Introduction

Depending on the context, plants have different strategies in the forest. Either they allocate their resources to the growth of their tissues, or they defend themselves against pests (Bryant et al. 1983; Herms & Mattson 1992, Barrere and al., 2019[1]). Herbivory is one of these pests. Over the last decades, ungulates populations have increased in western Europe. Since then, the impact of certain cervids on plants has been largely studied (Rackham, 2008; Bernard and al., 2017; Boulanger and al. 2018; Linnell and Zachos, 2011).

On a mixed forest ecosystem, *browsing* ungulates have direct and indirect effects *on* plants communities composition. They modify plant functional traits and species richness (Hester et al., 2006, Mason et al., 2010 ; Boulanger and al., 2018). Consequently, the palatable species eaten by cervids (*lead to) cause* the homogenization of the forests community by affecting the local extinction process and they *reduce* the inter-specific competition (Ronney, Wiegmann, Rogers, & Waller, 2004, Hester et al., 2006, results also confirmed by Boulanger 2018). They disturb the ecological role of those forests' ecosystems. Also, if the pressure of herbivory is too high, it can decrease or modify *the species richness in a community* (Côté, Rooney, Tremblay, Dussault, & Waller, 2004).

In the literature, the diversity of species *has been* used to analyze the evolution of the plants communities in presence or absence of wild ungulates. But the use of other characteristics of the plantscan be useful to investigate the impact of browsing and better understand the response of plant communities (Rooney & Waller, 2003). Some studies have shown that the density of one species increases. In the meantime, the diversity of the species decreases due to the browsing effect (Bernhardt-Römermann *et al.*, 2015). Besides, *using* functional traits can precise the impact of each species of ungulate on different forest layers. For example, the presence of wild boars is *more* favorable to therophytes species than other growth modes when the soil is disturbed (Boulanger and la., 2018). Deer and wild boars are dispersal agents for epizoochorous species which can enrich plant communities through the increase of species immigration (Albert et al., 2015; Heinken & Raudnitschka, 2002). These results show that each ungulate species can have different impacts on regeneration processes and the colonization of plants (Bradshaw & Waller, 2016). For example, species of Quercus are impacted in several steps of their life cycle and differently according to the cervid species. Acorns can be eaten by deer and wild boar, so less acorns will be available for the forest renewal. Also, seedlings can be eaten by red and roe deer but also dug up by wild boars that turn over the soil in search of food. Red and roe deer rub against the willows (Saïd and al. 2019).

Also, lots of studies observed that browsing has a different impact on overstory and understory forest communities (Boulanger and al., 2018; Faison et al., 2016). For example, some studies show that browsing pressure decreased species richness on trees and shrub layers but that profits to herbaceous layers which benefit from light penetration and increase their biomass (Gill and Beardall, 2001). Overstory layer can have a diluted or strengthened effect on understory diversity (Bernhardt-Römermann *et al.*, 2015).

Considering all these observations, we aim to address the following questions. What is the effect of total exclusion and selective exclusion of 3 species of ungulates (wild boars, red and roe deer) on herbaceous, low shrub, high shrub and tree layers in deciduous forest ecosystems? Is there an effect of site, and/or an effect on ungulates on these 4 different layers to explain the composition of the plant community with functional traits?

For our first hypothesis, we believe that species richness and light-preferring plants would be more important outside the exclosure and selective exclosure in the herb layer. For our second hypothesis, we think that the proportion of species with vital organs underground (chamaephyte, hemicryptohyte) would be more important in selective exclosure and outside the exclosure. For our fourth hypothesis, we think that the proportion of epizoochorous species would be more important outside the exclosure than in selective enclosures and pens. Finally, for our fourth hypothesis, we think that the phanerophyte species would be more important in the high shrub and tree stratum but that the specific richness would be less important than in the other strata in the presence of ungulates.

To answer these questions, we compared the effect of selective fencing of roe deer, red deer and wild boars separately, the presence of all these 3 species together and their absence on forest layer: herbaceous, low shrub, high shrub and tree layer on three different sites in northern east France between 2016 and 2022. Permitting one species to get in a patch can be useful to see precisely the impact of this species on forest layers, as the fencing process has been particularly useful for other studies (Waller, 2014, Boulanger and al., 2018).

Materials and methods

Study Area and Experiment Design:

The data were collected from three deciduous production forests located in northeastern France (figure 1). The first site, Arc-en-Barrois (Arc), is in the Marne department. The area is home to red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and wild boar (*Sus scrofa*). The substrate here is composed of limestone. The second site, Trois-Fontaines (TF), is in haute-Marne and also composed of limestone, but red deer are absent from this area. The sampling began in 2013 at LPP, in 2016 at TF, and in 2016 at Arc. Regularly employed for sampling at these three sites were one engineer, two permanent technicians, two non-permanent technicians, and students. At Arc, we compared 12 pairs of fenced/unfenced plots and 4 pairs at TF.

Vegetation Data

At Arc, we used data sampled in 2017, 2018, 2019, 2021, and 2022. For TF, there were samples in 2016, 2017, 2018, 2019, 2021, and 2022, and for LPP, there were samples in 2016, 2017, 2018, 2021, and 2022. From the EFFORT device, we used the data from 9056 quadrats for this study. In the herbaceous layer, *Carpins betulus* was the dominant species in terms of percentage of presence in all the quadrats at Arc and TF (only outside the exclosure) and in terms of percentage of mean abundance. *Rubus fruticosus* was the dominant species in fenced plots at TF. For the low shrub and high shrub strata, *Carpins betulus* was the dominant species at Arc and LPP, and at LPP, *Fagus sylvatica* was the dominant species. In the tree layer, Lari de (the exact name was not found in the database) was the most present species in all the sampled quadrats at Arc, but *Carpins betulus* had the highest mean abundance. At LPP and TF, *Fagus sylvatica* was the dominant species.

We compared 22 pairs of fenced-unfenced plots with the same area and characteristics in the same site to compare fenced and unfenced quadrats. In each pair, there were four unfenced quadrats of 314 square meters, and four fenced or selectively fenced quadrats of the same area (figure 1). We sampled 32 plots of 1 square meter in each quadrat, resulting in a total analysis of 9056 plots. For the fenced patches, four types of steel wire fence were installed around a quadrat of one square meter to protect the vegetation from browsing. For the roe deer enclosure, the fence was cut at 1.20 meters. For the red deer, we created a small opening in the fence. And for the wild boar, we established a heavy swinging door system. A fence was installed to prevent the three species from entering these quadrats. (When were the fences installed?) For each quadrat, one engineer and several technicians recorded the abundance-dominance of all plant species in spring using the Braun-Blanquet coefficient (see annex 3) according to the four different strata heights: herbaceous layer (<0.5 m), low shrub layer (0.5-1.3 m), high shrub layer (1.3-3 m), and tree layer (>3 m).

