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Influence of ant–grass association on soil microbial activity through organic matter decomposition dynamics in Lamto savannah (Côte d'Ivoire)

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Abstract

Ants are widely regarded as ecosystem engineers because of their effect on soil structure and on the flow of energy. However, little is known about their influence on the carbon flux in tropical humid savannah. Recent investigations in a humid savannah ecosystem in Lamto showed that ant nests' association with perennial grasses enhances their growth and productivity. This study aimed at understanding the influence of ant nests on soil micro-organism's activity beneath grass tufts. The kinetic of mineralisation was tested in laboratory conditions at various times (days 1, 2, 4 and 7) beneath three grass species associated and not associated with ant nests, following the CO_2 amount released at 30°C during soil respiration. The amount of CO_2 released from the soil is higher beneath grass tufts associated with ant nests compared with those not associated with ant nest. The highest amount of CO_2 released from the soil was found beneath *Hyparrhenia diplandra* tufts followed by *Andropogon schirensis* tufts and the lowest under *Loudetia simplex* tufts. This study has shown that ant nests' association with grass tufts enhances microbial activity in this savannah ecosystem.

KEYWORDS
ant nests, grass tufts, lamto savannah, microbial activity, soil CO_2 released

Résumé

Les fourmis sont largement considérées comme des ingénieurs de l'écosystème en raison de leur effet sur la structure du sol et sur le flux d'énergie. Cependant, on sait peu de choses sur leur influence sur le flux de carbone dans les savanes tropicales humides. Des études récentes dans un écosystème de savane humide à Lamto ont montré que l'association des nids de fourmis avec des herbes pérennes améliore leur croissance et leur productivité. Cette étude visait à comprendre l'influence des nids de fourmis sur l'activité des microorganismes du sol sous les touffes d'herbe. La cinétique de minéralisation a été testée en laboratoire à différents moments (jour 1, 2, 4 et 7) sous trois espèces de graminées associées et non associées à des nids de fourmis, en suivant la quantité de CO2 libérée à 30°C pendant la respiration du sol. La quantité de CO_2 libérée du sol est plus élevée sous les touffes d'herbes associées aux nids de fourmis que sous celles qui ne le sont pas. La plus grande quantité de CO_2

libérée du sol a été trouvée sous les touffes d'Hyparrhenia diplandra, suivie par les touffes d'*Andropogon schirensis* et la plus faible sous les touffes de *Loudetia simplex*. Cette étude a montré que l'association des nids de fourmis avec les touffes d'herbe améliore l'activité microbienne dans cet écosystème de savane.

1 | INTRODUCTION

Several studies were conducted in tropical humid savannah of Lamto Scientific Reserve (Côte d'Ivoire). In Lamto savannah, most of the soil organic matter is linked to silts and clays that physically prevent microbial attacks; moreover, its chemical composition makes it costly degradation for micro-organisms. Consequently, the average soil organic matter mineralisation is quite low, except where energy is supplied such as in the rhizosphere zone or sites where soil is rehandled by invertebrates. Villecourt (1973) estimated the yearly emission of carbon by soil in the grass savannah at 800 g/m²/year. Bauzon in Schaefer (1974) measured the field CO_2 production by Loudetia and Andropogoneae savannah soils in small chambers set up the soil surface. The soil respiration rate also shows variation in space; it is about 60% higher under than between grass tufts, suggesting a strong contribution of root respiration to total CO_2 emission. But this high heterotrophic activity under tuft can also result from a high dead root decomposition rate and a stimulation of soil organic matter mineralisation by root exudates. Field measurements of CO_2 emission by grassland soil have shown that the contribution of living roots to total respiration ranges from about 20% to 40%. One of the important implications of this research is that the biological aspects of soil fertility are a key component of sustainable productivity. Among the players involved in recycling, invertebrates play an important role in the mineralisation process of soil organic matter. Thus, in the ecosystem functioning, ant nests are considered hot spots for CO_2 production and metabolic activity because their activities contribute to the mixing and accumulation of soils of different layers and the transport of organic matter around the nest's entrance (Folgarait, 1998). In addition, ants can significantly reduce the carbon available in the nest by bringing above, the nutrients from deep layers. Hence, they can affect the physical, chemical and biological parameters of the soil in different ways under different conditions (Frouz & Jilcova, 2008). Despite this significance impact, the involvement of ants in mechanisms underpinning their substantial contribution to the production of soil carbon and nitrogen has received little attention. A recent study conducted in Lamto savannah showed that the association of some perennial grass species with ants nest resulted in the enhancement of their growth and productivity (Ouattara et al., 2018).

