European Journal of Soil Biology 75 (2016) 62-69

Contents lists available at ScienceDirect

# European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

# Effects of residue quality and soil mineral N on microbial activities and soil aggregation in a tropical sandy soil in Senegal



011

Saïdou Nourou Sall <sup>a, \*</sup>, Dominique Masse <sup>b</sup>, Ndèye Hélène Diallo <sup>b</sup>, Thierno M.B. Sow <sup>b</sup>, Edmond Hien <sup>c</sup>, Aliou Guisse <sup>d</sup>

<sup>a</sup> Université Gaston Berger, UFR des Sciences Agronomiques, de l'Aquaculture et des Technologies Alimentaires, Département de Productions Végétales et Agronomie, BP 234, Saint-Louis, Senegal

<sup>b</sup> UMR 210 Eco & Sols, Laboratoire d'Écologie Microbienne des Sols et Agrosystèmes Tropicaux, BP 1386 Dakar, CP 18524, Senegal

<sup>c</sup> Universite de Ouagadougou, UFR/SVT, 03 BP 7021 Ouagadougou 03, Burkina Faso

<sup>d</sup> Département de Biologie Végétale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, BP. 5005 Dakar-Fann, Senegal

# ARTICLE INFO

Article history: Received 22 December 2015 Received in revised form 21 April 2016 Accepted 26 April 2016 Available online 7 May 2016

Handling Editor: C.C. Tebbe

Keywords: Residue quality Mineral N Fungal activity Aggregation Sandy soil

# ABSTRACT

The role played by organic residues and exogenous mineral N in the formation of stable soil aggregates in nutrient-poor, tropical sandy soils of Senegal is relatively unclear. This study assessed the effect of two representative low and high quality residues (Zea mays and Crotalaria retusa respectively) on the formation and stability of soil aggregates. The formation and stability of aggregates, soil biomass, root biomass, fungal hyphae length, C mineralization and chitinase activity, as a specific biomarker of the activity of fungal populations, were measured under controlled conditions over 120 days. In both the control and amended soils, there were more macroaggregates (>2000  $\mu$ m) and mesoaggregates (250  $-2000 \ \mu\text{m}$ ) than microaggregates (50 $-250 \ \mu\text{m}$  and <50  $\mu\text{m}$ ). The formation of macroaggregates and stability (MWD) were not significantly affected by the quality of residues. Amendment with organic residues shifted the distribution of the aggregate fractions. The macroaggregates increased by 26% with crotalaria and by 35% with maize residues while mesoaggregates decreased by 18% with crotalaria and by 26% with maize residues and microaggregates decreased by 8% with crotalaria and by 9% with maize residues. This study also confirmed that macroaggregates are formed from micro- and mesoaggregates. The total microbial biomass was significantly higher in soil amended with maize residues compared to soil with crotalaria residues and the control soil although the fungal hyphae length decreased when the soil was amended with either crop residue. Chitinase activity is the most pertinent indicator associated with macroaggregation stability. Adding mineral N (equivalent to 120 kg N ha<sup>-1</sup> as urea) to the residue increased microbial biomass and reduced fungal hyphae length but had no effect on macroaggregate formation and fungal activity. These observations suggested that, for short term incubation of soil amended with residues, fungal activity plays a greater role in aggregation in sandy soils than the fungal population density.

© 2016 Elsevier Masson SAS. All rights reserved.

# 1. Introduction

The role of soil organic matter in the formation and stabilization of the structure of most soils has been well established [1]. The relationships between the aggregation factors resulting from decomposition of organic inputs (binding agents and biomass decomposers) and the stability of the aggregates formed have been widely studied (see reviews [2,3]). Recently, Cotrufo et al. [4]

\* Corresponding author. E-mail address: nourou.sall@ugb.edu.sn (S.N. Sall).

http://dx.doi.org/10.1016/j.ejsobi.2016.04.009 1164-5563/© 2016 Elsevier Masson SAS. All rights reserved. proposed a Microbial Efficiency-Matrix Stabilization (MEMS) framework and suggested that microbial products of decomposition would be the main precursors of stable SOM by promoting aggregation, through strong chemical bonding to the mineral soil matrix. However, soil matrix stabilization should be dependent on the specific characteristics of different types of soil and the MEMS framework does not apply in western central Senegal which is dominated by tropical sandy and mixed haplic ferric lixisols. This could be attributed to their higher soil pH, low Al-toxicity, low CEC, high permeability for roots, low C content and high concentrations of alkyl C [5].



It has been argued that, in tropical sandy soils, the aggregate stabilization is controlled mainly by the specific fungal taxa that act as aggregating agents in these soil systems [6] although this depends on the decomposability of the organic matter. The development of hyphal networks can aggregate large numbers of solid particles into macroaggregate structures, which can, in turn, affect more general soil properties such as water infiltration [7]. Roots can also provide the mechanical framework for the initial formation of macroaggregates by trapping particles and producing root exudates which act as binding agents, stimulating microbial activity [3].