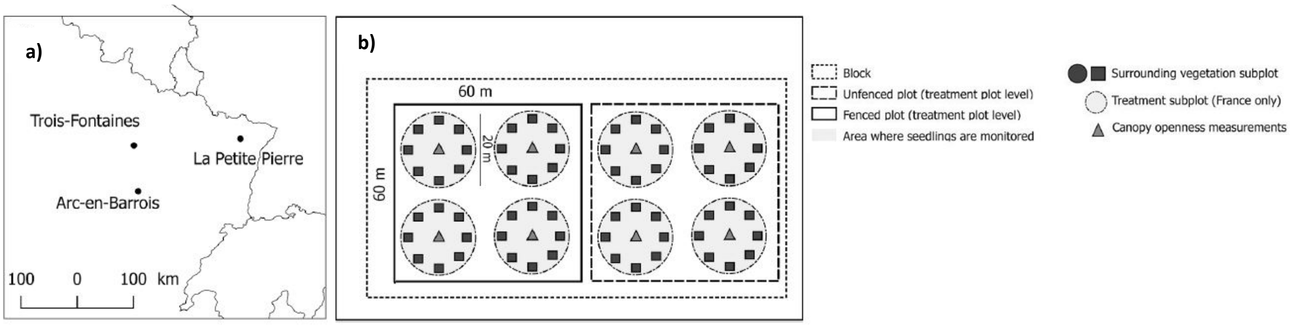


Figure 1. Location of the two study sites in France

We obtained species traits from the Baseeco database (cite year and reference) of the Mediterranean Institute of Biodiversity and Marine Ecology and Continental of Marseille for Raunkiaer's life form (growth form in the LEDA database), shade tolerance, and dispersal type of form. We used the LEDA Traitbase (Kleyer et al., 2008; last update) to complete the growth form to complete Raunkiaer's life form, and branching. Some features were missing for plants, so we supplemented this information with a few websites and scientific articles referenced at the end of this article. We chose only categorical data for functional traits, which were more generical and simple to have, as we didn't have it from individuals from the two forests analyzed.

The Raunkiaer's life form described the type of plant's growth, they were classified as follows: phanerophyte, hemicryptophyte, geophyte, therophyte, chamaephyte. The dispersal mode referred to the ability of plants to disperse their seeds. They have been classified among the following categories: hemerochor, zoochor, meteorochor, autochor, anemochor, nautochor. The shade tolerance has been defined as the capacity to growth in the shadow also named sciaphily. Finally, the capacity of branching was analyzed, but almost all the plants in our database were classified in the branching category. So, we decided to remove this trait from our analysis.

Statistical analysis

For every diversity parameter and functional traits analyzed, we established the number of species present in each layer (table 1). For each vegetation layer, we analyzed changes in species richness and Shannon index between exclosure or selective exclosure and control quadrat. Species richness was estimated at the quadrat level (1m², n = 5886) for each following year: 2016, 2017, 2018, 2019, 2020, 2021 and 2022. For species richness, we added up the number of species present in each quadrat. Then, we estimated the average of species present in each fencing modality for the four forest strata and each sampled year. To estimate the Shannon index, we converted the abundance-dominance coefficients sampled for species in each quadrat into a percentage (annexe 3, Boulanger and al, 2019). We calculated the Shannon index for each quadrat for all fencing modality of the four forest layers and each sampled year. With the Shannon index and species richness results obtained at quadrat level, we estimated the difference between exclosure or selective exclosure which means only red deer permitted or only roe deer permitted, or only wild board permitted, and the plots located outside the exclosure with the presence of the three ungulates. Finally, with an AIC test (annexe - tableau AIC) we selected the most parsimonious model between several combinations of linear regression and linear mixed model (LMM). We verified the assumption of homogeneity of variance by plotting the residuals against the predictors and the predicted values and we checked the distribution of residuals of each selected model. The most parsimonious test was a LMM to analyze the effect of the temporal change and the effect of each fencing modality on the differences of species richness and Shannon index at each layer forest between two modalities for all the sampled year. For the species richness, the LMM was the most parsimonious model for the herbaceous, low shrub and high shrub layer but a linear regression model (LM) with only the effect of years was the most parsimonious model (table 1). For the LMM, we included two fixed effects: time (7 levels: 2016, 2017, 2018, 2020, 2021 and 2022) and fencing (4 levels: exclosure – control; roe deer presence – control; red deer presence – control; wild board presence – control). The explicativee variables Fencing and Years, which had several levels of factors are not correlated based on the results of Cramer V test (~0.14) run for species richness and Shannon index and all functionals traits variables for the four-forests layer studied.

For each functional trait, we replaced the abundance-dominance coefficients sampled for species in each quadrat by its characteristics for each trait to have a categorical value. First, we selected species present at each forest layer (table S.1). We estimated the proportion of presence of each category for each functional trait. Then, we calculated the average percentage of presence of each category for each trait for the 4 different types of fencing and we estimated the difference between exclosure or selective exclosure which means only red deer permitted or only roe deer permitted, or only wild board permitted, and the plots located outside the exclosure with the presence of the three ungulates. With an the Akaike’s Information Criterion (AIC) test the most parsimonious model (table 1) and for completing this selection, we verified the assumption of homogeneity of variance by plotting the residuals of the selected test according to the predicted values and we checked the distribution of residuals of each selected model. a model of prediction we selected the model of regression which explains the effect of temporal changes and fencing modalities on each functional trait. Consequently, we chose a linear regression model with fencing as the explanatory variable at quadrat level at each forest layer.

Models	df	Herbaceous	Low shrubs	High shrubs	Tree
Lm(Shannon Index ~ Years * Fencing)	26	-64.3	-130.6	-177	-2302.7
Lm(Species richness ~ years * fencing)	26	23.3	1.5	-23.4	x
Lm(Species richness ~ years + fencing)	11	x	x	x	-21.9
Lm(Growth ~ Modalité)	5	-183.5	-121.5	-76.4	-88.3
Lm(Dispersal ~ Modalité)	5	-577.1	-232.9	-76.4	x
Lm(Dispersal ~ Years)	13	x	x	x	-47
Lm(sciaphily ~ Modalités)	5	-43	5	-7.8	-17.3

Table 1. Linear regression model selected by the AIC test

Legend: the results and details of variables and all the linear regression tested in the AIC tests are in the supplementary material (table S2).

