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Sampling techniques for the optimal measure of macromycetes diversity in the Soudano-Guinean ecozone (West Africa)

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Fungi range among the most important organisms in the world thanks to their ecological roles in the ecosystems and their socio-economic importance for human beings. Still, the global fungal species richness is full of uncertainties as evidenced by various estimates. Estimating fungal species richness and diversity is raising many questions related to the sampling effort (in space and time). This study aims to determine the effect of the sampling technique on the diversity measure and natural productions of wild macromycetes in the soudano-guinean forests. Six concentric plots different in the sizes and in the shape were installed in the Isoberlinia doka dominated woodlands. We recorded the number of fruit bodies and the fresh biomass of the species for each plot from June to October 2017. Dendrometric parameters were assessed by counting individual trees with dbh ≥ 10 within plots. A mixed linear model was applied through lme4 package to assess the influence of the size and shape of the plots on the abundance and species richness of macromycetes. An analysis of variances was used to assess the influence of the size and shape of the plots on the fresh biomass. Results showed that the abundance is higher in square plots at sizes 400 and 625m² but the biomass does not different significantly from one shape of plot to another (P = 0.228). Fresh biomass is higher in rectangular plots compared to the square and circular ones. Highest values of specie richness are obtained in the 25 m² for circular plots.

Key words: Diversity indices, sampling technique, macromycetes, natural production, Soudano-Guinean zone, Benin.

INTRODUCTION

Forest ecosystems of the world contain an extraordinary biological diversity. This biodiversity includes all the processes and functions that contribute to the maintenance of life and offering essential services to

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human beings (Tiecoura et al., 2015). Biodiversity measurement utilizes some techniques and methods that are specific to each taxonomic group of organisms, but the overall output depends upon the sampling effort invested. For each taxonomic group, sampling techniques (Picard, 2007) are defined by considering the specific objective of the study, the geographical and temporal heterogeneity and the human resources required to perform the sampling (Rondeux and Lecomte, 2002). Many others parameters related to the characteristics of the sampling units also should be considered. These include the nature, size, form and sometimes the orientation of the plots. Indeed, the minimal area approach is often used to identify the size of the sampling units in order to characterize plant communities (He and Legendre, 2002). The success of these techniques in the study of plant communities for example depends upon very precise choices of the shape, sizes and nature of the sampling units; whereas, the sampling frequency can vary from one observation per year (Straatsma et al*.,* 2001, Straatsma and Krisai-Greilhuber, 2003) to a weekly interval (Al-thani, 2010). In addition, it is shown that the size of the plots is inversely proportional to the precision of the estimation (Shearer et al*.,* 2007; Salako et al*.,* 2013; Houéto et al*.,* 2013).

Yet, there is no precise standardized technique for optimal measure of mycodiversity in the tropics (Vanié-Léabo et al*.,* 2017; Milenge et al*.,* 2018). Different sampling techniques are used to make an inventory of the fungal communities in an ecosystem (Martínez-Peña, 2008), ranging from opportunistic sampling for inventory purposes to a plots-based survey when it comes to monitoring. Sampling techniques for mycodiversity measures are either executed within permanent plots of square, circular or rectangular shapes, or based on transects of variable sizes (Yorou et al*.,* 2001; Mueller et al*.,* 2004; Hawksworth, 1991, 2003; Vanié-Léabo et al*.,* 2017; Hayward et al*.,* 2015).

Permanent plots of 900 to 2500 m_2 have been used in some mycodiversity studies in tropical Africa (Vanié-Léabo et al*.,* 2017; Milenge et al*.,* 2018; Yorou et al*.,* 2001; Kangas and Maltamo, 2006). Still, because the size of permanent plots differs and that the frequency of plots visited has changed from one investigation to another, it becomes difficult to make reliable comparisons even for similar ecosystems. Here we test the influence of plot size and shape on the species richness and fresh biomass of macromycetes in a tropical ecosystem.

MATERIALS AND METHODS

Study area

The Okpara woodland forest is located in the Northern zone of Benin, 15 km from Parakou City between 9° 18' and 9° 22' North latitude, 2° 33' and 2° 37' East longitude (Figure 1). It covers an area of 33,000 ha and peaks at an altitude of 295 m. It is a typically tropical climate with one rainy season (from May to October) that strongly contrasts with one dry season. The soil texture in this area is sandy, sandy-clay or loamy in places (Youssao et al*.,* 2000). The vegetation is dominated by *Isoberlinia doka* and *Uapaca togoensis*, two forest trees typical for the soudano-guinean zone (White, 1983).

