water absorption [50] and provided protection against some pathogens [51]. Moreover, hyphae of arbuscular mycorrhizal have a significant role in the formation and stability of soil aggregates [52]. These 3 microbial groups have been found in the runoff water treatments after Millet or *A. seyal* cultivation. However, their composition was highly dependent from the origins of runoff water. In our study, fluorescent pseudomonas were significantly more numerous in millet rhizosphere growing in a substrate inoculated with runoff water from a fallow plot. A larger actinomycete community was also recorded in the soil inoculated with groundnut runoff water after an *A. seyal* culture. In the soil without chitin, a positive effect of the runoff water on *A. seyal* growth was detected but no significant differences were recorded between the runoff water origins.

Chitin amendment has significantly decreased the growth of millet or *A. seyal*. It has been previously shown that chitin could be phytotoxic at levels greater than 0.8% when the chitin was mixed into soil for 10 weeks before planting [53]. This phytotoxicity could be due to the high level of NO_3^- measured in the soil of the control treatment soil with chitin. In contrast, no chitin phytotoxicity was recorded after runoff water inoculation. Runoff inoculations have also induced a large multiplication of actinomycetes. Since large part of chitin degradation is accomplished by actinomycetes [54], this organic amendment was largely of benefit to this microbial group. Unfortunately, it is well established that actinomycetes may affect arbuscular mycorrhizal fungus spore populations and the ability of hyphae to survive in the soil [55, 56] as chitin is an important structural constituent of the fungal cell walls [57]. This negative effect has been partially observed in this experiment. In addition, actinomycetes can produce some antibiotic compounds [58] which could inhibit the multiplication of other bacterial groups as it has been recorded with fluorescent pseudomonas in this experiment. An inhibition of nodule formation has also been observed in the treatments with chitin. This negative effect of the actinomycetes on nodule formation has been previously described by Meyer and Linderman [59].

Runoff water have also strongly influenced soil functioning described by enzymatic activities. In particular, two runoff water origins have significantly improved β-glucosidase and nitrate reductase activities in the soil amended with chitin. β-glucosidase depends generally on the quality and quantity of organic inputs [60, 61] but also on specific microbial communities. This result suggests that, at the end of the experiment, the microbial diversity was different in the soil of each treatment.

CONCLUSIONS

Five major types of runoff farming are generally distinguished: (1) micro-catchments system, (2) terraced wadi system, (3) hillside conduit system, (4) liman system and (5) diversion system [62]. Since, origins of runoff water are very important in terms of microbial communities carried, it could be possible to manage vegetable covers to enhance the development of specific microbial groups which could be beneficial to the plant growth and soil functioning in a sustainable agriculture. For instance, the plantation of leguminous plants or trees could be a source of mycorrhizal fungi with their associated microflora or rhizobia which could drive ecosystems functions such as plant biodiversity, productivity and variability.

REFERENCES

1. Albergel, J., M. Diatta, M. Grouzis, M, P. Perez and M. Sene, 1995. Réhabilitation d'un hydrosystème semi-aride au Sénégal par les aménagements du territoire. In: L'homme peut il refaire ce qu'il a défait ? Eds. R. Pontanier, A M'Hiri, N Akrimi, J Aronson and E. Le Floch. John Libbey Eurotext, Paris, pp: 293-306.