These findings occur in a paradoxical environment where the soil of Lamto humid savannah is known to have low agronomic value because of the rarity of organic matter (Abbadie, 1990), while previous studies reported the primary production of grasses to be among the highest in the world (Mordelet & Roux, 2006).

The aim of the current study was to gain a basic understanding of the CO_2 flux released from the soil resulting from the decomposition of organic matter by micro-organisms under perennial grass tufts associated and not associated with ant nests.

We hypothesised that ant-grass association increases the activity of soil micro-organisms and improves the release of nutrients through organic matter decomposition dynamics. Specifically, the amount of CO_2 released from the soil was used to assess micro-organism's activity, and then, the dynamics of organic matter decomposition under grass tufts was also analysed.

2 | MATERIALS AND METHODS

2.1 | Study site

The study was carried out in the Lamto Scientific Reserve located in Côte d'Ivoire at the southern edge of the 'V Baoulé'. The climate is intertropical with an annual temperature around 27.8°C. The annual rainfall average of 1200 mm is distributed in four seasons: two wet seasons which extend from March to July and from September to November, and two dry seasons with the first one occurring in August while the second spans from December to February (Lamotte & Tireford, 1988).

The vegetation is a forest-savannah mosaic. The savannah is dominated by the palmyra palm trees (*Borassus aethiopum* Mart) and perennial grasses and occurs on three soil types: red tropical ferruginous soil; ochre tropical ferruginous soil and hydromorphic pseudo-clay soil (Menaut & Cesar, 1979; Vuattoux et al., 2006). The study was conducted on three different sites, all located in savannah (Figure 1). These three different sites were chosen to show Lamto savannah heterogeneity.

The first site (N 06 12'54.3'' and W 005 01'06.4'') was a shrubby savannah dominated by grass species such as *Andropogon schirensis* Hochst. ex A. Rich, *Andropogon canaliculatus* Schumach., *Hyparrhenia diplandra* (Hack) Stapf, *Hyparrhenia smithiana* (Hook. f.) Stapf, *Loudetia simplex* (Nees) C.E. Hubb. The shrubby stratum is composed by *Piliostigma thonningii* (Schmach.) Milne-Redh., *Crossopteryx febrifuga* (Afz ex G. Don) Benth, *Borassus aethiopum*, *Cochlospermum planchoni* Hook. F. and *Tephrosia elegans* Wall.

The second site (N 06 12'19.3'' and W 005 00'17.8'') was a grassy savannah with scattered palm trees (*Borassus aethiopum*) dominated by grass species such as *Andropogon ascinodis*, *Andropogon schirensis*, *Hyparrhenia diplandra*, *Hyparrhenia smithiana*, *Hyparrhenia chrysargyrea* (Stapf) Stapf and *Loudetia simplex*.

FIGURE 1 Vegetation map of Lamto Scientific Reserve and localization of sampling sites (modified from Gauthier, 1990)

The third site (N 06 12'26.3'' and W 005 00'30.4'') was a grassy transition savannah dominated by species such as *A. schirensis*, *H. diplandra* and *L. simplex*. The shrubby stratum consisted of palm trees and woody species such as *Bridelia ferruginea* Benth, *Crossopteryx febrifuga* and *Tephrosia elegans*.

2.2 | Experimental design

Three perennial grass species: *A. schirensis*, *H. diplandra* and *L. simplex* (APG, 2009), which constitute a major component of savannah grass in Lamto reserve, were selected to carry out this study on the three study sites.

On each study site, a patch of one hectare was delimited. Each patch was divided into four plots of 2500 m² (50 m × 50 m), but three of them were used to perform different types of sampling. One of these plots of 2500 m² was further divided into 25 squares of 10m × 10m. Within each square, 6 grass tufts were selected. For each grass species, one tuft associated with ant nest and one other not associated with ant nest were selected. As such in each study site, in total 150 individual grass tufts were selected, totalising 450 grass tufts for the three study sites. Soil was taken with an auger at 0–2 cm depth below each selected tuft. Each sample was placed in a bag. The soil samples were then dried and reduced to fine soil using a 2-mm-diameter sifter at the laboratory. Samples of each grass species at each study site were then put together to form two composite samples (one with ant nest and the other without ant nest). In such way, a total of six soil samples are recorded at each study site.

The plot used to sample the soil (0–2 cm) to measure the quantity of CO_2 released was equally used to monitor the growth of these grass tufts over a period of two (2) years. The selection was made by the digging method of

grass tufts. It consisted of rummaging the ground below the tufts. When there is an ant nest, at the slightest disturbance of the tuft, some workers would appear to inquire about its origin in order to ensure safety. As soon as the first workers appear on all sides, the tuft is covered with soil to prevent dispersal of the individuals. The selection of the tufts not associated with an ant nest required careful observation. This consisted of observing all around the affected grass tufts, to make sure that there was no sign of the presence of ants engineering activity characteristic. Once reassured, the grass tuft is then selected and recorded to be used for sampling.