Collection, identification and preservation of specimens

Permanent plots of different shape and sizes were installed in one single vegetation type dominated by *I. doka*. Three shapes were used: Square (K), Circular (C) and Rectangular (R). For each shape, 6 concentric plots (25, 100, 225, 400, 625 and 900 m_2) were installed. Plots are labeled according to their shape, notably; K1 to K6 for square, C1 to C6 for circular and R1 to R6 for rectangular plots. The Geographic Positioning System Garmin Etrex 20™ was used for georeferencing each plot. The surveys were performed at a fixed frequency of 48 h between two surveys during the mycological season from June to October 2017. Thus, 45 visits were carried out per plot, which corresponds to a total of 810 surveys for all 18 plots. During surveys, all fruit bodies of any Ectomycorrhizal (EcM) fungi were harvested and arranged according to the sampling date, plots shape and size. The samples were then transported to the laboratory. The specimens were identified by means of a compound microscope type Leica LMD6 Microdissection Systems available at the Laboratory of Ecology of Botany and Plant Biology (LEB) of the University of Parakou, using some field guide manuals (De Kesel et al*.,* 2002, 2017, Eyi-Ndong et al*.,* 2011) and a catalogue with more than 1000 color pictures. After counting the number of carpophores and measuring the fresh weight with a Sunto™ electronic balance (0.01g precision), the specimens were assigned a unique voucher number. The best specimens of each species were sorted and placed in sieves before final preparation for drying using the Stocklii Dorrex™ brand electric dryer. Drying was done at a temperature between 40 and 45°C for 24 h. Dried specimens were preserved in plastic bags type minigrip and deposited at the Mycological herbarium of the University of Parakou and the dried specimens were kept desiccated in the laboratory.

Data processing and analysis

Dendrometric parameters

The floristic homogeneity of the site was tested by measuring the dendrometric parameters of the trees in each shape of the plots.

Tree density: This is the average number of trees per plot. It is expressed in stems / hectare and mainly concerns trees with a dbh ≥ 10: Ni = n / s, where n: the total number of trees with dbh ≥ 10 cm in the plots and s: the area of the plots (block 6 plots) (ha).

The basal area: It expresses the sum of the basal sections of all trees of dbh ≥ 10 cm found in each block of plots. It is expressed in m^2 / ha and is represented by the formula below:

$$G = \frac{\pi}{4s}\sum_{i=1}^{n} di^2$$

n: the total number of dbh trees ≥ 10cm in the plots; di: diameter of the tree i (m) and s: area of the plot (s = 0.01 ha).