2. Middleton, N.J., 1985. Effect of drought on dust production in the Sahel. Nature, 316: 431-434.
3. Barth, H.K., 1986. Wind erosion in the interior delta of the Niger in Mali. In: Physics of desertification. F. El Baz and M.H.A. Hassan (Eds.). Kluwer, Dordrecht, The Netherlands, pp: 274-289.
4. Roose, E. and J.M. Sarrailh, 1990. Erodibility of some tropical soils. Twenty years of records in some erosion plots under natural rainfall. Cahier ORSTOM Série Pédologie, 25: 7-30.
5. Albergel, J., P. Perez, M. Grouzis and M. Diatta, 1997. Rehabilitation of a semiarid ecosystem in Senegal Part 1. Hillside scale experiments. Agric. Ecol. Environ., 65: 95-106.
6. Le Houérou, H.N., 1995. Dégradation, régénération et mise en valeur des terres sèches d'Afrique. In: L'homme peut il refaire ce qu'il a défait ? R. Pontanier, A.M'Hiri, N. Akrimi, J. Aronson and E. Le Floch (Eds.). John Libbey Eurotext, Paris, pp: 65-102.
7. Evenari, M., L. Shana and N. Tadmor, 1982. The Negev: the challenge of a desert (2nd Edn.). Harvard University Press (1st Edn., 1971), Cambridge, Massach., London, UK, pp: 437.
8. El Amami, S., 1984. Les aménagements hydroliques traditionnels en Tunisie. Centre National de Recherches du Génie Rural, Tunis, pp: 69.
9. Roose, E., 1994. Introduction à la gestion conservatoire de l'eau, de la biomasse et de la fertilité des sols (CGES). Bulletin de Pédologie de la FAO, n°70. FAO, Rome, pp: 420.
10. Valentin, C., J. Collinet and J. Albergel, 1994. Assessing erosion in West African savannas under global change: overview and research needs. 15th World Congress of Soil Science, Acapulco, Mexico, pp: 255-274.
11. van der Hejden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken and I.R. Sanders, 1998. Mycorrhizal fungal diversity determines plant biodiversity ecosystem variability and productivity. Nature, 396: 69-72.
12. Duponnois, R., C. Plenchette and A.M. Bâ, 2001. Growth stimulation of seventeen fallow leguminous plants inoculated with *Glomus aggregatum* in Senegal. Eur. J. Soil Biol., 37: 181-186.
13. Ratnayake, M., R.T. Leonard and J.A. Menge, 1978. Root exsudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. New Phytol., 81: 543-552.
14. Linderman, G., 1988. Mycorrhizal Interactions with the Rhizosphere Microflora: The Mycorrhizosphere Effect. Phytopathology, 78: 366-371.
15. Founoune, H., R. Duponnois, A. Bâ, S. Sall, I. Branger, J. Lorquin, M. Neyra and J.L. Chotte, 2002. Mycorrhiza Helper Bacteria stimulate ectomycorrhizal symbiosis of *Acacia holosericea* with *Pisolithus alba*. New Phytol., 153: 81-89.
16. Ames, R.N., 1989. Mycorrhiza development in onion in response to inoculation with chitin-decomposing actinomycetes. New Phytol., 112: 423-427.
17. Hallmann, J., R. Rodriguez-Kabana and J.W. Kloepper, 1999. Chitin-mediated changes in bacterial communities of the soil, rhizosphere and within roots of cotton in relation to nematode control. Soil Biol. Biochem., 31: 551-560.
18. Cadet, P., O. Planchon, M. Esteves and J.M. Lapetite, 2002. Experimental study of the selective transport of nematodes by runoff water in the Sudano-Sahelian area. Appl. Soil Ecol., 19: 223-236.
19. Esteves, M., O. Planchon, J.M. Lapetite, N. Silvera and P. Cadet, 2000. The EMIRE large rainfall simulator, design and field testing. In: Rainfall Simulation in Geomorphology. Earth Surface Processes and Landforms? A. J. Parsons and Lascelles (Eds). Vol. 25: 681-690.
20. Richaume, A., C. Steinberg, L. Jocteur Monrozier and G. Faurie, 1993. Difference between direct and indirect enumeration of soil bacteria: influence of soil structure and cell location. Soil Biol. Biochem., 25: 641-643.
21. John, M.K., 1970. Colorimetric determination in soil and plant material with ascorbic acid. Soil Sci., 68: 171-177.
22. King, E.O., M.K. Ward and D.E. Raney, 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Medicine, 44: 301-307.
23. Williams, S.J. and F.L. Davies, 1965. Use of antibiotic for selective isolation and enumeration of actinomycetes in soil. J. Gen. Appl. Microbiol., 38: 251-261.
24. Amato, M. and J.N. Ladd, 1988. Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils. Soil Biol. Biochem., 20: 107-114.
25. Bremner, J.M., 1965. Inorganic forms of nitrogen. In: Methods in soil analysis. Part 2 Agronomy. C.A. Black, D.D. Evans, J.L. White, L.E. Endminger and F.E. Clark (Eds.). Monograph 9. ASA, Madison, Wisconsin, USA, pp 1179-1237.