2.3 | Data collection

2.3.1 | Sampling mode

Sampling is done on grass tufts that were used to monitor growth in three savannah types in Lamto (shrubby savannah, clear grassy and transition grassy savannah). The soil was removed with an auger from a depth of 2 cm below each selected tuft. Each sample was placed in a ziplock bag (resealable sealable bag) bearing the site, patch and tuft numbers, the symbol for the first letter of the grass species and the soil level collected. The samples were transported to the station for drying. After drying, they were reduced to fine soil using a 2-mm-diameter sieve in laboratory.

Samples from each grass were then put together to form two composite samples (one with a nest and another without ant nest). A total of six (6) soil samples were recorded at each study site. Eighteen (18) composite samples were thus obtained in the three study sites.

2.3.2 | Experimental protocol

Two treatments were clearly considered: (1) soil samples below grass tufts associated with ant nest (2) and soil samples below grass tufts not associated with ant nest.

The method of soil CO_2 release was used to determine micro-organism's activity following Nicolardo et al., (1982). We used a 15 g sample of dried soil and sieved to 2 mm, and then, the water content of the soil was adjusted to 20% (i.e., 0.2 ml of distilled water for the 15 g of dried soil) to be humidified. The vials each previously contained 5 ml of NaOH (0.2N) in pill boxes were also attached to the inside of each incubation flask. The CO_2 released during the incubation is trapped in the 5 ml of sodium hydroxide (NaOH, 0.2N) contained in the pill boxes put with the soil in the incubation flasks.

The flasks were hermetically sealed and placed in an incubator at 30°C (Memmert, Schutzart DIN 40050—IP 20). In order to follow the kinetics of the mineralisation, the flasks remained in the incubator for various periods (1, 2, 4 and 7 days). The CO_2 released in the flask was trapped by sodium hydroxide in the form of sodium bicarbonates (Na_2CO_3). At the end of each incubation period, the Na_2CO_3 formed was precipitated with barium chloride ($BaCl_2$, 20%) and a cloudy solution was formed. The residual amount of soda obtained was entirely assayed by titrimetry using a hydrochloric acid solution (HCl, 0.1N), in the presence of phenolphthalein. The presence of phenolphthalein gives a pink colour to the solution.

At the end of the assay, the solution returns to the starting colour (cloudy solution). The experiment was repeated three times with the same soil samples collected beneath grass tufts associated with ant nest and the other not associated with ant nest.

The quantity (Q) of mineralised carbon was obtained by calculation according to the following formula (Anderson & Ingram, 1996: TSBF handbook,).

$$Q\left(\frac{mg}{15\ g\ of\ soil}\right) = (V_{white} - V_{sample.}) \times 2.2$$

where

V_{white}: is the average volume of HCl for the control sample.

V_{sample}: is the average volume of HCl for the measured sample.

2.2: is a constant (the coefficient of the mineralisation speed of the mineralisable compartment per day).

Q is the amount of mineralised carbon; it is a bioindicator informing on microbial activity beneath grass tufts associated and non-associated with ant nest.

2.4 | Data analysis

An analysis of variance (ANOVA) made it possible to determine the variations in the flow of CO_2 released from the soil between the different study sites and to compare soil respiration under associated tufts and those not associated with ant nests. Student's *t*-test was used to compare soil respiration beneath associated tufts and those not associated with ant nests. The simple linear model allowed showing the arrangement of points in space, in order to reproduce the differences observed between the amounts of CO_2 released during soil respiration. This made it possible to establish the cause and effect relationship between the amounts of CO_2 released from the soil under the tufts of the various associated grass species and those not associated with ant nests. Past software was used to analyse the data.

3 | RESULTS

3.1 | Effect of ants on the amount of CO_2 released from the soil in Lamto savannah

3.1.1 | Amount of CO_2 released from the soil in the whole of habitats

In the whole of combined habitats, the amount of CO_2 released from the soil beneath grass tufts associated with ant nest was higher than those not associated with ant nests in the three types of savannah studied in Lamto. This amount is significantly higher under tufts associated with ant nests than those not associated with ant nests ($t = 9.262$, $N = 4$; $p = .003$).