Mycological data

The species richness, the number of fruit bodies, as well as

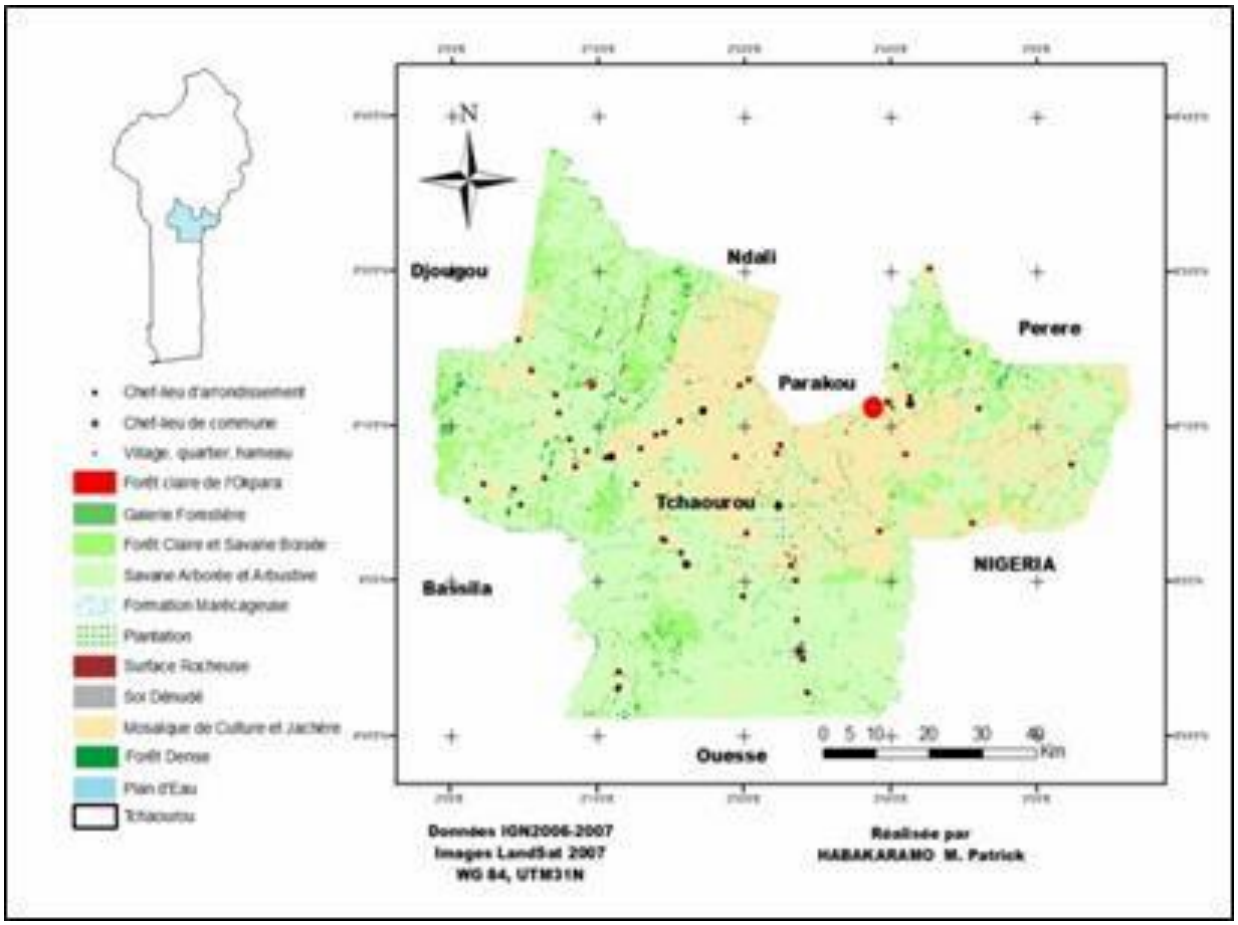

Figure 1. Okpara woodland forest (red point) in Tchaourou municipality.
Source: Authors.

the fresh biomass of each species is computed per sampling date, shape and size of the plot. Time series analysis was applied to assess the evolution of abundance, fresh biomass and specie richness by shape of plots as a function of time during the whole fruiting season. The statistical software R version 3.4.2 R was used (Oksanen et al*.,* 2017; R Core Team, 2017). The rank-frequency curves of the sampled species were constructed for each shape of the plots in order to choose the appropriate diversity index to assess the diversity of macromycetes in the ecosystem. The Shannon diversity index (H) and Pielou equitability index (P) were calculated using the vegan package (Oksanen et al*.,* 2017). Subsequently, the specific estimator (Chao1) was calculated under the Biodiversity R package (Kind and Coe, 2005). To assess the influence of the size and the shape of the plots on the number of fruit bodies and species richness of macromycetes, a Generalized Linear Mixed Effect Model (GLMM) was applied through the lme4 package™. To choose the appropriate minimum model, five models were compared on the basis of the Akaike Information Criterion (AIC). The chosen model was one with the lowest AIC. Analysis of variance (ANOVA) was used to assess the influence of the size and the shape on the fresh biomass of macromycetes by applying the statistical software R version 3.4.2 (R Core Team, 2017; Bates et al*.,* 2015). The temporal sampling effort was assessed by constructing the species accumulation curves as a function of the number of surveys according to the rarefaction method for each shape by using the vegan package (Walker et al*.,* 2008). The certification of floristic homogeneity of the site and the relationship between the dendrometric parameters and the fungal variables (number of fruit bodies, fresh biomass and specie richness) was made by the Spearman's correlation test in statistical software R version 3.4.2 (R Core Team, 2017).

RESULTS AND DISCUSSION

Specie richness of macromycetes by shape and size of the plots

During 19 weeks of investigations, 90 species of macromycetes sorted into 41 genera were harvested. The most represented genera in the ecosystem are essentially *Amanita* (11 species), *Russula* (9 species), *Termintomyces* (7 species) and *Lactifluus* (6 species). In particular, 63 species accommodated into 26 genera were listed in the square plots against 62 species accommodated into 32 genera within the circular and 60 species sorted into 25 genera in the rectangular plots. A large temporal variation of the specie richness of macromycetes among the plots was observed throughout