Recent studies have highlighted the importance of understanding how the quality of residues interacts with the use of mineral fertilizers to determine the structure of fungal communities and how this interaction affects the mechanisms of soil aggregation and decomposition [2,8]. The results presented in the literature are conflicting. There is extensive literature on the importance of mineral N input for fungal communities. It is recognized that, when applied with organic residues, mineral N can improve mineralization [9] through the stimulation of microbial activities. Other studies, however, have shown that the addition of exogenous N decreased microbial decomposition of residues [10,11] by reducing fungal communities [12].

This discrepancy arises largely from the diversity and quality of the organic residues [13], or the initial soil N content in the study [14,15]. It is, therefore, very important to understand the interaction between the quality of organic residues and mineral N on the formation and stabilization of soil aggregates, particularly in nutrient-poor, tropical sandy soils of western central Senegal, where soils are less structured. Very little is known about these soils and how root and fungal aggregation mechanisms are affected by mineral N fertilization. In these soils, the optimal management of organic resources is limited by a deficit in the amount and N content of available organic inputs [16]. Combining residues of different qualities with added mineral N to ensure the synchronization of nutrient release to plants has been suggested as a means of managing this deficit [8,13].

This study set out to: (1) investigate the relationships between organic residues and water stable aggregates; (2) determine the contribution of soil fungi and roots to these relationships and (3) assess the effects of mineral N input on the formation of soil aggregates.

## 2. Material and methods

The experiment was carried out over 120 days under glasshouse controlled conditions. Mineral N was added, at two different rates, to two biochemically different organic residues to quantify the effects on microbial activities and water-stable aggregation. The effect of roots as a factor controlling aggregation was determined by sowing maize (*Zea mays*) when the residue had been decomposing for 60 days. The maize was harvested 60 days after sowing.

# 2.1. Soil and organic residues

This study was conducted with soil from low-input agricultural systems near Nioro du Rip which is in the "peanut basin" in western central Senegal. In these low-input agricultural systems crop residues are removed from fields and the mineral fertilizer application rates are lower than the nutrient loss rates. Cultivation consists essentially of hand hoeing and planting with little or no mechanical tillage. The soil used in the experiment was taken in 2008 from the ISRA (Institut Sénégalais de Recherches Agricoles) experimental field research station (13°45 N, 15°47 W), at a height of 18 m above sea level. This area has a mean annual rainfall of 750 mm from July to September and mean air temperatures ranging from 20 °C to

35.7 °C. The soil is a loamy-sand, leached ferruginous tropical soil, known locally as a deck-dior soil [17], fine sandy, mixed Haplic Ferric Lixisol [18]. The soil samples were collected at depths of 0–10 cm and 10–30 cm in plots that had not been amended with organic or mineral fertilizer for 10 years. Several replicates were taken at each depth, pooled, air-dried, sieved to <2 mm and stored at room temperature pending processing. The soil composition was 48 g kg<sup>-1</sup> clay, 129 g kg<sup>-1</sup> silt and 823 g kg<sup>-1</sup> sand, the total C was 3 g C kg<sup>-1</sup> dw soil, the total N was 0.3 g N kg<sup>-1</sup> dw soil and the total P was 66.0 g P kg<sup>-1</sup> dw soil.

Two types of organic residue were used (*Crotalaria retusa* and *Zea mays*) that were representative of very different organic residue qualities in terms of nitrogen, lignin, and phenolic contents [19] with *Crotalaria retusa* being a high quality resource and *Zea mays* a low quality resource (Table 1).

# 2.2. Experimental design

The soil was placed in pots in a completely randomized design with two factors: organic residue type (crotalaria, maize and control) and mineral N amendment (ON and 120N). Each treatment was replicated 4 times. The experimental setup consisted of 24 pots filled with the soil samples from the 0–10 cm and 10–30 cm horizons. Each pot contained 10 kg of soil.

The organic residues ground to less than 3 mm, were mixed with the soil at a rate of 5 T ha<sup>-1</sup> (equivalent to 4.17 g kg<sup>-1</sup> soil in the top 10 cm soil). Mineral N was added as urea (46% N) at a rate of 120 kg N ha<sup>-1</sup>, in two doses of 40 kg N ha<sup>-1</sup> at the start of the experiment and 80 kg N ha<sup>-1</sup> before sowing the maize (60 days after the start of the experiment).

The soil was incubated for 120 days with the soil water content maintained between 30% and 40% (dry weight) by adding water two or three times a week. The maize was sown when the residue had been decomposing for 60 days. The plants were harvested 60 days after sowing, 120 days after the start of the experiment.

Additional laboratory incubation was undertaken to evaluate the fraction of the residues that had decomposed when the maize was sown (Supplementary Information). Carbon mineralization was measured in amended soil samples incubated in sealed glass jars (four replicates per treatment) kept at 28 °C for 60 days. Before sowing, the decomposition of crotalaria and maize residues without added mineral N was 58% and 63% of the added C and decomposition with added mineral N was 57% and 74% of the added C, respectively.

