

ORIGINAL ARTICLE

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Ant nests effect on organic matter, carbon, and nitrogen flux in the soil under grasses tufts in Lamto savannah (Côte d'Ivoire)

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Abstract

Ants are known to contribute to the physical and chemical improvement of the soil. In this context, the hypothesis put forward is that ants improve the nitrogen (N) supplementation necessary to ensure the high primary production measured in the Lamto savannah. Recent investigations in the humid savannah ecosystem showed that ant nests' association with perennial grasses enhances their growth, productivity, and microorganism activity. This study aimed at understanding the effect of ant nests on organic matter (OM), carbon (C), and N flux beneath grass tufts. Under each grass tuft chosen to carry out this study, soil samples were taken from the depths of 0–10 cm using an auger at shrubby, clear grassy, and transitional grassy savannah. The analytical method by incineration of the loss on fire was used to determine the OM amount. The Kjeldahl method was used to determine the total N amount in the soil under grass tufts. The results showed that these components' amount is higher beneath grass tufts associated with ant nests than those not associated with ant nests. The presence of ant nests increases OM and C amount in the soil under *Hyparrhenia diplandra* tufts than *Andropogon schirensis*, and *Loudetia simplex* tufts. In contrast, N amount is higher under *L. simplex* tufts than *A. schirensis*; but mean under *H. diplandra*. The carbon/nitrogen ratios less than 10 indicate high OM mineralization under grass tufts associated with ant nests. This provides the plants with an adequate supply of nutrients.

Abbreviations: C, carbon; C/N, carbon and nitrogen ratio; N, nitrogen; OM, organic matter; pH, hydrogen potential; TAN, tufts with ant nests; TAs, *Andropogon schirensis* tufts associated with ant nests; tAs, *Andropogon schirensis* tufts not associated with ant nests; THd, *Hyparrhenia diplandra* tufts associated with ant nests; tHd, *Hyparrhenia diplandra* tufts not associated with ant nests; TLs, *Loudetia simplex* tufts associated with ant nests; tLs, *Loudetia simplex* tufts not associated with ant nests; TWN, tufts without ant nests.

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1 | INTRODUCTION

Soil organic matter (SOM) is a primary indicator of soil quality (Rice, 2005).

The low clay and organic matter (OM) content lead to a low stability of the soil structure. Therefore, there is a high sensitivity of the soil to physical (erosion) and chemical degradations (Rice, 2005).

SOM is a complex, naturally occurring material and plays a critical role in soil fertility and the global carbon cycle. It is also a mixture of components of different forms and types of

biotic origin and can be broadly classified into living and non-living (Coleman et al., 1989). OM is particularly important as the prime habitat for immense numbers and a variety of soil fauna and microflora. These organisms play a critical role in the health and productivity of soils. They are highly susceptible to changes in land use and management and to changes in soil temperature and moisture.

In Lamto savanna Scientific Reserve, the soil is known to have a low agronomic value (Le Roux & Mordelet, 1995). These soils are poor in OM (Abbadie & Bismarck, 2006). That is probably due to the burning of most of the above-ground primary production, rapid decomposition of plant debris, and soil texture. Moreover, this OM is quite regularly distributed in a soil profile: at 1 m depth, the concentration of OM still amounts to 0.2%–0.3% versus 0.8%–0.9% in surface layers (Abbadie & Bismarck, 2006; Pirovano, 1996). However, according to Le Roux and Mordelet (1995), the primary production of grasses in this savannah is among the highest in the world. It is well known that nitrogen (N) is the main nutrient for plant growth and development. In the Lamto savanna, the availability of mineral N is a key determinant of primary production. The minimal flux of together herbaceous stratum rose to 77 N ha⁻¹ year⁻¹ but only 2–5 kg N ha⁻¹ year⁻¹ mineralized and put at plant available (Abbadie, 1990). Thus, to investigate the origin of the surplus, Abbadie (1990) showed that it exist a shorter N cycle other than OM mineralization under grass tufts. This shorter cycle is based on the decomposition of older roots under the grass's tufts, their mineralization, and rapid recycling by living roots (Abbadie, 1990).