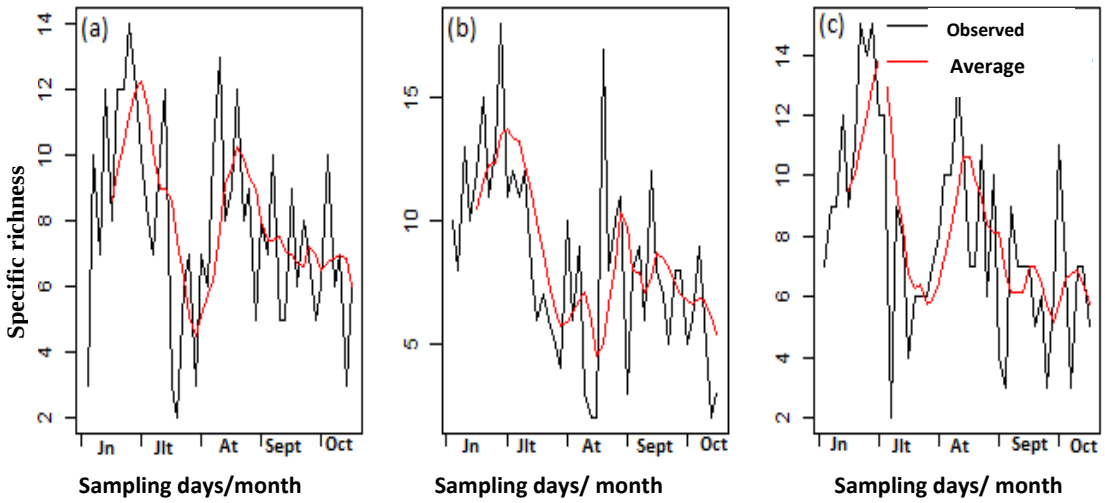

Figure 2. Temporal variation of the specie richness of macromycetes by the shape of plots: (a) circular, (b) square, (c) rectangular.
Source: Authors.

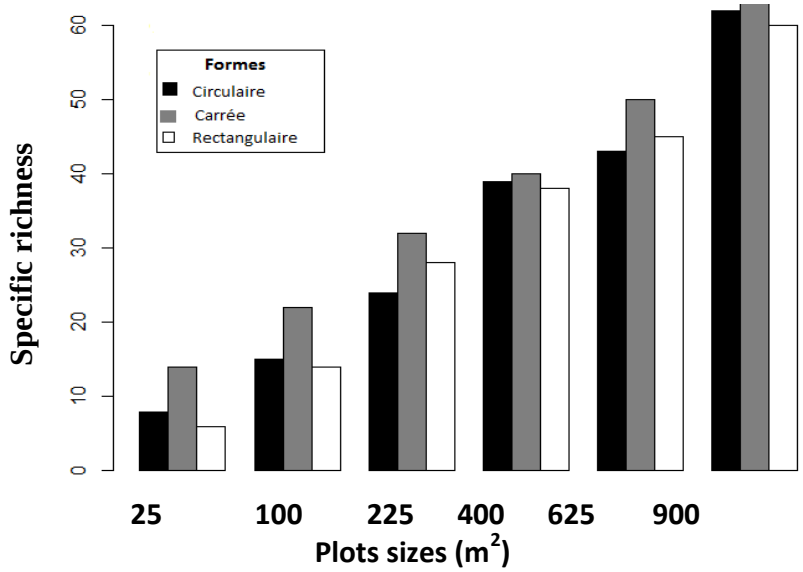

Figure 3. Spatial variation in the species richness of macromycetes by size and shape of plots.
Source: Authors.

the mycological season. This taxonomic richness recorded is smaller than those reported from similar but close to other forest ecosystems. Yorou et al. (2001), identified 126 EcM species after various surveys in different regions of Benin. Furthermore, in the Comoé National Park in the Ivory Coast, Vanié-Léabo et al. (2017) mentioned 123 species distributed in 23 genera; while Kamou et al. (2017) identified 179 species and 52 genera in the Fazao - Malfakassa National Park in Togo. Although these variations in species richness among studies can be due to the years covered by each of those surveys, it can obviously be explained by climatic variability, soil composition and also differences in host tree composition.

The standardized average curve in red (Figures 2 and 3) shows that maximal species richness is recorded in June with a peak observed towards the end of June (25 to 30); whilst lowest richness is recorded in July (15 to 25). We found out that the species richness increases with the size of the plots regardless of the shape (Figures

Table 1. Variation in the specie richness of macromycetes depending on the shape and the size of the plots.

	Coefficient (β)	Error	Statistics (z)	Probability
Constance		0.1587	-0.4940	0.6213
FC	**2.0822**	0.1595	13.0540	**<0.0001**
FR	**0.2587**	0.2002	1.2920	**0.1962**
225m²	0.8979	0.1784	5.0320	<0.0001
25m²	**-1.0761**	0.2983	-3.6080	**0.0003**
400m²	1.4785	0.1667	8.8710	<0.0001
625m²	1.8215	0.1621	11.2360	<0.0001
900m²	2.1101	0.1593	13.2500	<0.0001
FC:225m²	-0.8979	0.1936	-4.6380	<0.0001
FR:225m²	-0.2048	0.2409	-0.8500	0.3953
FC:25m²	**1.0761**	0.3076	3.4990	**0.0004**
FR:25m²	-0.0225	0.3985	-0.0560	0.9550
FC:400m²	-1.4785	0.1828	-8.0870	<0.0001
FR:400m²	-0.1839	0.2237	-0.8220	0.4108
FC:625m²	-1.8215	0.1787	-10.1950	<0.0001
FR:625m²	-0.2735	0.2179	-1.2560	0.2092
FC:900m²	-2.1101	0.1761	-11.9840	<0.0001
FR:900m²	-0.2235	0.2133	-1.0480	0.2945

Source: Authors.