# 2.3. Analysis

At the end of the experiment, the roots were washed gently, dried at 65 °C for 1 week and weighed. One 5 cm cube of soil was removed intact from the 0-10 cm horizon in each pot, close to the

#### Table 1

Characteristics of organic residues used. Values represented are the mean of 4 replicates. Values followed by different letters in each line are significantly different at p < 0.05 using Fisher's LSD test.

Characteristics	Organic residues		
	Crotalaria	Maize	
Soluble (% OM) Hemicellulose (% OM) Cellulose (% OM) Lignin (% OM) Total Nitrogen (%)	$70.34 \pm 0.1$ b $15.28 \pm 0.3$ a $10.83 \pm 0.1$ a $3.55 \pm 0.5$ a $2.57 \pm 0.1$ b	$17.76 \pm 0.1 a$ $31.85 \pm 0.3 b$ $45.12 \pm 0.4 b$ $5.27 \pm 0.0 b$ $0.74 \pm 0.2 a$	
Total Carbon (%) C:N ratio	$2.57 \pm 0.10$ 38.58 ± 0.0 a 15.11 ± 0.9 a	$43.63 \pm 1.5 \text{ b}$ $58.75 \pm 1.6 \text{ b}$	

roots for laboratory analysis of soil aggregation and microbial properties (C mineralization, microbial biomass, fungal hyphae length and chitinase activity).

The distribution of soil aggregates was measured by sieving in water using a series of sieves (2, 0.25 and 0.05 mm). An intact 50 g soil sample was separated into macroaggregates (>2000  $\mu$ m), mesoaggregates (250–2000  $\mu$ m), microaggregates (50–250  $\mu$ m) and silt and clay fractions (<50  $\mu$ m). These aggregates were separated successively by 50 vertical oscillations with an amplitude of 3 cm for two minutes as described by Elliott [20]. The aggregate classes that were separated were all dried at 50 °C and weighed. The stability of the aggregates was estimated using the mean weight diameter (MWD), using the formula proposed by van Bavel [21]:

$$MWD = \sum_{i=1}^{n} X_i W_i$$

where  $X_i$  is the mean weight diameter of each fraction *i*,  $W_i$  is the proportion of the aggregate in fraction *i*, and *n* is the number of total fractions.

Carbon mineralization was measured by incubating 10 g from the sampled cube in sealed vessels (120 mL) at 28 °C over 7 days and collecting headspace gas samples using a glass syringe and injecting the gas into a micro gas chromatograph (MTI P200, MTI Analytical Instruments, Fremont, CA.) with a TCD detector, using helium as the carrier gas.

The fungal hyphae length was determined after extracting and filtering hyphae as described by Hanssen et al. [22]. An intact 2 g soil sample was placed on a 100  $\mu$ m sieve and sieved for 30s in 500 mL of deionized water. Thirty mL of the resulting supernatant was removed using a pipette and placed on a 1.2  $\mu$ m pore diameter filter. The extracted fungal hyphae on the filter were colored with Trypan blue (0.05%) and then mounted in glycerol (50%). The total length of fungal hyphae, expressed in m g<sup>-1</sup> of dry soil, was measured by microscope using the gridline intersection method [23].

Chitinase activity was determined by measuring the amount of  $\beta$ -N-acetylglucosamine liberated from the soil [24]. A 100 mg sample of soil was incubated with 400  $\mu$ l of N-acetyl- $\beta$ -D-glucosaminide and 400  $\mu$ l of a 0.12 M phosphate buffer for 2 h at 37 °C. The para-nitrophenyl (*p*-NP) liberated was measured at a 400 nm by spectrophotometry (Ultrospec 3000, Pharmacia Biotech).

Microbial biomass C was determined by fumigation-extraction [25]. Ninhydrin-reactive N compounds were extracted from soils with 1 M KCl before and after 10 days fumigation and measured by colorimetric flow injection (Evolution II, Alliance-Instruments, France). Microbial biomass C was estimated from the increase in ninhydrin-reactive N after fumigation, multiplied by 21 [25].

#### 2.4. Statistical analysis

The principal effects of each treatment and their interactions were tested using one-way and two-way ANOVA using XLSTAT (Addinsoft version 2008.7.03). The probability level of  $p \leq 0.05$  was considered significant for Fisher's LSD test. Pearson linearity tests were performed to determine the correlation between microbial variables and soil aggregation measurements. Linear regression was used with the amendment factors (no residues, crotalaria or maize residues, without or with mineral N) as independent variables. F values were considered significant at 0.05 (\*), 0.01 (\*\*) and 0.001 (\*\*\*) probability levels. In addition, a principle component analysis (PCA) was carried out to investigate the possible relationships between the biological properties as active variables

and the aggregate fractions as supplementary variables. The PCA was performed using the XLSTAT (Addinsoft version 2008.7.03).