26. Gerdemann, J.W. and T.H. Nicolson, 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans. Brit. Mycol. Soc., 46: 235-244.
27. Phillips, J.M. and D.S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc., 55: 158-161.
28. Brundrett, M.C., Y. Piche and L. Peterson, 1985. A developmental study to the early stages in vesicular-arbuscular mycorrhizal formation. Can. J. Bot., 63: 184-194.
29. Hayano, K., 1973. A method for the determination of β-glucosidase activity in soil. Soil Sci. Plant. Nutr., 19: 103-108.
30. MacIlvain, T.C., 1921. In: Methods in Enzymology. P. Sydeney, N. Colovick and O. Kaplan (Eds.) Vol. 1. Academic Press, New York, pp: 833.
31. Somogyi, M., 1945. Determination of blood sugar. J. Biol. Biochem., 160: 61-68.
32. Nelson, N., 1944. A photometric adaptation of Somogyi method for the determination of glucose. J. Biol. Chem., 153: 375-380.
33. Ladd, J.N., J.H.A. Butler, 1972. Short-term assay of soil proteolytic enzymes using proteins and dipeptide derivatives as substrate. Soil Biol. Biochem., 4: 19-30.
34. Abdelmagid, H. M. and M.A.Tabatabai, 1987. Nitrate reductase activity of soils. Soil Biol. Biochem., 19: 421-427.
35. Kandeler, E. and H. Gerber, 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol. Fert. Soils, 6: 68-72.
36. Tabatabai, M.A. and J.M. Bremner, 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem., 1: 301-307.
37. Tabatabai, M.A., 1994. Enzymes. In: Methods of soil analysis. Part 2. Microbiological and biochemical properties, N°5. R.W. Weather, S. Augle, P.J. Bottomly, Q. Berdicek, S. Smith, A. Tabatai and A. Wollum (Eds.) Soil Science Society of America, Madison, pp: 775-783.
38. Pimentel, D., J. Allen, A. Beers, L. Guinaud, R. Linder, P. McLaughlin, B. Meer, D. Musonda, D. Perdue, S. Poisson, S. Siebert, K. Stoner, R. Salazar and A. Hawkins, 1987. World agriculture and soil erosion: Erosion threatens world food production. BioScience, 37: 277-283.
39. Dregne, E., 1990. Erosion and soil productivity in Africa. J. Soil Water Conserv., 45: 431-436.
40. Lal, R., 1993. Soil erosion and conservation in West Africa. In:World soil erosion and conservation. D. Pimentel (Ed.). Intl. Union for Conservation of Nature and Natural Resources, Switzerland, pp: 7-26.
41. Chepil, W. and N.P. Woodruff, 1963. The physics of wind erosion and its control. Adv. Agron., 15: 211-302.
42. Yeates, G.W., R.E. Stannard and G.M. Barker, 1983. Vertical distribution of nematode populations in Horotiu soils. New Zealand Soil Bureau Scientific Report, 60: 1-14.
43. Cadet, P. and J. Albergel, 1999. Passive transport of phytoparasitic nematodes by runoff water in the Sudano-Sahelian climatic area. J. Hydrol., 214: 91-102.
44. Burr, T.J. and A. Caesar, 1984. Beneficial plant bacteria. Crit. Rev. Plant. Sci., 2: 1-20.
45. Bull, C.T., D.M. Weller and L.S. Thomashow, 1991. Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. tritici by *Pseudomonas fluorescens* strain 2-79. Phytopathol., 81: 954-959.
46. Davison, J., 1988. Plant beneficial bacteria. Biotechnol., 6: 282-286.
47. Williams, S., S. Lanning and E.M.H. Wellington, 1984. Ecology of actinomycetes. In: The Biology of the Actinomycetes. M. Goodfellow, M. Mordarski and S.T. Williams. Academic Press, London, pp: 481-528.
48. Plenchette, C. and J.C. Fardeau, 1988. Prélèvement du phosphore par les racines et les mycorhizes. C. R. Acad. Sci. Agric. Fr., 75: 23-29.
49. Barea, J.M., F. El Atrach and R. Azcon, 1991. The role of VA mycorrhizas in improving plant N acquisition from soil as assessed with ^{15}N. In: The use of Stable Isotopes in Plant Nutrition, Soil Fertility and Environmental Studies. C. Flitton 'Ed.). Joint IAEA, FAO Division, Vienna, pp: 677-808.
50. George, E., K. Haussler, G. Vetterlein, E. Gorgus and H. Marschner, 1992. Water and nutrient translocation by hyphae of *Glomus mosseae*. Can. J. Bot., 70: 2130-3137.
51. Dehne, H.W., 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathol., 72: 1115-1119.
52. Hamel, C., Y. Dalpé, V. Furlan and S. Parent, 1997. Indigenous populations of arbuscular mycorrhizal fungi and soil aggregate stability are major determinants of leek (*Allium porum* L.) response to inoculation with *Glomus intraradices* Schenck and Smith or *Glomus versiforme* (Karsten) Berch. Mycorrhiza, 7: 187-196.