TABLE 1 Amount of CO_2 released from the soil according to the three habitats and grass species associated and those not associated with ant nests (±Standard deviation)

Study sites	samples number	*Andropogon schirensis*		*Hyparrhenia diplandra*		*Loudetia simplex*		Mean CO_2 released/ Site (mg/g of soil)
		T As	t As	T Hd	t Hd	T Ls	t Ls	
Shrubby savannah	6	0.16 ± 0.02	0.12 ± 0.02	0.21 ± 0.02	0.15 ± 0.03	0.14 ± 0.04	0.12 ± 0.03	0.15 ± 0.03
Grassy savannah	6	0.15 ± 0.01	0.13 ± 0.02	0.17 ± 0.02	0.12 ± 0.02	0.11 ± 0.03	0.08 ± 0.02	0.13 ± 0.03
Grassy transition savannah	6	0.15 ± 0.02	0.13 ± 0.01	0.21 ± 0.03	0.14 ± 0.02	0.12 ± 0.03	0.12 ± 0.03	0.14 ± 0.03
Mean CO_2 released (mg/g of soil)	6	0.15 ± 0.003	0.13 ± 0.003	0.2 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.14 ± 0.03

Abbreviations: T As, *Andropogon schirensis* tufts associated with ant nests; t As, *Andropogon schirensis* tufts not associated with ant nests; T Hd, *Hyparrhenia diplandra* tufts associated with ant nests; t Hd, *Hyparrhenia diplandra* tufts not associated with ant nests; T Ls, *Loudetia simplex* tufts associated with ant nests; t Ls, *Loudetia simplex* tufts not associated with ant nests.

3.1.2 | Amount of CO_2 released from the soil in shrubby savannah, grassy savannah and transitional grassy savannah

Between the three different savannah habitats, the amount of CO_2 released from the soil (Table 1) showed shrub savannah (0.15 mg/g of soil ± 0.033), grassy savannah (0.13 mg/g of soil ± 0.031) and transition grassy savannah (0.145 mg/g of soil ± 0.034). The amount of CO_2 released from the soil was significantly higher under grass tufts associated with ant nests than those not associated with ant nests in the three study sites ($F = 12.8$; df = 5; $p = .023$). A comparison of the amount of CO_2 released from the soil showed that it was significantly higher beneath grass tufts associated with ant nests than those not associated with ant nests in shrubby savannah (S1: $t = 5.96$; $N = 4$; $p = .01$), grassy savannah (S2: $t = 7$; $N = 4$; $p = .006$) and transitional grassy savannah (S3: $t = 5.75$; N = 4; $p = .01$). This amount was higher in shrubby savannah than in the other savannah types (Table 1).

3.1.3 | Amount of CO_2 released from the soil beneath grass tufts

According to the perennial grass species, in the whole of three savannah types, the results showed that *Hyparrhenia diplandra* associated with ant nest recorded higher values of the CO_2 released from the soil than *A. schirensis* and *L. simplex* tufts associated with and not associated with ant nests (Table 1). Also, there was a significant difference between tufts associated and those not associated with ant nests of each grass species (t = 11,5; $p = .007$). The amount of CO_2 released from the soil under tufts differs significantly depending of the grass species ($F = 15.29$; df = 8; $p = .004 < .05$). The amount of CO_2 released from the soil beneath grass tufts with ant nests was significantly greater than those without ant nests by *Andropogon schirensis* ($t = 4.612$, $p = .044$); *Hyparrhenia diplandra* ($t = 11.5$, $p = .007$). In contrast, there was no significant difference between *Loudetia simplex* tufts with/without ant nests ($t = 2.395$; $p = .139$).

The linear model results showed that in the whole of the three habitats studied, *H. diplandra* associated with ant nests was recorded the highest overall amount of CO_2 released from the soil. On the other hand, *L. simplex* tufts associated and those not associated with ant nests recorded the lowest values of CO_2 released from the soil (Figure 2).

3.2 | Dynamic of micro-organism's activity during incubation time

3.2.1 | Between the three different habitats

In the combined habitats, this study showed that the amount of CO_2 released varied during the incubation time. Overall, the amount of CO_2 was high at day 1 of incubation time. At day 2, the amount of CO_2 released from the soil decreased. Then, after 4 days of incubation, the amount of CO_2 released has increased again and decreased

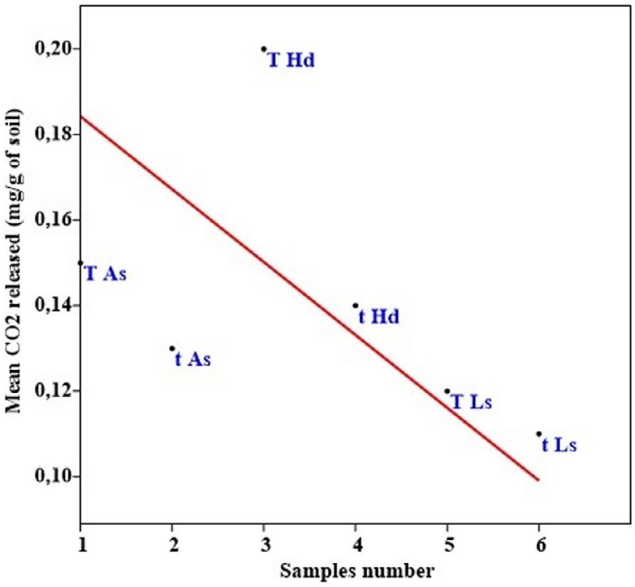

FIGURE 2 Mean amount of CO2 released from the soil according to the three perennial grasses

when the incubation period became longer at 7 days. This pattern was identical for the three habitats types (Table 2).