# 3. Results

## 3.1. Effect on aggregation

After 120 days of incubation, the organic inputs had a significant effect on the quantity of macroaggregates and mesoaggregates as well as on the mean weight diameter (Table 2 and Fig. 1A). In non-amended soil, the quantity of mesoaggregates ( $250-2000 \mu m$ ) was higher than the other classes of aggregates ( $>2000 \mu m$ ,  $50-250 \mu m$  and  $<50 \mu m$ ).

The application of residues significantly increased the macroaggregates (>2000  $\mu$ m) by an average of 26% for crotalaria and 35% for maize compared with the control, while the mesoaggregates (250–2000  $\mu$ m) decreased by an average of 18% for crotalaria and 26% for maize. The difference between the residues was not significant (Table 2). The silt and clay fraction (<50  $\mu$ m) was significantly lower in the amended soils (8% for crotalaria and 9% for maize). The results showed a significant positive correlation between the MWD and quantity of macroaggregates (r = 0.99; p < 0.0001) and a negative correlation between the MWD and the quantities of smaller aggregates (See Table SI).

Mineral N had no significant effect on the formation of aggregates and the MWD for any organic residue amendment (Table 2 and Fig. 1A).

#### 3.2. Effect of organic residue amendment on microbial properties

Organic residue amendment had a significant effect on microbial biomass and fungal hyphae length: the microbial biomass was significantly higher and the fungal hyphae length was significantly lower (Figs. 2B and 1B). The soil amended with crotalaria had a higher microbial biomass and a lower fungal hyphae length than the soil amended with maize.

The C mineralization was similar between the amended and non-amended soils (Fig. 2A).

Organic residue amendment had a significantly increased chitinase activity (Fig. 2C). However, there was no significant difference between the soils amended with crotalaria and maize residues.

Mineral N increased the microbial biomass in the soils amended with organic residues (Fig. 2B). However, this increase was only significant in soils amended with maize. Mineral N significantly decreased the fungal hyphae length in the control soil and soil amended with maize residue and there was a significant interaction between organic and mineral N amendments.

Mineral N did not have any effect on chitinase activity in soils with organic residues (Fig. 2C). However, chitinase activity in the control soil increased significantly with mineral N amendment.

The root biomass was not significantly affected by organic residues or mineral N at the end of the experiment (Fig. 1C).

# 3.3. Relationships between aggregate formation and microbial properties

A principle components analysis was carried out to study the relationships between aggregate formation and microbial properties (Fig. 3). The first axis explained 71% of the variance and separated soils amended with residues and the control soil. This separation was explained primarily by the higher macroaggregate content in soils amended with residues and the higher meso- and microaggregate contents in the control soil. The second axis explained 16% of the variance with a gradient between organic

# Table 2

Aggregate fraction distribution (% dw soil) of control soil and soil amended with crotalaria or maize, alone or in combination with 120 kg ha<sup>-1</sup> of urea fertilizer. Values within a column comparing treatments with and without urea, followed by the same lower case letter, are not significantly different at p < 0.05. Values within a column comparing different treatments, preceded by same upper case letter, are not significantly different at p < 0.05 using Fisher's LSD test. Mean  $\pm$  SE, n = 4.

Treatment	Aggregates	Aggregates			
	>2000 µm	250–2000 μm	50–250 μm	<50 μm	
Control	<sup>A</sup> 4.97 ± 0.17 a	$^{\rm B}$ 55.84 $\pm$ 5.63 a	<sup>B</sup> 32.71 ± 4.69 a	<sup>B</sup> 6.31 ± 0.86 a	
Control + Urea	$4.12 \pm 0.85$ a	58.94 ± 3.32 a	31.13 ± 3.60 a	5.63 ± 0.92 a	
Crotalaria	<sup>B</sup> 31.26 ± 3.30 a	<sup>AB</sup> 37.96 ± 12.42 a	<sup>AB</sup> 27.36 ± 3.10 a	<sup>A</sup> 3.31 ± 0.91 a	
Crotalaria + Urea	34.12 ± 2.35 a	34.71 ± 3.01 a	27.48 ± 3.00 a	3.58 ± 1.22 a	
Maize	<sup>B</sup> 39.69 ± 15.82 a	<sup>A</sup> 30.02 ± 5.59 a	<sup>A</sup> 26.56 ± 0.47 a	$^{\rm A}$ 3.63 $\pm$ 0.42 a	
Maize + Urea	24.97 ± 6.84 a	41.43 ± 8.37 a	29.33 ± 2.18 a	3.84 ± 0.43 a	
Residue <sup>a</sup>	9.01**	6.32**	2.51ns	8.85**	
Urea	0.92ns	1.17ns	0.01ns	0.37ns	
Residue*Urea	0.57ns	0.28ns	0.49ns	0.23ns	

<sup>a</sup> F values are significant 0.05 (\*), 0.01 (\*\*) and 0.001 (\*\*\*) probability levels; ns is not significant.