53. Godoy, G., R. Rodriguez-Kabana, R.A. Shelby and G. Morgan-Jones, 1983. Chitin amendments for control of *Meloidogyne arenaria* in infested soils. 2. Effects of microbial populations. Nematropica, 13: 63-74.
54. Mitchell, R. and M. Alexander, 1962. Microbiological processes associated with the use of chitin for biological control. Soil Soc. Amer. Proc., 26: 556-558.
55. Ross, J.P., 1979. Factors affecting sporulation of endomycorrhizal fungi. In: Program and abstracts of the Fourth North American Conference on Mycorrhiza. A meeting held in Fort Collins, Colorado, USA.
56. Ross, J.P. and B.A. Daniels, 1982. Hyperparasitism of endomycorrhizal fungi. In: Methods and Principles of Mycorrhizal Research. N.C. Schenck (Ed.). The American Phytopathological Society Press, St Paul, MN,USA., pp: 55-58.
57. Weijman, A.C.M. and H.L.C. Meuzelaar, 1978. Biochemical contributions to the taxonomic status of the Endogonaceae. Can. J. Bot., 57: 284-291.
58. Ames, R.N., K.L. Mihara and H.G. Bayne, 1989. Chitin-decomposing actinomycetes associated with a vesicular-arbuscular mycorrhizal fungus from a calcareous soil. New Phytol., 111: 67-71.
59. Meyer, J.R. and R.G. Linderman, 1986. Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. Soil Biol. Biochem., 18: 191-196.
60. Nannipieri, P., L. Muccini and C. Ciardi, 1983. Microbial biomass and enzyme activities: production and persistence. Soil Biol. Biochem., 15: 679-685.
61. Miller, M. and R.P. Dick, 1995. Thermal stability and activities of soil enzymes as influenced by crop rotations. Soil Biol. Biochem., 27: 1161-1166.
62. Bruins, H.J., M. Evenari and U. Nessler, 1986. Rainwater-harvesting agriculture for food production in arid zones: the challenge of the African famine. Appl. Geography, 6: 13-32.