3.2.2 | In shrubby savannah, grassy savannah and transitional grassy savannah

The amount of CO_2 released from the soil was also observed during the incubation periods within the three different savannah types. The amount of CO_2 released was highest at 4 days of incubation time in shrubby and grassy savannah, while it was highest at 7 days in grassy transition savannah (Table 2). In the whole, the *p*-values showed that there was no significant difference between the amount of CO_2 released from the soil beneath grass tufts associated with ant nests in transitional grassy savannah during the incubation periods (1, 2, 4 and 7 days). Similarly for tufts not associated with ant nests in grassy savannah (Table 2).

3.2.3 | Beneath perennial grass species

According to the perennial grass species, the amount of CO_2 released from the soil under grass tufts associated with ant nest was the highest at 4 days of incubation time beneath *Hyparrhenia diplandra* (0.24 mg/g of soil) *Loudetia simplex* tufts (0.19 mg/g of soil) and *Andropogon schirensis* (0.18 mg/g of soil) in the whole of the three habitats (Table 3). Also, beneath the tufts not associated with ant nests, the CO_2 released was the highest at 4 days of incubation time and this amount was equally higher beneath *H. diplandra* (0.19 mg/g of soil) tufts than *A. schirensis* (0.16 mg/g of soil) and *L. simplex* tufts (0.16 mg/g of soil).

In shrubby savannah, the amount of CO_2 released was higher at 4 days of incubation time beneath *H. diplandra* (0.28 mg/g of soil), *L. simplex* tufts (0.23 mg/g of soil) and *A. schirensis* (0.21 mg/g of soil) associated with ant nest than those without ant nests. Beneath the tufts not associated with ant nests, the amount of CO_2 released from the soil was also higher at 4 days of incubation time. This amount was higher beneath *H. diplandra* (0.24 mg/g of soil) than *L. simplex* tufts (0.18 mg/g of soil) and *A. schirensis* (0.17 mg/g of soil).

In grassy savannah, the amount of CO_2 released from the soil was also the highest at 4 days of incubation time beneath the whole of grass tufts. This amount was higher beneath *H. diplandra* (0.21mg/g of soil) tufts associated with ant nest compared with those of *L. simplex* (0.19 mg/g of soil) and *A. schirensis* (0.17 mg/g of soil).

Beneath tufts not associated with ant nests, the CO_2 released was the highest at 4 days of incubation time. This amount was higher beneath *H. diplandra* tufts and *A. schirensis* (both 0.18 mg/g of soil) compared with those of *L. simplex* (0.14 mg/g of soil).

In grassy transition savannah, the amount of CO_2 released was the highest at 7 days of incubation time for all grass species. This amount was higher beneath *H. diplandra* (0.27 mg/g of soil) tufts associated with ant nests compared with those of *A. schirensis* (0.19 mg/g of soil) and *L. simplex* (0.18 mg/g of soil). Under the tufts not associated with ant nests, the amount of CO_2 released was the highest at 7 days of incubation time for all grass species. This amount was higher beneath *H. diplandra* (0.19 mg/g of soil) tufts not associated with ant nest compared with those of *A. schirensis* (0.18 mg/g of soil) and *L. simplex* (0.17 mg/g of soil).

The *p*-values showed that there was no significant difference between the amount of CO_2 released from the soil beneath *Andropogon schirensis* and *Hyparrhenia diplandra* tufts associated with ant nests during the incubation periods (1, 2, 4 and 7 days). Contrary, there was a significant difference between the amount of CO_2 released from the soil beneath *Loudetia simplex* tufts associated with ant nests (Table 3). For tufts not associated with ant nests, there was no significant difference between the amount of CO_2 released from the soil during the incubation periods (1, 2, 4 and 7 days) only beneath *Hyparrhenia diplandra* (Table 3).