2 and 3). Whatever the size of a square plot is, higher specie richness was recorded in square-shaped plots, while the low scores are observed on the rectangular plots.

Effect of the shape and size of the plots on the species richness of macromycetes

The GLMM results (Table 1) show that the species richness of macromycetes is significantly higher in circular plots (β = 2.0822, p <0.0001) compared to square plots, but there is no difference between the rectangular (β = 0.2587, p = 0.1962) and square plots. Considering the sizes, the richness is significantly lower (β = -1.0761, p = 0.0003) at 25m² for all three shapes of plots. The interdependent effect between the size and the shape of the plots indicate that the greatest richness was noticed for the circular shape compared to the square one (β = 1.0761, p = 0.0004). Above this size, the richness becomes significantly low for circular plots. Guinberteau and Courtecuisse (1997) indicated that the number of fungal taxa collected depends on the sampling period and the size of the area (plot) as well as the survey frequency during the sampling campaign.

Diversity of macromycetes by shape and size of plots

Our results indicate the predominance of rare species than abundant in all blocks of plots (Figure 4). The Shannon diversity index was used for the specific diversity and Piélou's equitability index for the distribution of fruit bodies within the species (Table 2). The Shannon index shows that as the size of the plots increases, the diversity of species becomes more important regardless of the shape of the plots with a high diversity recorded at 900 m². Thus, circular plots harbor the highest diversity index (3.05) for 900 m_2 followed respectively by the rectangular (2.95) and the square plots (2.85).

Species diversity estimated in the different plots is calculated with Chao 1 index from the effective specie richness observed in the field (Table 3). The specie richness and the estimated diversity are higher in square-shape plots. Species recovery from our sampling effort is higher in circular (84.64%) compared to rectangular (83.20%) and square (64.81%) plots. This attests that the fruit bodies are less distributed in the circular plots compared to the other two forms.

Abundance of macromycetes by shape and size of plots

We collected 7499 fruit bodies from all plots during the whole mycological season. Among these, 2782 were recorded in square plots, 2584 in rectangular and 2133 in circular plots. The standardized average curve of the abundance of fruit bodies shows almost the same dynamic regardless the shape and size of the plot (Figure

Table 2. Shannon (H) and Piélou equitability (P) index of the different forms and sizes of the plots.

Sizes (M^2)	Circular plots		Square plots		Rectangular plots	
	H	P	H	P	H	P
25	1.29	0.62	1.39	0.63	1.29	0.57
100	2.02	0.75	2.11	0.68	2.36	0.6
225	2.17	0.68	2.26	0.65	2.5	0.6
400	2.51	0.69	2.44	0.66	2.68	0.63
625	2.61	0.69	2.57	0.66	2.92	0.68
900	3.05	0.74	2.85	0.69	2.95	0.75

Source: Authors.

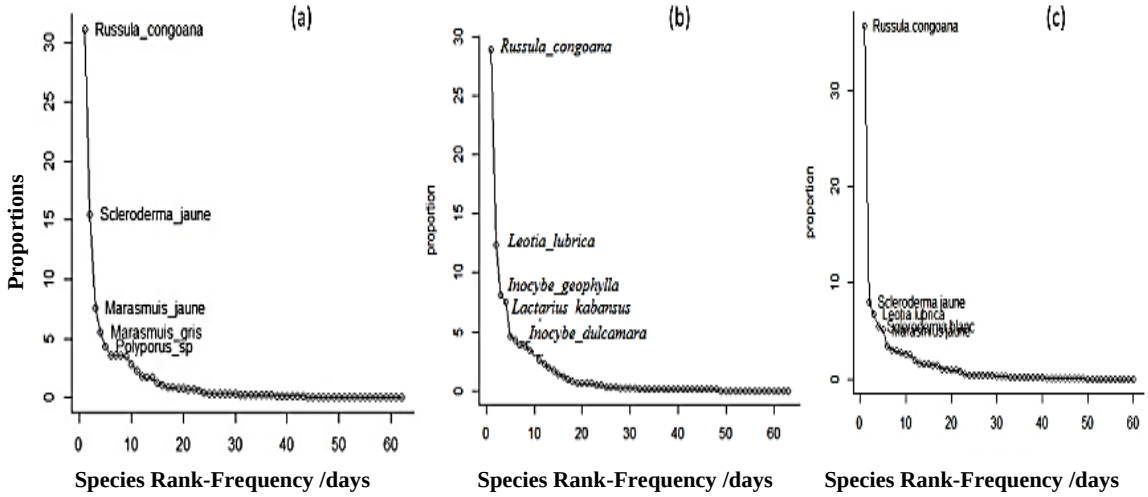

Figure 4. Frequency rank of species by form of plots: (a) Circular, (b) Square, (c) Rectangular.
Source: Authors.