The invertebrate can control SOM stability (Wolters, 2000). Thus, these organisms at higher trophic levels create feedback mechanisms that modify the spatiotemporal framework in which the micro-food web affects SOM stability. Several studies are showing that ants are known as important ecosystem engineers for their potential in modifying the energy flows and nutrient cycles of soil (Wang et al., 2017).

Most ant species can build corridors and galleries in their nests above or below ground (Gosselin et al., 2016). Their ability to build biogenic structures is a foundation of the soil engineering concept (Bottinelli et al., 2015; Franco, 2015). The processes of nest-building involve the mixing and gathering of soils from different sources, horizons, and soil vertical layers. Also, the transport of organic material from the surroundings into the nests as food or building material. The activities of ant nesting can greatly affect soil's physical, chemical, and biological properties, which are closely associated with the size and composition of soil C and N pools (Wang et al., 2017; Wu et al.,). This study aims to assess the OM, C, and N content in the Lamto savanna ecosystem. And the interactions between soil processes, ant nests, and perennial grass cover.

Core Ideas

- Ants improve the nitrogen (N) supplementation necessary to ensure high primary production measured in the Lamto savannah.
- This study aimed at understanding the effect of ant nests on organic matter (OM), carbon (C), and N flux beneath grasses.
- The carbon/nitrogen ratios less than 10 indicate high OM mineralization under grasses associated with ant nests.
- OM capacity to decompose more or less rapidly and the absorption of N is released into the soil under grass tufts.

2 | MATERIAL 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 of 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 pseudoclay soil (Menaut & Cesar, 1979; Vuattoux et al., 2006). The study was conducted on three different sites, all located in the 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., *H. diandra* (Hack) Stapf, *Hyparrhenia smithiana* (Hook. f.) Stapf, *Loudetia simplex* (Nees) C.E. Hubb. The shrubby stratum is composed of *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 ascinioidis*, *A. schirensis*, *H. diandra*, *Hyparrhenia smithiana*, *Hyparrhenia chrysargyrea* (Stapf) Stapf and *L. simplex*.