Results

1. Diversity of forest communities

For the diversity at every forest layer, we didn't observe a significative difference of Shannon index and species richness between fenced and unfenced plots. At the herbaceous layer, there is no statistical results that valid the difference according the two diversity variables we choose between unfenced and one of the fenced plots over the years (table 2). The effect of certain years coupled paired to the presence of one ungulate has an impact on the number of species and their abundance Nevertheless, for the Shannon index, at this layer the difference of abundance and the number of species is higher than other forest layers. The fence appears to be gradually reducing the abundance and shade of herbaceous and low shrub species (figure 2). At the low shrub layer, wild boar populations impact the most negatively the number and the abundance of species compare to the selective presence of the two other ungulates or the presence of the three species of ungulates all together (table 2 et figure 2). Even if the impact of wild boar is the worst with the Shannon index among the types of modalities, this difference remains very small between the exclosure and the presence of wild boar alone (Estimate = 0.2250; Standard Error = 0.0541; p value = 0.0059). For the tree and high shrub layer, the trend is reversed compared to the two previous layers. The number of species and their abundance are higher in the selective fenced plots or in the absence of ungulate. At high shrub layer, it's the wild board which has the most positive effect on number and abundance of species (figure 2, table 2). At tree layer, the presence of just one of these three herbivores or the absence of these three species contribute to more species of tree with a higher abundance to plots with the presence of the three species together (figure 2, table 2). Finally, at the herbaceous and low shrub layer, the plots where all the three large herbivores are present have more species with an abundant population than totally or selective fenced plots. At superior forest layer, particularly at the tree layer, the plots where only one ungulates is present have also more species with more abundance than plots without fence.

table 2. Linear Regression of selected test

Response Variable	Forest layer	Explicative variable	Estimate	Standard Error	t value	p value
Shannon index	Low shrub	2021: sélectif cerf_exclos)	**0.1300**	0.0501	2.5957	0.0409
		2020: selectif chevreuil_exclos	0.1350	0.0541	2.4956	0.0468
		2021: selectif chevreuil_exclos	0.1400	0.0501	2.7953	0.0314
		2020 : selectif sanglier_exclos	0.2250	0.0541	4.1593	0.0059
	High shrub	2020 : selectif cerf _exclos	-0.080	0.0244	-3.2797	0.0220
		2020 : selectif chevreuil_exclos	0.080	0.0244	3.2797	0.0220
		2021 : sélectif sanglier_exclos	-0.125	0.0226	-5.5351	0.0026
	Tree	2021 : selectif cerf _exclos	0.05	0	5.395501e+15	$< 2^{e-16}$
		2021 : selectif chevreuil_exclos	0.06	0	6.474601e+15	$< 2^{e-16}$
		2020 : sélectif sanglier_exclos	0.00	0	-4.023600e+00	0.0101
		2021 : sélectif sanglier_exclos	0.07	0	7.553702e+15	$< 2^{e-16}$
Species Richness	Low shrub	2019 : selectif sanglier_exclos	-0.7917	0.2923	-2.7088	0.0423
	High shrub	2019 : selectif sanglier_exclos	-0.7917	0.2923	-2.7088	0.0423

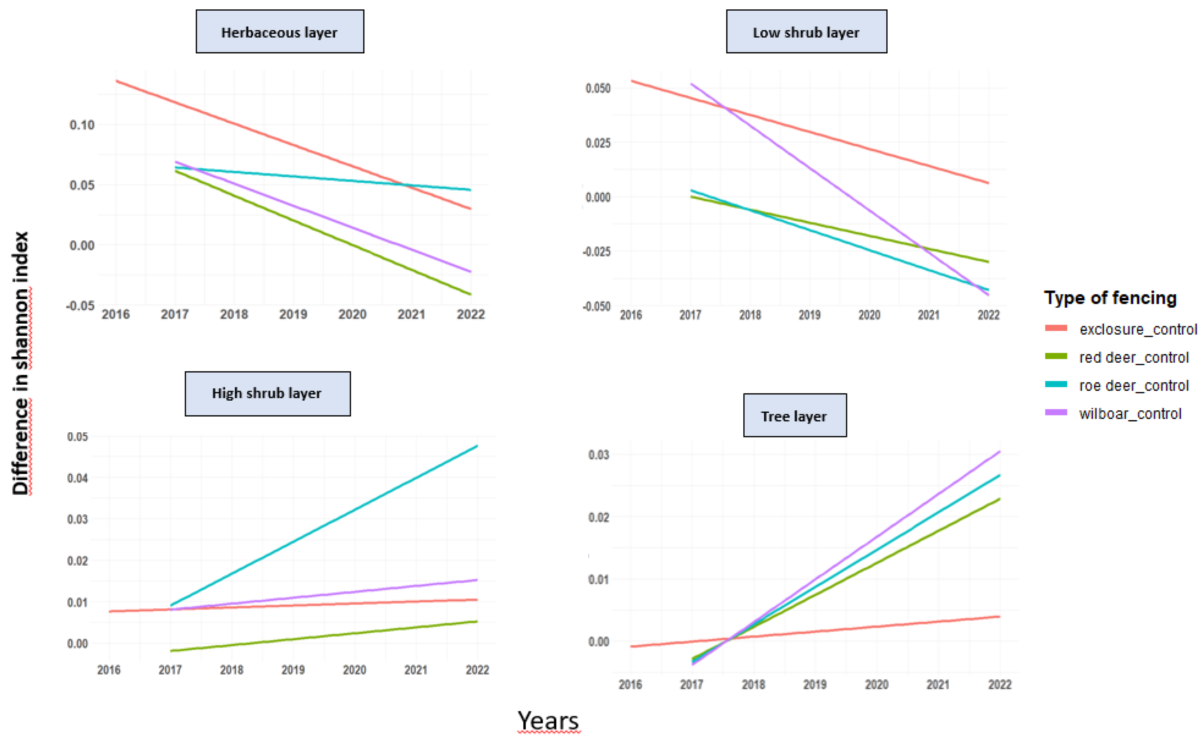


Figure 2. Linear regression of the difference of Shannon index between fenced and unfenced plots between 2016 and 2022 at TF and Arc.