**Fig. 1.** Mean weight diameter (A), fungal hyphae length (B) and root biomass (C) of control soil and soil amended with crotalaria or maize, with (+Urea) and without urea (- Urea). Soil amendments without urea marked with the same upper case letter are not significantly different at p < 0.05. Urea treatments for each residue amendment marked with the same lower case letter are not significantly different at p < 0.05. Weans  $\pm$  SE, n = 4.

residue amendments, with and without mineral N (Fig. 3A). Maize residue amended soil was separated from all other treatments

along this axis. The microbial biomass was higher in soils amended with residues and mineral N than in soils amended only with



**Fig. 2.** C mineralization (A), microbial biomass (B) and chitinase activity (C) of control soil and soil amended with crotalaria or maize, with (+Urea) and without urea (- Urea). Soil amendments without urea marked with the same upper case letter are not significantly different at p < 0.05. Urea treatments for each residue amendment marked with the same lower case letter are not significantly different at p < 0.05. Means  $\pm$  SE, n = 4.

residues (Fig. 3B). Chitinase activity was higher in maize amended soils than in crotalaria amended soils. Chitinase, root biomass and microbial biomass were closely correlated with the macroaggregates Ag > 2000 and MWD, whereas the hyphae length was closely correlated with microaggregates Ag < 50. Macroaggregates Ag > 2000 were correlated with C mineralization (r = 0.88), root biomass (r = 0.68), microbial biomass (r = 0.60) and chitinase activity (r = 0.55) (See Table SI). There was also a negative correlation between the microbial biomass and fungal hyphae length (r = -0.71). These results suggest that the formation of macroaggregates was related to chitinase activity rather than fungal hyphae length.

# 4. Discussion

#### 4.1. Effect of residues quality on aggregation

The effect of residues on the formation of macroaggregates

could be assessed by comparing the difference in macroaggregate content in the control soil (5%) with the soil amended with residues (31% and 40% for crotalaria and maize, respectively). This shows that organic residue amendment resulted in an increase in macroaggregate content and stability (i.e. greater MWD). It is known that organic residue amendment stimulates microbial activity and soil aggregate formation and stability [2,26]. However, few studies have considered how the initial biochemical characteristics of different organic residues affect aggregate formation. Martens [27] reported that there was a correlation between the quantity of macroaggregates and the abundance of easily decomposable material, such as protein. However, our results showed that the formation of macroaggregates and stability (MWD) were not controlled by the quality of crop residue added, even though the soluble material in crotalaria was 4 times higher than in maize. This agrees with the results reported by Abiven et al. [28] for a loamy soil amended with a range of organic residues (composts, manure, crop residue and urban waste). However, in a long-term study, Chivenge



Fig. 3. Principle Components Analysis (PCA) of aggregate fractions, Mean weight diameter and microbial properties of residue amendments and control with and without urea. Vectors show the correlations between variables.

et al. [8] found that the proportion of large macroaggregates and MWD was higher with low quality residues (maize) than with high quality residues (Tithonia). These authors found that maize residue decomposed slowly and macroaggregates accumulated over 8 months. The influence of a high quality residue should be observable in short-term accumulation of aggregates but disappear over time owing to the macroaggregate turnover. This effect was not seen in our study, possibly because the period of incubation might not have been long enough to allow the appearance of significant differences. We took samples 120 days after incorporation and found more macroaggregates in soil amended with maize, although the difference was not significant. Continuing the experiment for 6 or 8 months might show more accumulation of macroaggregates with maize (low quality) and thus significant differences compared to crotalaria (high quality) as found by Chivenge et al. [8]. It was interesting that, as the percentage of macroaggregates increased, so the percentage of mesoaggregates and microaggregates decreased. Tisdall and Oades [1] previously showed that macroaggregates were formed from microaggregates. They suggested that microaggregates are the "building blocks" for macroaggregate formation. Bossuyt et al. [6] showed that a larger amount of macroaggregates could be formed with the incorporation of mesoggregates (250–2000 µm). Recently, Gunina and Kuzyakov [29] guantified the C flows within the aggregates and SOM density fractions based on  $\delta^{13}$ C values, and showed that the most intensive C flows occur in the small macro and microaggregate size classes, which are formed inside the macroaggregates.

# 4.2. Importance of microbial properties on aggregate formation

The total microbial biomass was significantly higher in the soil amended with high quality residue than in the soil amended with low quality residue. It has been shown that microbial biomass was stimulated by the addition of litter [11] and that this could be attributed to zymogenous microorganisms, such as bacteria, known to respond very promptly to the addition of energy-rich compounds. However, fungi are known for their ability to degrade low quality residues [30]. This could explain the higher fungal hyphae length in the soil amended with low quality residue than in the soil amended with high quality residue, giving rise to a negative correlation between fungal hyphae length and microbial biomass. Thus, the correlation between microbial biomass and macroaggregate content could be due to the fungal biomass which may not have been directly related to the fungal hyphae length. No conclusion could be drawn about the higher fungal hyphae length in the control soil. However, it is possible that the endogenous recalcitrant substrates in the soil could increase the fungal biomass. Consequently, the presence of fungal hyphae could provide physical binding for particles as previously demonstrated but it could not wholly explain the formation and stabilization of aggregates [31]. In addition, it is known that roots and hyphae act together as binding agents between particles [32]. In this study, root biomass was correlated with the macroaggregate content and stability (MWD) whereas the fungal hyphae length was not.