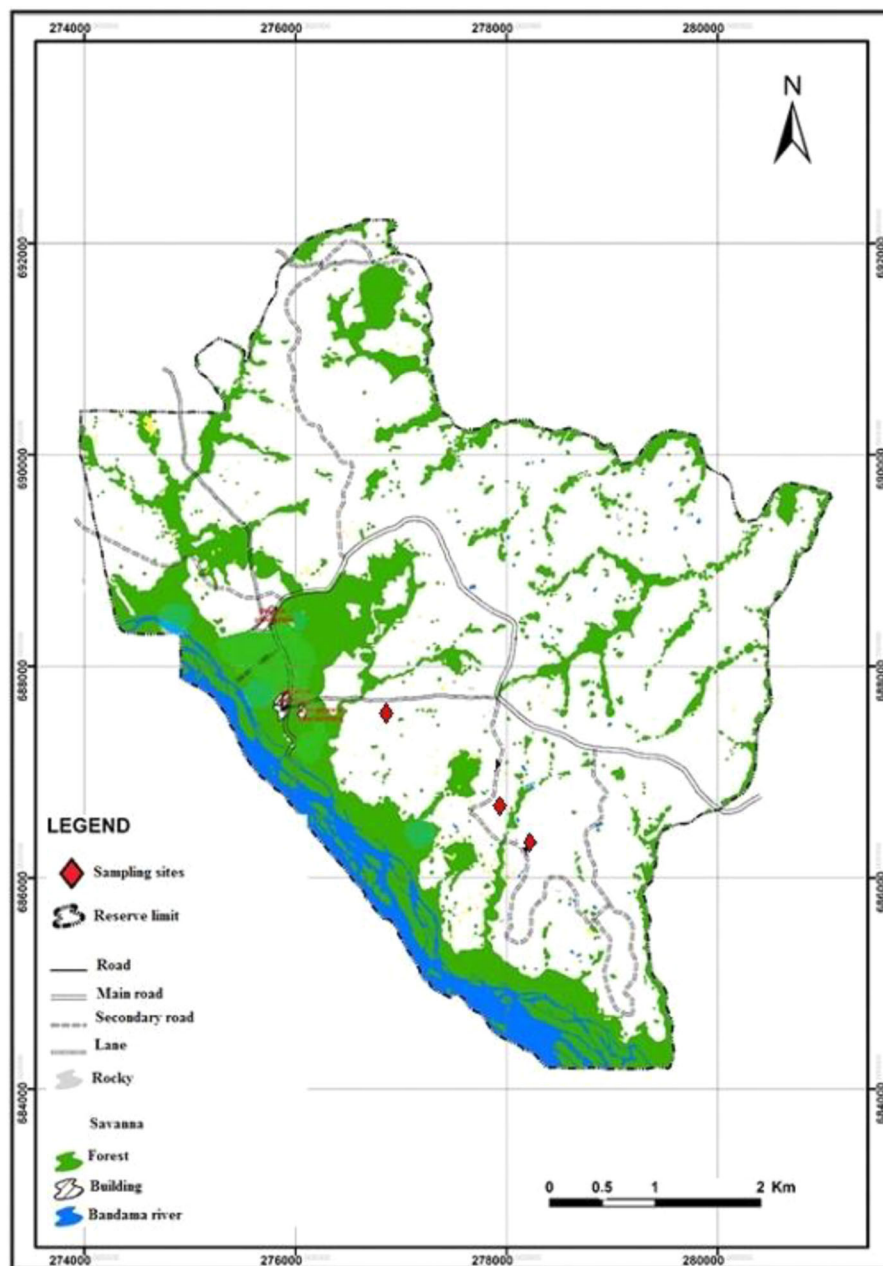


FIGURE 1 | Vegetation map of Lamto Scientific Reserve and localization of sampling sites (modified from Gautier, 1990).

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 | Soil sampling method for chemical analysis

Three perennial grasses species: *A. schirensis*, *H. diplandra* and *L. simplex* (APG, 2009), which constitute

a major component of savannah grasses in Lamto reserve, were selected to carry out this study on the three 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. On a 2500 m² plot in each site, the plots located at the four corners and the central plot, five in total, were selected for soil sampling. Under two tufts of each grass (one associated and one not associated with ant nests), soil samples were taken from depths of 0–10 cm using an auger (Edoukou, 2015). Soil samples were stored in labeled bags with the site name, plot name, tuft name, grass letter, and soil

depth (0–10 cm). A total of six soil samples are recorded from each study site. From this, 18 composite samples are obtained from the three study sites.

2.3 | Soil analytic method

2.3.1 | Method of total nitrogen analysis

The method used to determine total N is the Kjeldahl method (C. Gerhardt Compendium, 2015). This method is carried out in three steps: mineralization, distillation, and finally titration and calculation.

2.3.2 | Method of organic matter analysis

The analytical method used to determine the OM by incineration was that of the loss on fire. It was described by the Quebec Center of Expertise in Environmental Analysis (MA 1010-PAF 1.0 edition: 2003-03-27). Organic carbon = OM (LFO) / 1.724

2.4 | Statistical analysis of soil nitrogen, organic matter, and carbon content

2.4.1 | Results calculation and expression

The OM rate from the soil was determined according to the following expressions:

$$\%OM = C \times 1.724$$

Where

OM, organic matter; C, carbon, g, gram; %, percentage

2.4.2 | Data analysis of soil nitrogen, organic matter, and carbon content

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

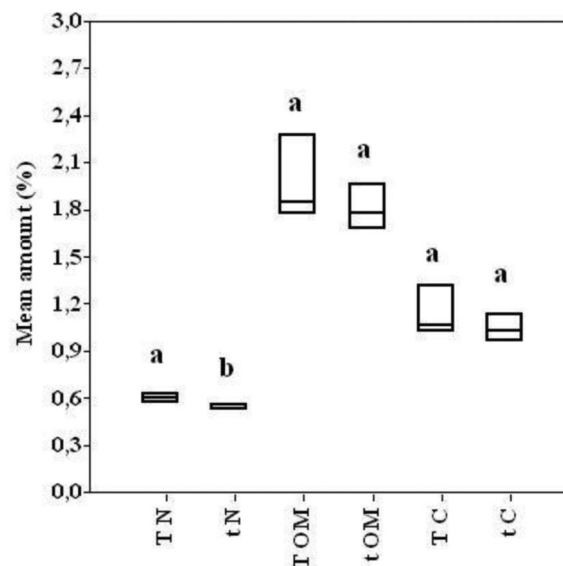


FIGURE 2 Mean amount of nitrogen, organic matter and carbon from soil according to the all three savannah types; N = nitrogen; OM = organic matter; C = carbon; T = tufts with ant nest; t = tufts without ant nest.

3 | RESULTS

The results showed that N amount is equal in the three study sites beneath tufts associated with ant nests (shrubby savannah = 0.61%; grassy transition savannah = 0.61% and clear grassy savannah = 0.61%). The comparison of N amount, by Student's *t*-test, showed that it is significantly higher under tufts associated with ant nests than those not associated with ant nests ($t = 7$; $p = 0.02$). On the other hand, OM ($t = 1.76$; $p = 0.22$) and C ($t = 1.78$; $p = 0.22$) amounts from soil did not vary significantly between tufts associated and not associated with ant nests in all habitats. Analysis of variance showed that the OM amount was significantly higher than that of N in all three savanna types ($F = 221.2$; $p = 0.01$). The boxplot model showed N, OM, and C amount variation under grasses tufts in all three savanna types (Figure 2). The results show that despite the small variations, the amounts of OM and C are higher under tufts associated than those not associated with ant nests in all habitats.

In all three savannah types, N amount is equally in soil under grasses tufts associated with ant nests (ANT = 0.61%) (Table 1).

In the shrubby savannah, analysis of variance showed that the OM amount is significantly higher than that of N ($F = 233.3$; $p = 0.01$). N amount is significantly higher under the tufts associated than those not associated with ant nests (Student's *t*-test; N: $t = 5.398$; $p = 0.003$). In contrast, there was no significant difference between soil OM amount ($t = 0.48$; $p = 0.68$) under grasses tufts associated and those not associated with ant nests.

TABLE 1 Soil elements amount in Lamto savannah according to the study sites (soil samples were taken from depths of 0–10 cm using an auger).

Grass tufts	Soil elements				
	N (%)	OM (%)	C (%)	pH	C/N
TAN					
Shrubby savannah	0.61	2.62	1.52	6.03	2.5
Clear grassy savannah	0.61	1.55	0.9	5.96	1.47
Transitional grassy savannah	0.61	1.75	1.01	5.87	1.65
TWN					
Shrubby savannah	0.51	2.52	1.46	6.09	2.86
Clear grassy savannah	0.54	1.4	0.82	6	1.52
Transitional grassy savannah	0.61	1.52	0.88	6.04	1.44
<i>t</i>	1.91	4.23	4.09	–2.23	
<i>p</i>	0.19	0.05	0.05	0.15	

Abbreviations: C = carbon; N = nitrogen; OM = organic matter; *p* = associated probability; pH = hydrogen potential; *t* = Student's *t*-test; TAN = tufts with ant nests; TWN = tufts without ant nests.

In clear grassy savannah, analysis of variance showed that OM amount was significantly higher than that of N ($F = 54.81$; $p = 0.01$). Student's *t*-test showed that there was no significant difference between N amount ($t = 1.73$; $p = 0.22$) under grasses tufts associated and those not associated with ant nests. The same result was true for the OM amount ($t = 1.96$; $p = 0.19$).

In transitional grassy savannah, N is equal amounts under grasses tufts associated and not associated with ant nests (respectively: TAN = 0.61% and TWN = 0.61%).

On the other hand, the OM amount was significantly higher under grasses tufts associated than those not associated with ant nests (Student's *t*-test; OM: $t = 7$; $p = 0.02$). Soil carbon/nitrogen (C/N) ratio and pH are indicators of soil functioning. The pH and C/N ratio values of each sample are grouped by habitat and recorded in the table (Table 1). The pH ranges from 5.80 to 6.10 in soil under grasses tufts in all three savannah types.