Legend : For all the figure I this article, on the x-axis, "exclosure" represent the plots not accessible by ungulates, red deer represent the plots only accessible to red deer, roe deer represent the plots only accessible to roe deer, wild boar represent the plots only accessible to wild board. Control represent the unfenced plots.

As the other indicator of diversity, we used the species richness. There are no marked differences between the 5 types of management (figure 3), all the results are around zero (figure 3). But statistically there are two significative results if we paired the effect of 2019 with the effect of wild boar at the low and high shrub layer (table 2). When the wild board is alone to access to the fenced plots, the number of low shrub species decreased whereas at the high shrub layer the number of species increased, and it continue to increase at the tree layer. The low shrub layer seems to be the forest strata the most impacted by the wild board populations (figure 3). The herbaceous species diminished in plots in absence of ungulates or if only red deer population are present. The low shrub species only increased if the fenced plots are only accessible to red deer or if all the species are present. For the high shrub species are more numerous when the plots are only accessible to wild boar, red deer. The exclusion of all ungulates seems also to have a positive effect with more species in these areas. Finally, trees species are more numerous in fenced plots accessible to roe dear or wild board only (figure 3). The presence of only wild board and roe deer in plots are also supported by more species in the high forest strata whereas the red deer has more impact on the two other strata particularly the low shrub one.

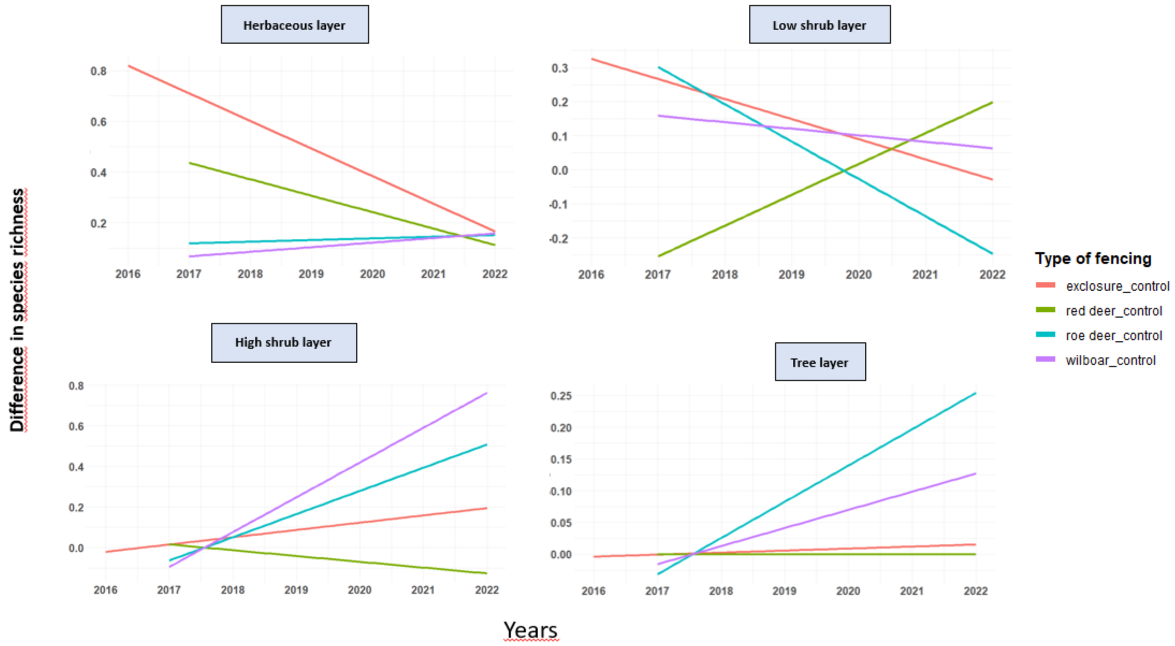


Figure 3. Linear regression of the difference of species richness between fenced and unfenced plots between 2016 and 2022 at TF and Arc.