Table 3. Specie richness observed, estimated diversity and recovery within various forms of plots.

Forms of plots	Observed Specie richness	Estimated specific Diversity, Chao1	Recovery of samples (%)
Squares	63	97.20	64.81
Circulars	62	73.25	84.64
Rectangulars	60	72.11	83.20

Source: Authors.

5). June constitutes the period of highest abundance with a peak observed on mid-June (10 to 15) for all plots. The other months (July to October) recorded lower abundance.

Effect of the shape and size of the plots on the species abundance

According to the GLMM, the results show that the date of the survey impacted on the abundance at 50% and accounts for the random effect. The fruit bodies abundance is significantly higher in square plots (β =0.1939, p = 0.0297) compared to circular plots, but this difference was not significant between rectangular and square plots (Table 4). However, our results also indicate an interdependent effect between the size and the shape of the plots. The higher abundance observed in square plots compared to circular plots is observed especially for 400 m² (β = 0.2769, p = 0.0052), and 625 m² (β = 0.2705,

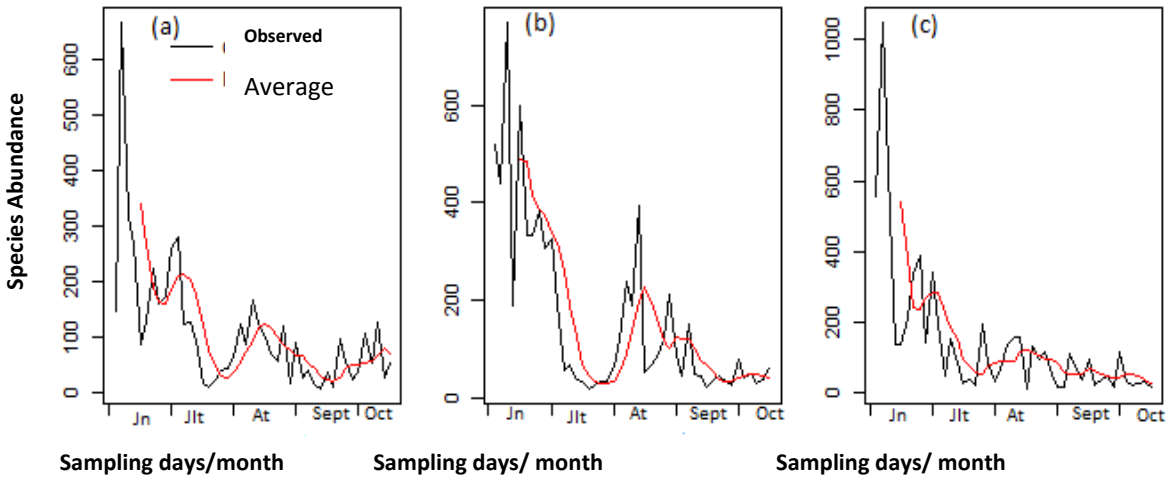

Figure 5. Temporal variation of fruit bodies abundance in the plots: (a) Circular, (b) Square, (c) Rectangular.
Source: Authors.

Table 4. Variation of macromycetes abundance by shape and size of the plots.

	Coefficient (β)	Error	Statistics (z)	Probability	Average
Constance	1.1968	0.1503	7.9600	<0.0001	18.22222
FK	**0.1939**	0.0892	2.1700	**0.0297**	26.09259
FR	0.1037	0.0911	1.1400	0.2550	23.90000
225 m²	0.7546	0.0801	9.4200	<0.0001	13.4666667
25 m²	-1.3228	0.1440	-9.1900	<0.0001	1.39259267
400 m²	1.3267	0.0743	17.8600	< 0.0001	25.474074
625 m²	1.6112	0.0723	22.2700	< 0.0001	34.9111113
900 m²	2.2316	0.0695	32.1100	< 0.0001	55.548148
FK:225 m²	0.2026	0.1067	1.9000	0.0576	16.088889
FR:225 m²	0.1166	0.1095	1.0600	0.2870	13.488889
FK:25 m²	-0.3158	0.2069	-1.5300	0.1270	1.200000
FR:25 m²	0.0760	0.1958	0.3900	0.6982	1.622222
FK:400 m²	**0.2769**	0.0992	2.7900	**0.0052**	30.711111
FR:400 m²	0.2210	0.1014	2.1800	0.0294	26.533333
FK:625 m²	**0.2705**	0.0968	2.7900	**0.0052**	40.555556
FR:625 m²	0.3136	0.0987	3.1800	0.0015	38.688889
FK:900 m²	0.0717	0.0937	0.7700	0.4441	61.822222
FR:900 m²	0.0881	0.0957	0.9200	0.3568	57.422222

Source: Authors.

p = 0.0015) sizes. The same results are noted by Engeman et al. (1994), Kangas and Maltamo (2006), mycological studies, and in several floristic studies reported by Houéto et al. (2013) and Salako et al. (2013) which recommend square plots.