The mean amount of N and OM from the soil according to the three perennial types of grass associated and not associated with ant nests (Figure 3). The Figure 3a shows that these amounts under the tufts associated with ant nests are located above the equation right (THd, TAs, and TLs) and those not associated with ant nests are below it (tHd, tAs, and tLs) (Figure 3a).

In the whole of the three habitats studied, *H. diplandra* tufts associated with ant nests recorded the highest overall amount of SOM (Table 1) with an average amount of N (THd: OM = 2.28%; N = 0.58%). In contrast, *A. schirensis*

(TAs: OM = 1.78%; N = 0.61%) and *L. simplex* (TLs: OM = 1.85%; N = 0.63%) tufts associated with ant nests recorded the highest amounts of N and a low amount of OM (Figure 3a).

In shrubby savannah, *H. diplandra* tufts associated with ant nests recorded the highest amount of SOM (S1THd: OM = 3.15%); and also the highest amount of N under *L. simplex* tufts associated with ant nests (S1THd: N = 0.63% and S1TLs: N = 0.63%).

The lowest amount of N was recorded under *L. simplex* tufts not associated with ant nests (tLs: N = 0.42%). *A. schirensis* tufts associated with ant nests (TAs: OM = 2.25%; N = 0.56%) recorded the lowest amount of OM and a medium amount of N (Figure 3b).

In clear grassy savannah, *H. diplandra* tufts associated with ant nests recorded the highest amount of SOM and an average amount of N (S2 THd: OM = 1.9% and N = 0.56%). In contrast, *L. simplex* tufts associated and those not associated with ant nests had the highest amount of N (S2TLs N = 0.7% and S2tLs N = 0.7% respectively) and the lowest OM. *A. schirensis* tufts associated and not associated with ant nests recorded average amounts of OM and tufts not associated with ant nests recorded the lowest amount of N (Figure 3c).

In transitional grassy savannah, *H. diplandra* and *L. simplex* tufts associated with ant nests had the highest SOM and mean N rate but this amount is the lowest under tufts not associated with ant nests in this habitat (S3THd and S3TLs respectively: OM = 1.8% and N = 0.56%).

A. schirensis tufts associated and those not associated with ant nests recorded the highest amount of N (respectively: S3 TAs = 0.7% and S3 tAs = 0.7%).

However, *A. schirensis* tufts not associated with ant nests recorded the lowest amount of OM in the habitat (Figure 3d).

4 | DISCUSSION

4.1 | Amount of nitrogen, organic matter, and carbon recorded in the soil according to savannah types and grass species studied in Lamto

The habitat type could influence ants action on soil physical and chemical properties due to environment heterogeneity. OM amount is higher under grasses tufts in all habitats. This could be due to the accumulation of root debris and dead leaves under grasses tufts not associated with ant nests and in addition bioturbation action and transport of building materials under tufts associated with ant nests.

Furthermore, this result corroborates with that of Ouattara (2020) and Ouattara et al. (2021) that showed microorganisms activity was more intense beneath perennial grasses