4 | DISCUSSION

4.1 | Effect of ants on the amount of CO_2 released from the soil in Lamto savannah

The results show that in the whole of the three habitats studied, the amount of CO_2 released from the soil was higher beneath tufts associated with ant nests compared with those not associated with ant nests. Soil animals stimulate soil microbial activity by providing low-molecular-weight carbon products (Abbadie, 1990). These could induce a strong and localised degradation of soil organic matter. Otherwise, the accumulation of food and waste generated by plants improves soil fertility more than that of animal origin (Farji-Brener, & Werenkraut, 2017).

So, ants living beneath the grasses, through the mechanism of 'bioturbation' (Nkem et al., 2000), and the transport of organic

TABLE 2 Mean amount of CO_2 released from the soil according to incubation time in the three study sites beneath grass tufts associated and not associated with ant nests. (±Standard deviation)

	Incubation time				Statistical values	
Mean CO_2 (mg/g of soil)/habitats	**1**	**2**	**4**	**7**	***F***	***p***
Mean T CO_2 (mg/g of soil) S1	0.15 ± 0.02	0.1 ± 0.03	0.24 ± 0.02	0.19 ± 0.01	5.85	.02
Mean T CO_2 (mg/g of soil) S2	0.16 ± 0.02	0.12 ± 0.02	0.19 ± 0.01	0.12 ± 0.02	14.44	.001
Mean T CO_2 (mg/g of soil) S3	0.16 ± 0.02	0.1 ± 0.03	0.17 ± 0.02	0.21 ± 0.03	3.21	.08
Combined habitats T CO_2 (mg/g of soil)	0.16 ± 0.01	0.11 ± 0.01	0.2 ± 0.04	0.17 ± 0.05	7.19	.001
Mean t CO_2 (mg/g of soil) S1	0.11 ± 0.01	0.08 ± 0.01	0.2 ± 0.02	0.14 ± 0	5.64	.02
Mean t CO_2 (mg/g of soil) S2	0.12 ± 0.01	0.08 ± 0.03	0.17 ± 0.01	0.08 ± 0.01	3.35	.08
Mean t CO_2 (mg/g of soil) S3	0.12 ± 0.01	0.08 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	21.45	0
Combined habitats t CO_2 (mg/g of soil)	0.12 ± 0.01	0.08 ± 0.00	0.17 ± 0.02	0.13 ± 0.05	13.06	0

Abbreviations: S1, shrubby savannah; S2, grassy savannah; S3, grassy transition savannah; T CO_2, CO_2 released beneath grass tufts associated with ant nests;t CO_2, CO_2 released beneath grass tufts not associated with ant nests.

TABLE 3 Amount of CO_2 released from the soil per grass species associated and not associated with ant nests according to the incubation time (Mean ± standard deviation)

	Mean CO_2 released (mg/g of soil) / Incubation time (days)					
Grass tufts	**1**	**2**	**4**	**7**	***F***	***p***
T As	0.15 ± 0.02	0.12 ± 0.01	0.18 ± 0.03	0.17 ± 0.03	3.117	.09
T Hd	0.2 ± 0.0	0.15 ± 0.1	0.24 ± 0.04	0.21 ± 0.06	2.803	.11
T Ls	0.12 ± 0.01	0.05 ± 0.02	0.19 ± 0.04	0.14 ± 0.05	7.376	.01
t As	0.12 ± 0.01	0.09 ± 0.02	0.16 ± 0.02	0.14 ± 0.04	4.862	.03
t Hd	0.13 ± 0.01	0.1 ± 0.01	0.19 ± 0.04	0.14 ± 0.05	3.623	.06
t Ls	0.1 ± 0.00	0.04 ± 0.01	0.16 ± 0.02	0.12 ± 0.06	6.383	.02

Abbreviations: T As, *Andropogon schirensis* tufts associated with ant nests; t As, *Andropogon schirensis* tufts not associated with ant nests; T Hd, *Hyparrhenia diplandra* tufts associated with ant nests; t Hd, *Hyparrhenia diplandra* tufts not associated with ant nests; T Ls, *Loudetia simplex* tufts associated with ant nests; t Ls, *Loudetia simplex* tufts not associated with ant nests.

material, could make the nutrients necessary for the growth of heterotrophic micro-organisms available. This could stimulate and increase micro-organism's activity but also the microbial biomass and could make it possible to provide a large amount of CO_2 for the plants, promoting their rapid growth and development. Ant nests are hot spots for CO_2 production and metabolic activity in the ecosystem (Frouz & Jilcova, 2008). Microbial activity can be substantially higher in the ant nests than in the surrounding soil because of the surplus of available nutrients and because of the adequate conditions of humidity and temperatures (Frouz, 2000). Likewise, Fernández et al., (2014) demonstrated that *Acromyrmex lobicornis* (leaf-cutting ant) in the refuse dumps enhances the activity of soil biota in arid regions of Patagonia, and demonstrated that a small quantity of water enhances this effect. Microbial biomass could be mainly concentrated in the upper layers of nests where temperature and humidity were higher. Some animal and plant groups in the soil including protozoa were more abundant in ants' nests than in the surrounding soil (Frouz & Jilcova, 2008; Zaragoza et al., 2007).