Natural production of macromycetes

The natural production of all species harvested amounts to 92.95 kg/ha per year during the mycological season. The rectangular plots with the highest fresh biomass represent 95.53 kg/ha per year, 94.30 kg/ha and 89.04 kg/ha per year, respectively, for the square and circular plots (Figure 6). Highest fresh biomass was recorded in June (10 to 15) with a highest peak in the circular and rectangular plots and at the end of June (25 to 30) for the square plots. Our results are similar to those mentioned by Yorou et al. (2001) in Wari-Maro forest, but different than those noted by Vanié-Leabo et al. (2017). The

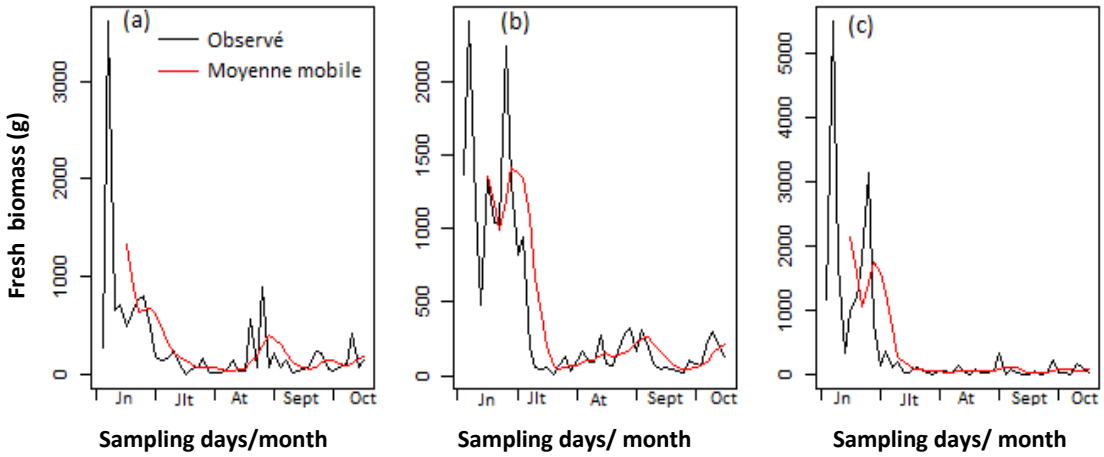

Figure 6. Temporal variation of the fresh biomass of macromycetes by form of the plots: (a) circular, (b) square, (c) rectangular.
Source: Authors.

Table 5. Variance test of the fresh biomass of macromycetes within the different shapes and sizes of plots.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Shapes	2	73802	36901	1.482	0.228
Sizes	5	3128783	625757	25.140	<2e-16
Shapes: Sizes	10	24522	2452	0.099	1.000
Residuals	792	19713743	24891		

Source: Authors.

difference observed in natural production of macromycetes in this study can be explained by the biotic and abiotic characteristics of the habitats as also noted by Ducousso et al. (2002) and Bonet et al. (2004). In accordance with Straatsma et al. (2001), the fresh biomass and specie richness of macromycetes show several fluctuations from one year to another and it is subjected to habitat fragmentation and climatic fluctuations. In addition, natural production as well as number of fruit bodies shows strong intra-annual fluctuation as already reported in similar ecosystems (Yorou et al., 2001, 2017). In the Soudano-guinean zone, on one hand, macromycetes are very abundant from mid-June to the end of the same month. On another hand, macromycetes are very few (abundance, specie richness and fresh biomass) between the end of July and the end of September during the mycological season.

Effect of the shape and size of the plot on the fresh biomass of macromycetes

According to the ANOVA test, only the size of the plot has a significant effect on the fresh biomass of macromycetes (Table 5). At identical size, the fresh biomass does not differ significantly from one shape of plot to another (p = 0.228); whereas, it does vary significantly according to the size of the plots regardless of the shape (p = <2e-16).