The role of fungal activity was demonstrated by this study. There were significant correlations between microbial biomass, C mineralization, chitinase activity and macroaggregate content and stability. The correlation between macroaggregate stability (MWD) and C mineralization after 120 days of incubation showed that the increases in C mineralization were associated with increases in aggregate stability [32]. This suggested that most of the aggregate-associated C was easily mineralized by the microbial community.

It has been established that chitinase activity reflects the activity of fungal biomass [33]. Our results suggest that fungal activity plays a major role in the formation of macroaggregates in the sandy soil studied. This is supported by Cosentino et al. [34] who used ergosterol as an indicator of fungal biomass. They, as well as several other authors, showed that fungi had a more prominent role in aggregation than bacteria, as fungi contributed to three aggregation mechanisms: physical entanglement, production of hydrophobic substances and production of extracellular polysaccharides. In our study, the correlation between chitinase activity and macroaggregate stability is consistent at least with the role of production of extracellular polysaccharides. Chitinase is an enzyme that hydrolyzes chitin, a polymer of N-acetylglucosaminide, a structural substance that can be found in the cell wall of fungi. Therefore, we suggest that, the chitinase activity is the most pertinent indicator associated with aggregation.

#### 4.3. Effect of mineral N

In this study, the addition of exogenous mineral N to soil amended with residues was not found to have any significant effect on macroaggregate formation. Bossuyt et al. [6], however, showed a significant decrease in the formation of large macroaggregates (>2000  $\mu$ m) measured 14 days after the incorporation of low

quality residue. These authors considered that exogenous N decreased microbial activity and, therefore, inhibited the production of microbial metabolites, the binding agents responsible for the formation of large macroaggregates. Similar results were found by Chivenge et al. [8] who explained the decrease in aggregate stability when mineral N was added to a soil amended with a low quality residue (maize) as being due to increased aggregate turnover as a result of the exogenous N accelerating the decomposition of the residue. One possible explanation for the difference between their results and our results could be the process of aggregate formation over the period of the experiment and the type of soil. When residues are incorporated into soil, there may be rapid immobilization of the N during the early stages of decomposition. The mineral N may form complexes with the binding agents, preventing them from forming aggregates [11]. At a later stage, there will be a steady increase in mineral N thereafter, indicating that mineralization is greater than immobilization. Furthermore, the initial soil and exogenous N availability for the residue, expressed as the proportion (residue + soil) N/total residue dry matter, varied from 1.6% to 4.5%. These values are above the threshold value (1.1%-1.2% of residue dry matter) proposed by Recous et al. [14] for the overall N concentration limiting C decomposition. This is supported by the fact that increasing the soil N content had no effect on residue C mineralization [15].

The addition of mineral N increased the microbial biomass for all treatments. Microbial biomass has been found to increase in response to short-term amendment [35,11]. It has been established that rapidly proliferating bacteria play an important role during the initial attack on easily degradable molecules, while fungi are the major decomposers of low quality recalcitrant compounds [36]. The added mineral N may have increased the bacterial biomass significantly by increasing the amount of ammonifying and proteolytic bacteria [37] as well as increasing the root biomass and, therefore, the exudation [38]. As both fungi and bacteria contribute to the measurements of total microbial biomass, an increase in total biomass at the end of the experiment could indicate an increase in fungal biomass. Interestingly, whereas adding mineral N increased microbial biomass, our results showed a decrease in fungal hyphae length. Fungi are often the group of microorganisms most affected by N fertilization [39]. Because the fungal hyphae length was measured after plants had been grown in the soil, we do not know to what extent this reduction in fungal biomass concerned saprophytic or mycorrhizal fungi. No conclusions can, therefore, be drawn on the negative relationship between fungal biomass and mineral N amendment.

Although fungal hyphae length was reduced by mineral N in the control and maize treatments, chitinase activities were not affected in the same way. Chitinase is an enzyme that hydrolyzes chitin, an important source of N in soil and chitinase activity may increase in low N conditions [40]. This could correspond to the control soil having a limiting N content, whereas no significant effect was observed in soil amended by residues where the N content was higher. This observation that N amendment does not affect chitinase activity when the N content is non-limiting agrees with the effect of mineral N amendment on soil aggregation found in this study. Consequently, measuring the fungal hyphae length is not a good indicator of the quantity of aggregates and does not represent the importance of the hyphae in fungal activity.