In shrubby savannah, through microflora and macrofauna activities, the presence of litter and other organic matter is incorporated into the soil. This improves soil conditions, both physically and chemically (Anderson, 1986). According to Belsky et al., (1989), this activity has a longer duration under trees because of a stabilised microclimate and the accumulation of organic matter. In Lamto, it has been shown that tree / grass interaction leads to improved grass water status due to the shading effect (Mordelet & Le Roux, 2006). This could therefore promote the growth of micro-organisms in shrubby savannah.

The activity of ants could lead to an increase in substrate proportion quickly assimilable by the micro-organisms present in the environment. In addition, in terms of mineralisation, microbial activity could immobilise nitrogen in the microbial biomass. Furthermore, it has been suggested that nutrients available to plants are mainly released after deadly desiccation of micro-organisms and rewetting of soil (see Bernhard-Reversat, 1982). All these processes could be an explanation why the highest and best results of released CO_2 were obtained in shrubby savannah.

In grassy savannah, the lower amount of CO_2 released from the soil could be explained by the fact that the vegetation consists only of grass species. This savannah ecosystem is a clearing between a shrubby savannah and a large open grassy savannah dominated by *L. simplex*. The soil on which this savannah is situated was much sandier. The type of soil and vegetation could therefore restrain

the activity of ants as well as micro-organisms living in this area. Although the quantity of CO_2 is low in grassy savannah, it is even higher under grass tufts associated with ant nests than those not associated with ant nests. That is due to the effect of ants beneath grass tufts. According to Fernández et al., 2014, ants nest have a potential importance to nutrient cycling, the maintenance of plant cover and the carbon balance in the arid regions.

In grassy transition savannah, the sampling took place has scattered thickets interspersed with a few shrubs and was surrounded by a woody savannah and opens to a *Loudetia simplex* savannah. This localisation could create a specific microclimate favouring the cohabitation of micro-organisms specific to the surrounding habitats, as well as the species of ants living in the area. That could be inducing the same microclimate conditions to the two savannah types.

According to the perennial grass species, *H. diplandra* recorded the highest amount of CO_2 released from the soil, followed by *A. schirensis* and finally *L. simplex*. Probably, *H. diplandra* grass provides to micro-organisms a microclimate favourable for their development. Activity beneath *L. simplex* was less explicit in this study. Soil animals are likely to stimulate soil microbial activity by providing low-molecular-weight carbon products (Abbadie, 1990). These could induce a strong and localised degradation of soil organic matter. In fact, this was in particular the case for *H. diplandra* associated with ant nests. *A. schirensis* tufts showed the greatest species richness of ant nests in Lamto savannah (Ouattara et al., 2018). *L. simplex* grass recorded the lowest values of CO_2 amount released from the soil.

The hydromorphic characteristics of this grass could be an explanation for its weak activity of micro-organisms. In addition, nutrient uptake is optimised by grass tuft architecture and spatial aggregation of roots (Abbadie & Lata, 2006). Otherwise, a low coverage of *Loudetia* and, contrary, a good development of *Hyparrhenia* showed that the tufts of *Hyparrhenia* provide a remarkable screen against direct insolation (Monnier, 1968). This could lower soil temperature and evapotranspiration, which could then increase the moisture content of the soil beneath the tufts of *H. diplandra*. This mechanism may favour the development and intensity of micro-organism activity beneath *H. diplandra* tufts, especially those associated with ant nests. As for *A. schirensis*, the appearance of roots in the form of moss could give it a microclimate favourable to ant nidification.

4.2 | Dynamic of micro-organism's activity during incubation time

The amount of CO_2 released gives an indication of the quantities of mineralised carbon (Abbadie, 1990; Dommerges, 1968). The significant CO_2 production observed (at 24 h from the beginning of incubation) could be explained by a strong microbial respiration (Zaouchi, 2015). Indeed, micro-organisms consume oxygen, produce CO_2 from their respiratory activity and heat through their metabolic reactions as well as by the decomposition of organic matter (Finstein & Morris, 1975). On the other hand, before incubation, the soil samples have become desiccated since they have been subjected to different treatments (transport, sieving and storage). As a result, they were brought back to a humidity corresponding to 80% of that equivalent. This desiccation could result in a high microbial mortality causing an increase in organic molecules (Jedidi, 1998). After moistening of the soil samples, microbial activity and CO_2 production were resumed; this could be intensified by the rapid mineralisation of these organic molecules (Bernard, 1981). In addition, moisture and temperature control processes within the soil could influent decomposition rates of organic matter, denitrification and nitrification (FAO, 2003). The incubation of soil samples at a temperature of 30°C and their moistening up to 80% at the corresponding humidity (oxygen-moisture equilibrium) could also promote the intense recovery of microbial activity. The decrease in daily CO_2 production after 24 h of incubation could be related to the adaptation of microbial populations to the new conditions of temperature and humidity (Bernal et al., 1998). Hence, the readaptation to microclimate conditions can result in an accelerated recovery of their activity. This could explain the rapid evolution of the amount CO_2 released from the soil after 48 h of incubation, specifically below grass tufts with ant nests.