Optimal mycodiversity measure: Temporal sampling effort

Figure 7 illustrates the species accumulation curves as a function of the number of surveys (frequency of visits) within the plots. These curves have almost the same temporal sequential trends. The accumulation curves are all ascending and have not reached an asymptote; the temporal sampling effort was not satisfactory because the specie richness increases during the new visit. As noticed by Vanié-Léabo et al. (2017) and Tedersoo et al. (2007), the species accumulation curves, performed as a function of frequency of visits, did not reach a horizontal asymptote. This would indicate the highest species richness of the study area. It means that the visit frequency is an important factor for estimating the species richness of fungal communities in order to have a standardized temporal sampling effort. Many other studies demonstrated that the mycodiversity studies

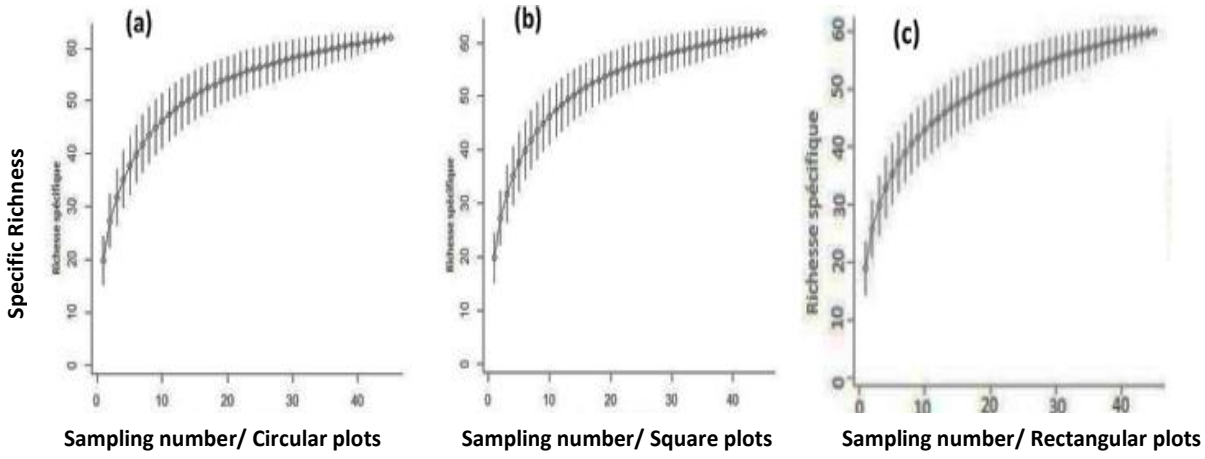

Figure 7. Accumulation and rarefaction curve of macromycetes per sampling number for each form of plots.
Source: Authors.

Table 6. Correlation matrix between floristic and fungal parameters.

Floristic parameter`	Macromycetes parameter		
	Species richness	Abundance	Fresh biomass
Trees density	p = 0.01344	p = 0.0609	p = 0.1434
Basal area of trees	p = 0.02434	p = 0.09756	p= 0.2626

Source: Authors.

require several years (3, 8-12, 10 and 21 years, etc.) of sampling to reach the asymptotic curve and that this should be conditioned by standardization of sampling techniques (Guinberteau and Courtecuisse, 1997; Lodge et al*.,* 2004; Mueller et al., 2004; Smith et al*.,* 2011; Straatsma et al*.,* 2001, Straatsma and Krisai-Greilhuber, 2003).

Effect of dendrometric parameters on fungal communities

We recorded 18 tree species ≥ 130 cm in height in the rectangular plots against 17 and 13 species within respectively the square and circular plots. The total density of trees in our inventory plots was 4,811 stems/ha, of which 488 stems/ha were in rectangular plots, 478 stems/ha in square and 333 stems/ha in circular plots. As for the total basal area, it was 943.36 m_2/ha; or 977.45 m_2/ha for rectangular plots, 923 m_2/ha and 647.21 m_2/ha, respectively, for square and circular plots. It therefore appears that the density and the basal area differ from one form of plot to another. The results of Spearman's test shows that there is no correlation between the dendrometric parameters (density and basal area of trees) and those of fungi (abundance and fresh biomass of macromycetes) with the exception of species richness (Table 6). Indeed, the species richness is correlated with the density (p = 0.01) and the basal area (p = 0.02) of trees (Spearman test).

Conclusion

The main results of this research showed that the abundance is higher in square plots at sizes 400 and 625 m^2, but the biomass does not differ significantly from one form of plot to another (p = 0.228). Fresh biomass is higher in rectangular plots compared to the square and circular ones. The highest values of species richness were obtained in the 25 m^2 for circular plots. The information and data collected within this research will allow, in addition to extend the scientific knowledge on fungi (macromycetes) biodiversity, to provide a good methodology and appropriate sampling unity and frequency as well as planning and management of the soudano-guinean tropical forest heritage. We would like to investigate and monitor the aboveground mycodiversity of the Okpara woodland forest for several years using constant sampling units and increasing the number of surveys. It is also desirable to further integrate the floristic, soil and microclimatic parameters to identify all

the factors that govern the fruiting of macromycetes in the soudano-guinean zone.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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