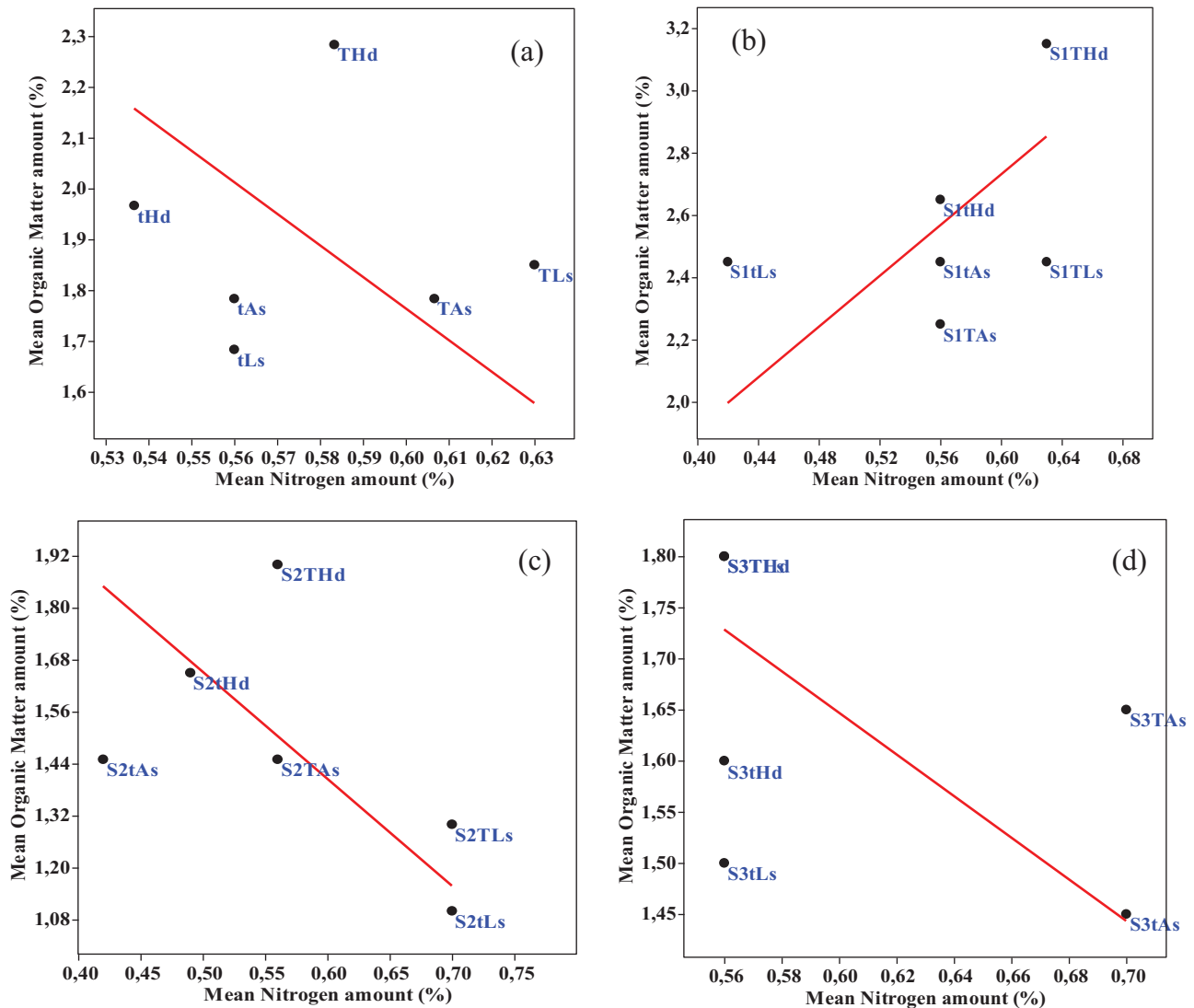


FIGURE 3 Mean amount of nitrogen and organic matter from the soil according to the three perennial grasses associated and not associated with ant nests. **(a)** In the all three savannah types; **(b)** Shrubby savannah. **(c)** Clear grassy savannah; **(d)** Transitional grassy savannah. **TAs**, *Andropogon schirens* tufts associated with ant nests; **tAs**, *Andropogon schirens* tufts not associated with ant nests; **THd**, *Hyparrhenia diplandra* tufts associated with ant nests; **tHd**, *Hyparrhenia diplandra* tufts not associated with ant nests; **TLs**, *Loudetia simplex* tufts associated with ant nests; **tLs**, *Loudetia simplex* tufts not associated with ant nests.

associated with ant nests than those not associated with ant nests.

Ant nests associated with grasses tufts in shrubby savannah could be more conducive to SOM availability than in clear grassy savannah and transitional grassy savannah.

The high amount of OM and C in the soil under *H. diplandra* tufts associated with ant nests could be the reason for the growth and increased microorganism populations. The amount of N, recorded under this grass is also important (Ouattara, 2020 & Ouattara et al., 2021). The amount of N, OM, and C recorded under *H. diplandra* tufts could come from external soil substrates, rejected by ants during bio-turbation, transport of leftover organic material (food scraps

or building material), nest cleaning, and bacterial corpses degradation.