2. Functional traits

2.1. Growth mode

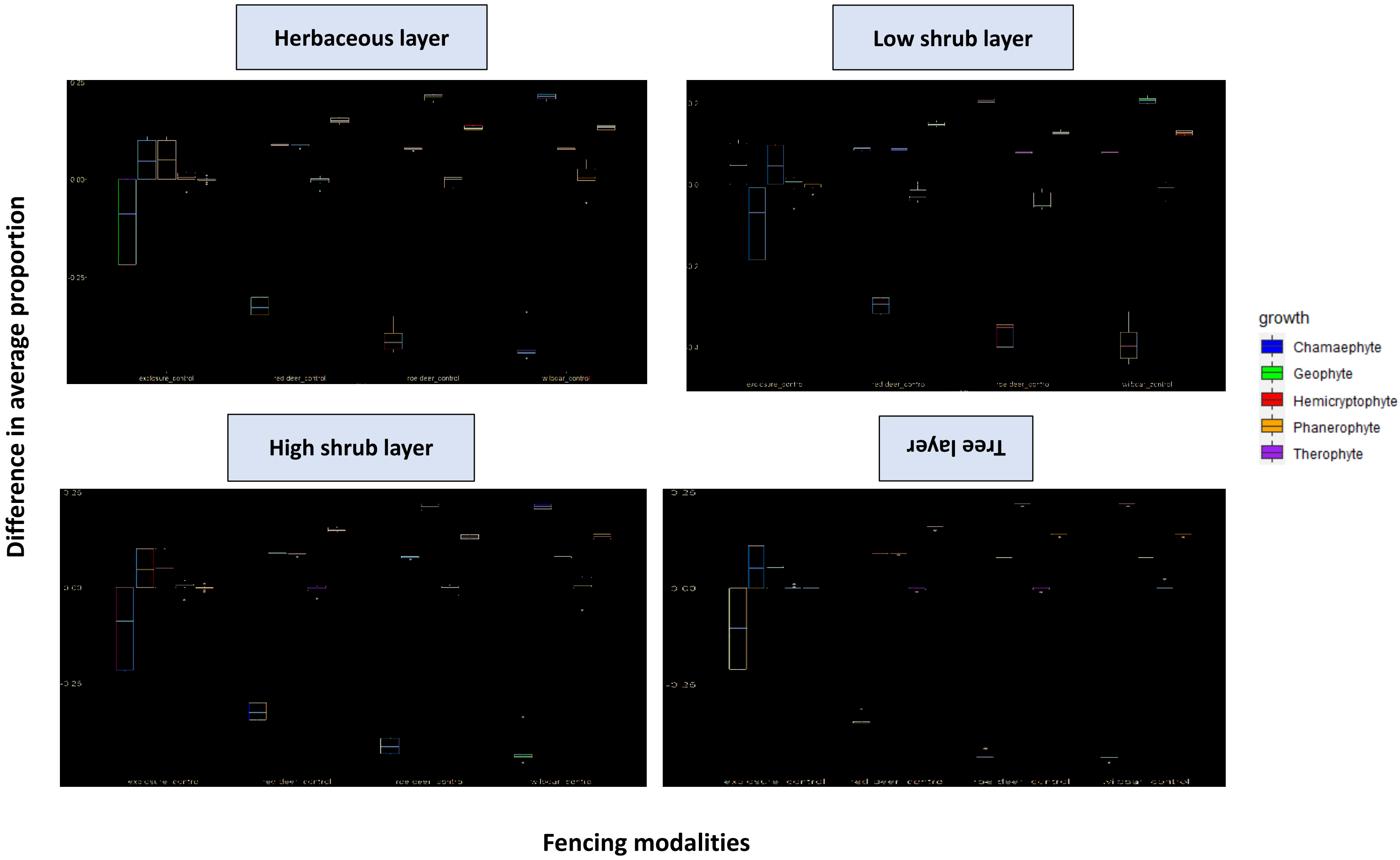


Figure 4. Distribution of the difference of repartition of 5 types of plants growth between fenced and unfenced plots at TF and Arc between 2016 and 2022

For all the functionals traits, there is no statistical effect overs years or fenced modality between selective or total exclosure and the presence of the three herbivores together. Regarding the AIC test, the most parsimonious model was a linear regression with only the effect of the different type of fencing on plants community (table S.2). Also, we decided to focus on the distribution of differences of repartition between fenced and unfenced plots for every category on each functional traits to have a first comprehension of the effect of fencing on plants communities traits we selected. In these distributions (figure 3, 4 and 5), the size of sample has also a huge impact on descriptive statistics we obtained. At the same time, the size of the boxplots indicates that the higher the vegetative strata, the fewer the data. Thus, the sites studied are richer in herbaceous and low shrub species and seem to have few arborescent species. Also, the different modality of fencing are only present at Arc, a factor that reduce once more the size of sample to have exhaustive results.

Despite this, the statistic descriptive on growth mode show like the species richness and the Shannon index that the difference of average proportion of each growth mode are quite similar between the 4 types of pairs of modalities. At every forest layer, the difference of mean proportion of chamaephyte species seems be more important for the chamaephyte species than the other types of growth in plants communities (figure 4).

2.2. Dispersal Mode

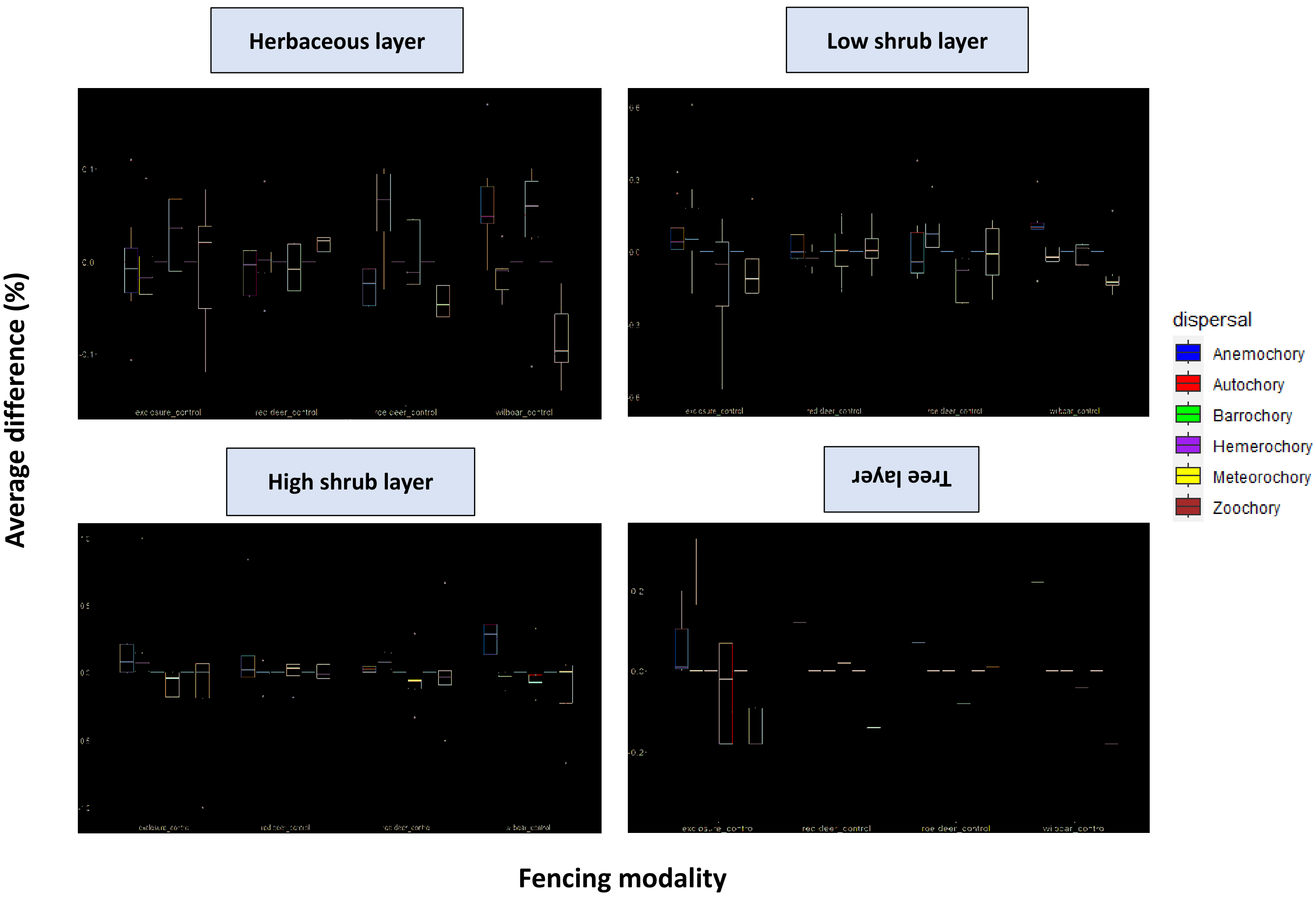


Figure 5. Distribution of the difference of repartition of 5 types of plants growth between fenced and unfenced plots at TF and Arc between 2016 and 2022