The results of shrubby and grassy savannah showed that the activity of micro-organisms was more intense in the soil at 4 days and then becomes weak later. This could mean that the activity time of micro-organisms is not long enough. This could be due to the rapid mineralisation of soil organic matter by micro-organisms. The decrease in CO_2 production observed after 4 days of incubation can probably be linked to the depletion of the stock of organic molecules following the consumption of micro-organisms for their metabolism. This could also be related to the adaptation of microbial populations to new conditions of temperature and humidity, which can result in a slowing down of this microbial activity (Bernal et al., 1998; Zaouchi, 2015).

In grassy transition savannah, the activity of micro-organisms was intense in the soil up to 7 days of incubation. An analysis of the physical properties of soil (unpublished data) indicated that this savannah has a mean conductivity of the soil equal to 34.23 S/m which is lower than that of shrubby savannah (average conductivity of the soil equal to 52.2 S/m) and grassy savannah (average conductivity of the soil equal to 49.52 S/m). Otherwise, the conductivity will be more important and higher there as the humidity of the soil is high and it is rich in salts (Api, 2016). This could mean that the moisture is lower in grassy transition savannah compared with that of shrubby and grassy savannah. These conditions could be favourable to the life of micro-organisms living in this environment, thus allowing them to be active over long periods. May another explanation should be searched in the quality of exudates produced by the grassroots. In addition, during the sample time, we remarked that grasses at grassy transition savannah appeared to be less stressed during the dry season compared with that of shrubby savannah and grassy savannah. In shrubby savannah, a prolonged effect of drought was observed. The ground becomes more compact and harder when there was a lack of water. This could also limit microbial activity. In soil, microbial activity depends on physical and chemical factors controlled partly by the specific composition of vegetation and its biomass as well as

by wildlife activity (Abbadie & Lata, 2006). Locally, microbial activity can be strongly regulated by the amount of organic matter due to the high spatial and temporal variability of this factor.

At Lamto, the intensity of micro-organism activity depends on the microclimatic conditions offered by each habitat type. This makes it possible to perceive the heterogeneity of the habitat. *H. diplandra* was the grass species that released the highest amount of CO_2 from the soil in all types of savannah (shrubby savannah, grassy savannah and grassy transition savannah).

Also, the amount of CO_2 released from the soil was high under *A. schirensis* only in grassy savannah, where the lowest values of CO_2 released from the soil were obtained. The amount of CO_2 released from the soil elevated at 4 days of incubation in the shrubby and grassy savannah might be explained by the availability and quality of organic matter in these environments. May be also the type of micro-organisms responsible for the mineralisation of organic matter are important and especially the physicochemical parameters which were a factor limiting the activity of these soil organisms.

In the transitional grass savannah, *H. diplandra* associated and not associated with ant nests showed a high amount of CO_2 released from the soil equally at 7 days of incubation. This could be due to lie at the microclimate conditions created and ant activities in this savannah which favour the long time activity of micro-organisms.

5 | CONCLUSION

The amount of CO_2 released from the soil showed that micro-organism's activity was more intense beneath perennial grasses associated with ant nests than those not associated with ant nests. Their activity was more intense in the soil beneath *H. diplandra* tufts associated with ant nest, followed by those of *A. schirensis* associated with ant nest and *L. simplex* associated with ant nests. On the other hand, the microbial activity is low beneath *L. simplex* associated and/or not associated with ant nests. Ants through their engineering promote the growth of micro-organisms (increase in microbial biomass). Micro-organisms stimulate the decomposition of pre-existing organic matter in the soil (humified organic matter) that induces the rapid mineralisation of organic matter beneath these plants. Ant-grass association increases effectively the activity of soil micro-organisms and improves nutrient release via organic matter decomposition. The variations in the amount of CO_2 released between incubation periods in different habitats indicate the heterogeneity of the soil and vegetation in Lamto savannah. Further investigations will allow assessing the quantity and quality of nutrient released by ants beneath grass tufts.

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DATA AVAILABILITY STATEMENT

Data will be available only upon request.

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