In clear grassy savannah, N measured under *L. simplex* tufts associated and not associated with ant nests was equal. During sampling, grasses tufts not associated with ant nests were collected just upstream of an *L. simplex* savannah. Indeed, the studies of Rambelli (1971), showed that *L. simplex* has hydromorphic characteristics. In addition, these studies have shown that *L. simplex* grass established a relationship in symbiosis with the mycorrhizal fungi. These fungi have as a food source the dead roots and the exudates. This could suggest that *L. simplex* tufts may have two sources of nutrients: ants and arbuscular mycorrhizal fungi. These mycorrhizal fungi form

an external and permanent mycelial network, linking plants together and allowing them to better exploit nutrient resources (Grime et al., 1987; Sanon, 2005). These fungi colonize the roots of most plant species and thus improve the acquisition of mineral resources from their hosts. In addition, *L. simplex* is the grass least associated with ant nests (Ouattara et al., 2018) and grows best in hydromorphic environments. This could explain the low amount of OM and C in the soil under this grass. Furthermore, studies by Rambelli (1971) showed that mycorrhizal fungi are much more abundant in savannahs, especially under *L. simplex* tufts. In addition, these fungi have as food sources dead roots and exudates. The soil pH is close to neutral.

According to Edoukou (2015), the pH is slightly acidic under the shrubby and grassy savannas. In the transitional grassy savannah, the OM amount in the soil is high under *H. diplandra* (OM, C) and *L. simplex* tufts. This could be due to the soil type.

4.2 | Amount and availability of soil nitrogen under grass tufts in habitats types

Overall, the results of this study showed that the distribution of soil N content under grass tufts depends on savannah type and grasses species. *A. schirensis* recorded the highest amounts in transitional grassy savannah; *H. diplandra* recorded the highest amounts in shrubby savannah. And *L. simplex* recorded the highest amounts in clear grassy savannah. This variation in N amount could be due not only to environment heterogeneity but also to microorganisms' adaptation to OM quality available under grasses tufts.

4.3 | The C/N ratio

The C/N ratio between the quantity of C and N makes it possible to judge OM ability to decompose more or less rapidly in the soil.

$C/N < 15$: N production, and decomposition speed increases; it is at its maximum for a C/N ratio = 10 (Giroux & Audesse, 2004).

The results showed that all three types of savannah have a C/N ratio below 10.

Indeed, when the C/N is lower than 10, microorganisms actively transform OM to make mineral elements bioavailable.

In all three savannah types, the C/N ratio is below 10, which could reflect a strong mineralization of OM that makes a satisfactory nutrient amount available to plants. Ants, therefore, bury OM and bring to the surface a fragmented soil in small particles containing nutrients available for the growth of grass tufts.

5 | CONCLUSION

This study shows that in the three savannah types studied in Lamto, OM, C, and N amounts are higher in soil under grass tufts associated with ant nests than those not associated with ant nests.

Soil OM and C amounts are higher in shrubby savannah than in transitional grassy savannah and low in clear grassy savannah. N is in equal amounts in all three habitats.

In clear grassy savannah, the N available under *L. simplex* comes from a source of supply in addition to that of ant nests; but also due to the low activity of soil microorganisms under this grass.

In transitional grassy savannah, OM amount is high under *H. diplandra* and *L. simplex* tufts associated with ant nests. Thus, OM, available C, and N assimilated by perennial grasses show that ants also improve nutrient quality.

The C/N ratios less than 10 indicate high OM mineralization under grass tufts associated with ant nests. This provides the plants with an adequate supply of nutrients.

AUTHOR CONTRIBUTIONS

Kaly Ouattara: Conceptualization; software; data curation; investigation; writing –original draft. **Kolo Yeo**: Conceptualization; software; data curation; investigation; writing –original draft. **Lombart M. M. Kouakou**: Conceptualization; software; data curation; investigation; writing –original draft. **Wouter Dekoninck**: Conceptualization; software; data curation; investigation; writing –original draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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