2.3. Sciaphily

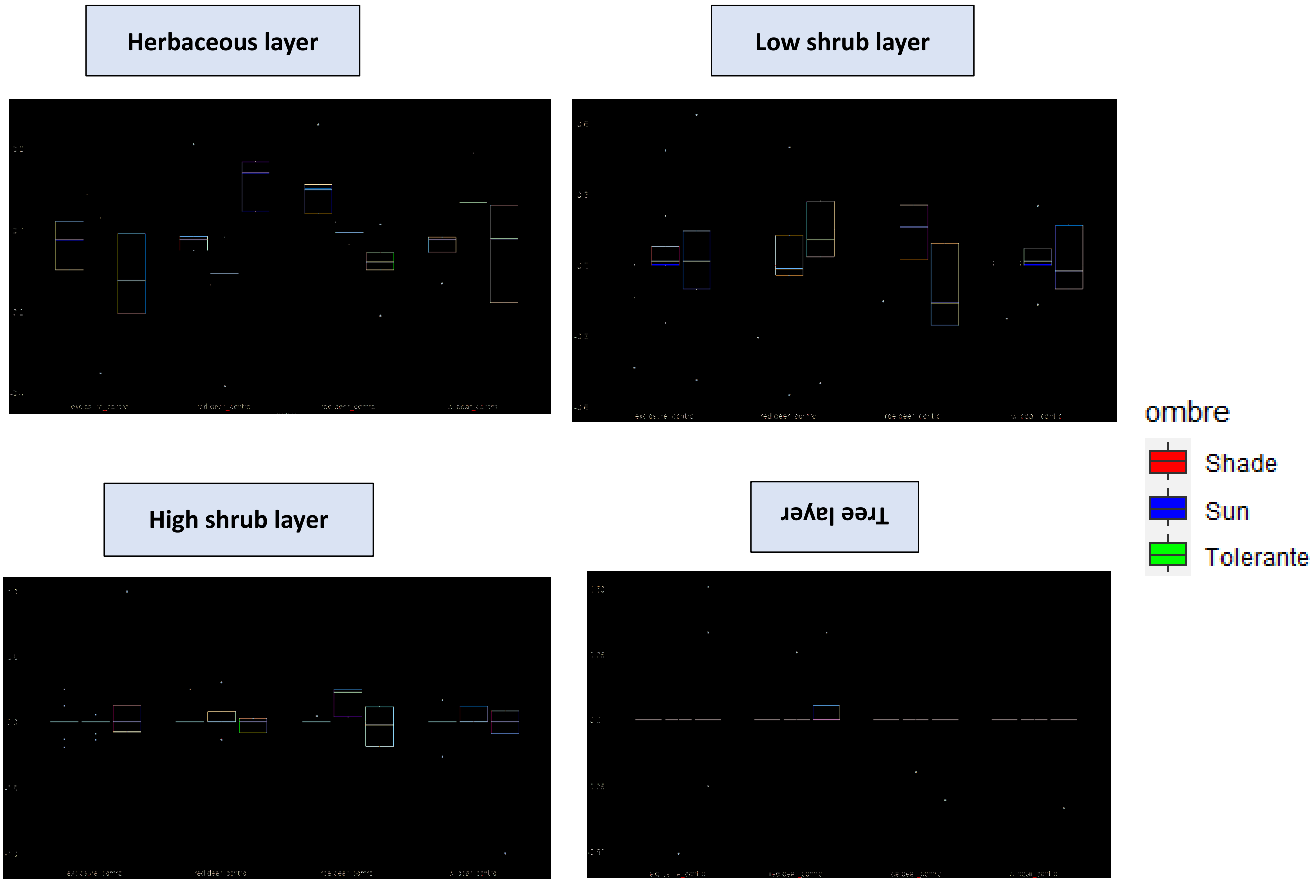


Figure 6. Distribution of the difference of repartition of 5 types of plants growth between fenced and unfenced plots at TF and Arc between 2016 and 2022

A FAIRE :

Discussion :

A high degree of canopy openness often leads to an increase in species richness (Alexander and Mack, 2017) in the forest understory. An increase in canopy openness may also, however, increase browsing pressure in forest gaps by providing a higher quantity and diversity of forages (Kuijper et al., 2009; Mangas and Rodríguez-Estival, 2010).

The top-down approach not seems to be the best way to analyze the recruitment or regeneration process of forest. (inspiré de la première phrase de l'article de barrere and al. 2021).

The light (Ericsson et al., 1996; Giertych et al., 2015 cité par Barrere and al. 2021) and herbivory regulate plant biomass (Martin et al., 2010 cité par Barrere and al. 2021).

Faire une analyse sur des traits fonctionnels quantitatif comme SLA (composition en azote, carbone) : Other studies have documented that plants growing under canopy gaps may have a different chemical composition than plants growing in the shade (Agrell et al., 2000; Giertych et al., 2015), and hence a different palatability for ungulates (Tucker et al., 1976). For instance, light-demanding species are believed to be more palatable than shade-tolerant species due to a higher nutrient content and fewer chemical defenses (e.g. phenolic compounds) (Giertych et al., 2015; Kitajima et al., 2012).

- Effet modalité seule pour growth / dispersal / ombre mais ça reste très faible comme effet quand on regarde l'estimé de chaque facteur. **Lire l'article sur Suède et France car n'a pas trouvé de résultats concluants. Regarder qui l'a sité après et qui lui il a cité.**

- Effet interaction modalité / année : species richness et indice de shannon : faible effet aussi quand on regarde l'estimée

- Le site semble avoir plus d'effet que la présence de populations d'ongulés.

- Perspectives de recherches : comparer le site de LPP avec TF et Arc. Le type de sol étant le même à Arc et TF (calcaire donc sol plus riche) qu'à LPP (grès vosgien), peut être qu'une analyse comparée de site avec un type de sol différentes serait plus pertinent.

- Pour les strates, catégoriser les plantes selon leur catégorie et non par taille, cé éviterait d'intégrer des plantes arborescentes dans les herbacées.

- Analyser la dynamique des populations d'arbres comme le chêne, érable, être pour observer l'abondance par strate, permettrait d'observer les tendances d'abondance, si a une étape de leur croissance, les espèces sont plus impactés par les ongulés.