#### 5. Conclusions

The results from this short-term experiment clearly indicate that the organic residues amendments shifted the distribution of the aggregate fractions. However, the quality of residue and fungal hyphae length are not the main drivers for aggregates formation although fungal activity, such as the production of chitinase, may play an important role in the formation of aggregates in sandy soil. Adding exogenous mineral N decreased the fungal hyphae length but did not affect the fungal activity or macroaggregate formation. Overall, this study confirms that macroaggregates (>2000  $\mu$ m) are formed from micro and mesoaggregates and shows the importance of fungal activity rather than the fungal hyphae length. However, previous authors have suggested that the polysaccharide compounds of residues and fungal populations play a major role in the formation of aggregates. Further research into the links between such compounds and the composition of fungal communities in a long-term experiment is required to give a better assessment of their effect on aggregation in the nutrient-poor, tropical sandy soils of Senegal. Furthermore, the effect of organic inputs and their decomposition on aggregate formation and aggregate turnover should be investigated using a long-term experiment under natural conditions.

## Acknowledgements

This work was funded partly by a grant from Agence Universitaire de la Francophonie (AUF). We should like to thank Lamine Sagna, Mayecor Diouf, Moutapha Sané and Oumar Faye for laboratory analyses of microbial biomass and activity in the Laboratoire d'Ecologie Microbienne des Sols et Agrosystèmes Tropicaux (LEM-SAT). In addition, we thank the two anonymous reviewers and the editor Y. Kuzyakov for constructive comments on the manuscript.

# Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2016.04.009.

#### References

- J.M. Tisdall, J.M. Oades, Organic matter and water-stable aggregates in soil, J. Soil Sci. 33 (1982) 141–163.
- [2] S. Abiven, S. Menasseri, C. Chenu, The effects of organic inputs over time on soil aggregate stability – a literature analysis, Soil Biol. Biochem. 41 (2009) 1–12.
- [3] J. Six, C. Feller, K. Denef, S.M. Ogle, J.C. de Morales Sa, A. Albrecht, Organic matter, biota and aggregation in temperate and tropical soils: effects of notillage, Agronomie 22 (2002) 755–775.
- [4] M.F. Cotrufo, M.D. Wallenstein, C.M. Boot, K. Denef, E. Paul, The microbial efficiency- matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob. Change Biol. 19 (2013) 988–995.
- [5] C. Rumpel, I. Kögel-Knabner, Deep soil organic matter a key but poorly understood component of terrestrial C cycle, Plant Soil 338 (2011) 143–158.
- [6] H. Bossuyt, K. Denef, J. Six, K. Paustian, Influence of microbial populations and residue quality on aggregate stability, Appl. Soil Ecol. 16 (2001) 195–208.
- [7] M.C. Rillig, F. Mardatin, M. Antunes, Mycelium of arbuscular mycorrhizal fungi increases soil water repellency and is sufficient to maintain water-stable soil aggregates, Soil Biol. Biochem. 42 (2010) 1189–1191.
- [8] P. Chivenge, B. Vanlauwe, R. Gentile, J. Six, Comparison of organic versus mineral resource effects on short-term aggregate carbon and nitrogen dynamics in a sandy soil versus a fine textured soil, Agric. Ecosyst. Environ. 140 (2011) 361–371.
- [9] B. Berg, E. Matzner, Effect of N deposition on decomposition of plant litter and soil organic matter in forest ecosystems, Environ. Rev. 5 (1997) 1–25.
- [10] E. Bremer, W. Van Houtoum, C. Kessel, Carbon dioxide evolution from wheat and lentil residues as affected by grinding, added nitrogen and the absence of soil, Biol. Fertil. Soils 11 (1991) 221–227.
- [11] S.N. Sall, D. Masse, F. Bernhard-Reversat, A. Guisse, J.L. Chotte, Microbial activities during the early stage of laboratory decomposition of tropical leaf litters: the effect of interactions between litter quality and exogeneous inorganic nitrogen, Biol. Fertil. Soils 39 (2003) 103–111.
- [12] L.M. Donnison, G.S. Griffith, R.D. Bardgett, Determinants of fungal growth and activity in botanically diverse hay meadows: effects of litter type and fertilizer additions, Soil Biol. Biochem. 32 (2000) 289–294.
- [13] B. Vanlauwe, A. Bationo, P.L. Woomer, Integrated soil fertility management: operational definition and consequences for implementation and dissemination, Outlook Agric. 39 (2010) 17–24.
- [14] S. Recous, D. Robin, D. Darwis, B. Mary, Soil inorganic N availability: effect on

maize residue decomposition, Soil Biol. Biochem. 27 (1995) 1529–1538.