Figure Indice de Shannon : Strate herbacée

b) c)

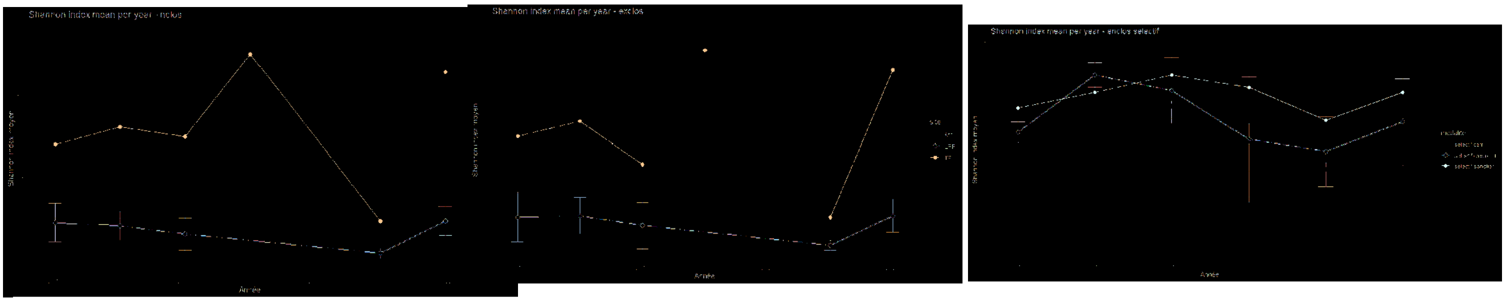
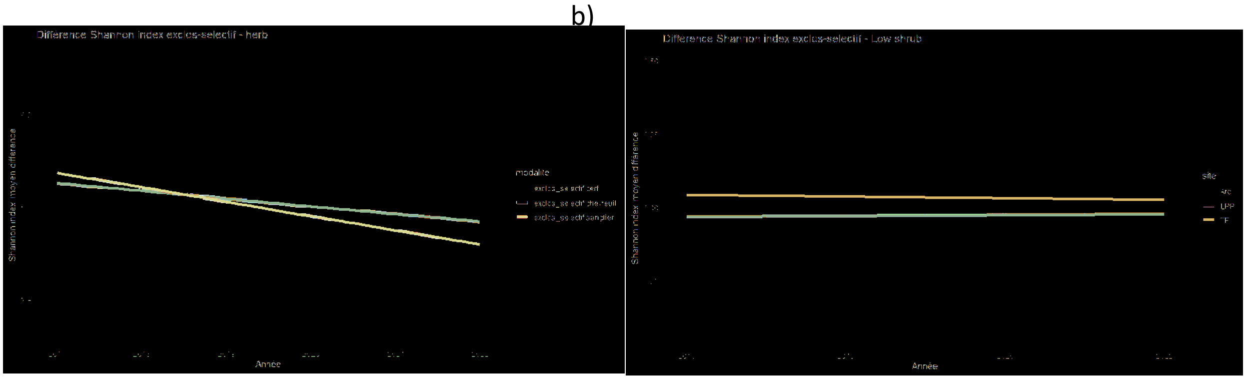


Figure 1. Indice de Shannon en enclos (a), exclos (b) et en enclos sélectif (c) entre 2016 et 2022 à Arc, TF et LPP

a) b)



a) b) c)

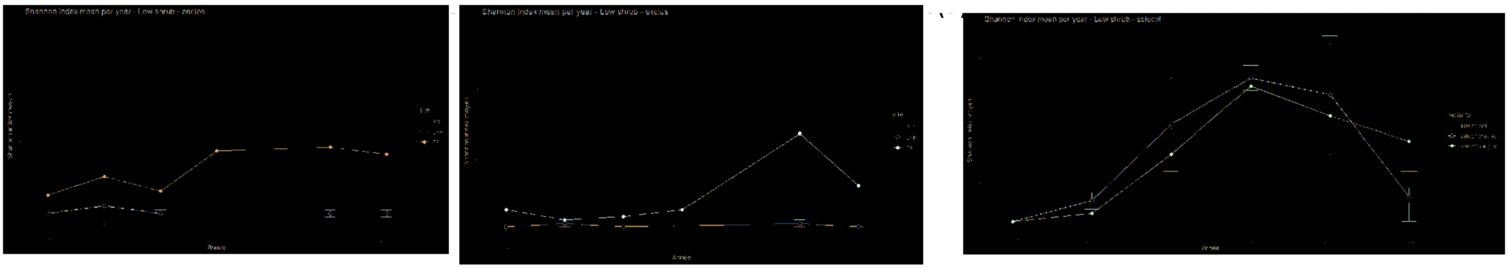


Figure 3. Indice de Shannon en enclos (a), exclos (b) et en enclos sélectif (c) entre 2016 et 2022 à Arc, TF et LPP

a) b)

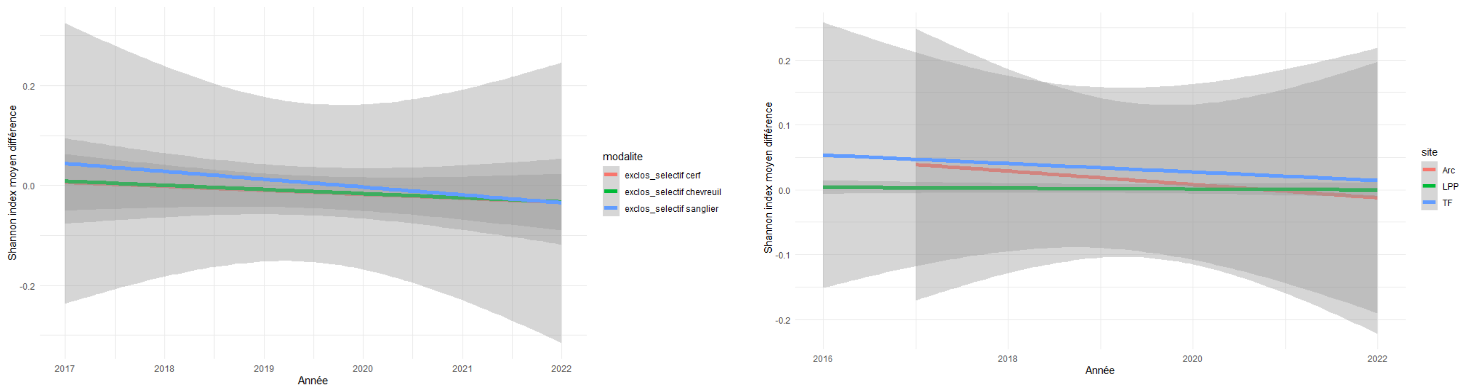


Figure 4. Différence de l'indice de Shannon entre les enclos sélectif-exclos(a) et enclos-exclos(b) entre 2016 et 2022 à Arc, TF et LPP