- [15] S.N. Sall, I. Bertrand, J.L. Chotte, S. Recous, Separate effects of the biochemical quality and N content of crop residues on C and N dynamics in soil, Biol. Fertil. Soils 43 (2007) 797-804.
- [16] N. Sanginga, O. Lyasse, J. Diels, R. Merckx, Balanced nutrient management systems for cropping systems in the tropics: from concept to practice, Agric. Ecosyst. Environ. 100 (2003) 99–102.
- [17] A.N. Badiane, M. Khouma, M. Sene, Region de Diourbel: gestion des sols, in: Drylands Research Working Paper 15, 2000, p. 25. Drylands Research, Somerset, England.
- [18] FAO, World Reference Base for Soil Resources, World Soil Resources Reports, Food and Agricultural Organization, Rome, 1998, p. 98.
- [19] B. Vanlauwe, C. Gachengo, C.A. Palm, Laboratory validation of a resource quality-based conceptual framework for organic matter management, Soil Sci. Soc. Am. J. 69 (2005) 1135–1145.
- [20] E.T. Elliott, Aggregate structure and carbon, nitrogen and phosphorus in native and cultivated soils, Soil Sci. Soc. Am. J 50 (1986) 627–633.
- [21] C.H.M. Van Bavel, Mean-weight diameter of soil aggregates as a statistical index of aggregation, Soil Sci. Soc. Am. J. 14 (1950) 20-23.
- [22] J.F. Hanssen, T.F. Thingstad, J. Goksoyr, Evaluation of hyphal lengths and fungal biomass in soil by a membrane filter technique, Oikos 25 (1974) 102–107.
- [23] R. Malcová, M. Gryndler, M. Vosátka, Magnesium ions alleviate the negative effect of manganese on Glomus claroideum BEG23, Mycorrhiza 12 (2002) 125–129.
- [24] K. Hayano, A method for determination of  $\beta$ -glucosidase activity in soil, Soil Sci. Plant Nutr. 19 (1973) 103–108.
- [25] M. Amato, J.N. Ladd, Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils, Soil Biol. Biochem. 20 (1988) 107–114.
- [26] S. De Gryze, J. Six, C. Brits, R. Merckx, A quantification of short-term macroaggregate dynamics: influences of wheat residue input and texture, Soil Biol. Biochem. 37 (2005) 55–66.
- [27] D.A. Martens, Plant residue biochemistry regulates soil carbon cycling and carbon sequestration, Soil Biol. Biochem. 32 (2000) 361–369.
- [28] S. Abiven, S. Menasseri, D.A. Angers, P. Leterme, Dynamics of aggregate stability and biological binding agents during the decomposition of organic material, Eur. J. Soil Sci. 58 (2007) 239–247.
- [29] A. Gunina, Y. Kuzyakov, Pathways of litter C by formation of aggregates and

SOM density fractions: implications from <sup>13</sup>C natural abundance, Soil Biol. Biochem. 71 (2014) 95–104.

- [30] E.J. Lundquist, L.E. Jackson, K.M. Scow, C. Hsu, Changes in microbial biomass and community composition, and soil carbon and nitrogen pools after incorporation of rye into three California agricultural soils, Soil Biol. Biochem. 31 (1999) 221–236.
- [31] J.L. Chotte, Importance of microorganisms for soil aggregation, in: F. Buscot, A. Varma (Eds.), Microorganisms in Soils: Roles in Genesis and Functions, Soil Biology, vol. 3, 2005, pp. 107–119, http://dx.doi.org/10.1007/3-540-26609-7\_
- [32] C.J. Bronick, R. Lal, Soil structure and management: a review, Geoderma 124 (2005) 3–22.
- [33] M. Miller, A. Palojärvi, A. Rangger, A. KjØller, The use of fluorogenic substrates to measure fungal presence and activity in soil, Appl. Environ. Microbiol. 64 (1998) 613–617.
- [34] D. Cosentino, C. Chenu, Y. Le Bissonnais, Aggregate stability and microbial community dynamics under drying-wetting cycles in a silt loam soil, Soil Biol. Biochem. 38 (2006) 2053–2062.
- [35] N. Ghoshal, K.P. Singh, Effects of farmyard manure and inorganic fertilizer on the dynamics of soil microbial biomass in a tropical dryland agroecosystem, Biol. Fertil. Soils 19 (1995) 231–238.
- [36] M.J. Swift, O.W. Heal, J.M. Anderson, Decomposition in Terrestrial Ecosystems, in: Studies in Ecology, 5, Blackwell Scientific Publications, Oxford, 1979, p. 372.
- [37] L. Schmitt, K. Muller, E. Ahrens, Chemical and Microbiological Changes in a Regosol after Mineral Fertilization in Long-term Field Experiments and Shortterm Aerobic Incubation Trials, 1991.
- [38] E. Blagodatskaya, S. Blagodatsky, M. Dorodnikov, Y. Kuzyakov, Elevated atmospheric CO<sub>2</sub> increases microbial growth rates in soil: results of three CO<sub>2</sub> enrichment experiments, Glob. Change Biol. 16 (2010) 836–848, http:// dx.doi.org/10.1111/j.1365-2486.2009.02006.x.
- [**39**] L.O. Nilsson, H. Wallander, Production of external mycelium by ectomycorrhizal fungi in a Norway spruce forest was reduced in response to nitrogen fertilization, New Phytol. 158 (2003) 409–416.
- [40] R.L. Sinsabaugh, K. Antibus, A.E. Linkins, C.A. McClaugherty, L. Rayburn, D. Reperd, T. Weiland, Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity, Ecology 74 (1993) 1586–1593.