Strate high shrub

a b) c)

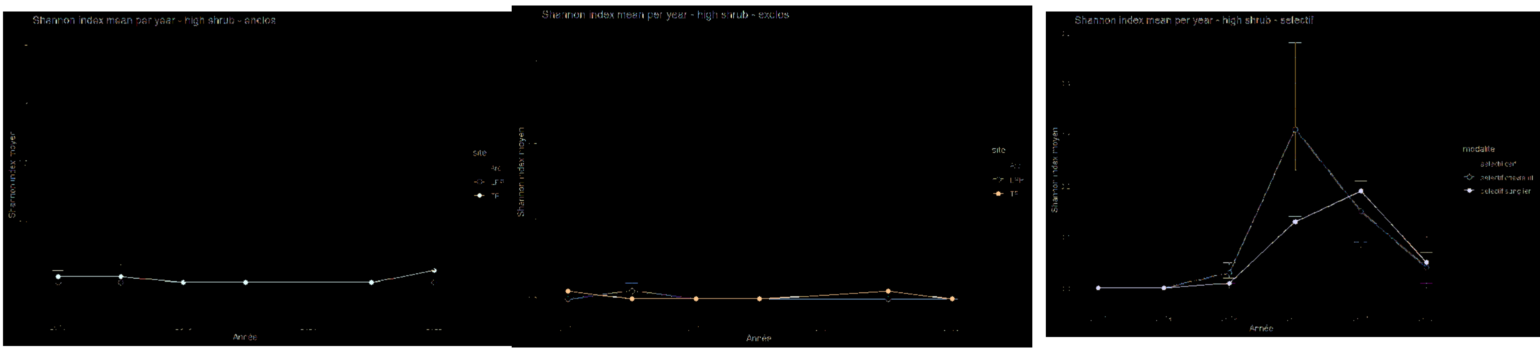


Figure 5. Indice de Shannon en enclos (a), exclos (b) et en enclos sélectif (c) entre 2016 et 2022 à Arc, TF et LPP

a) b)

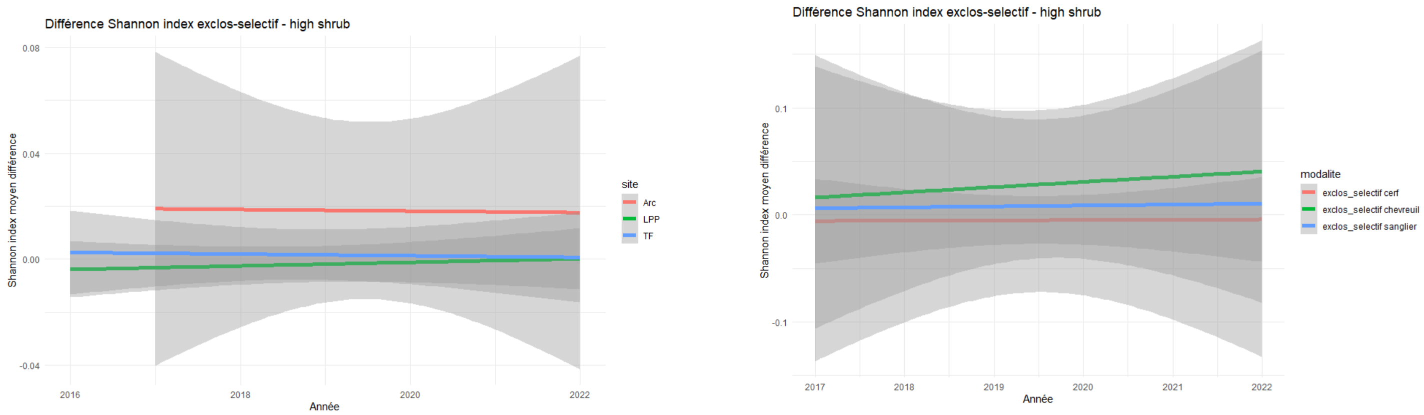


Figure 6. Différence de l'indice de Shannon entre les enclos sélectif-exclos(a) et enclos-exclos(b) entre 2016 et 2022 à Arc, TF et LPP

Strate arborescente

a b) c)

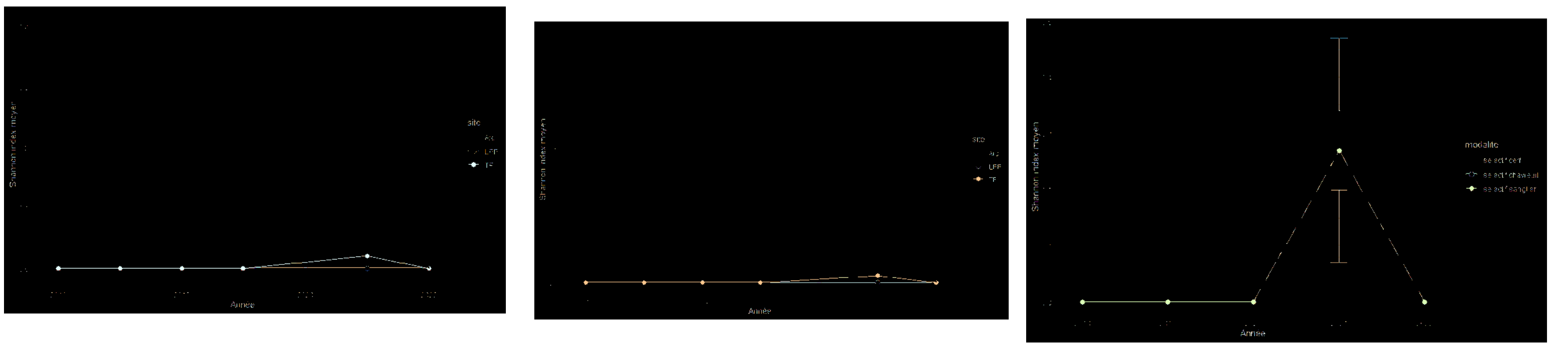


Figure 7. Indice de Shannon en enclos (a), exclos (b) et en enclos sélectif (c) entre 2016 et 2022 à Arc, TF et LPP

a) b)

Figure 8. Différence de l'indice de Shannon entre les enclos sélectif-exclos(a) et enclos-exclos(b) entre 2016 et 2022 à Arc, TF et LPP