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Les nématodes, des bio-indicateurs en lien avec l'invasion ligneuse : cas de l'invasion de l'*Acacia Sieberiana* dans les pâturages d'Afrique du Sud

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2015-2016

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- Accroître leurs compétences en matière de pratique professionnelle par la mobilisation de connaissances et de techniques, dont les fondements et contenus ont été explorés le plus finement possible afin d'en assurer une bonne maîtrise intellectuelle et pratique,
- Accroître la capacité des ingénieurs en génie de l'aménagement à innover tant en matière de méthodes que d'outils, mobilisables pour affronter et résoudre les problèmes complexes posés par l'organisation et la gestion des espaces.

La formation par la recherche inclut un exercice individuel de recherche, le projet de fin d'études (P.F.E.), situé en dernière année de formation des élèves ingénieurs. Cet exercice correspond à un stage d'une durée minimum de trois mois, en laboratoire de recherche, principalement au sein de l'équipe Ingénierie du Projet d'Aménagement, Paysage et Environnement de l'UMR 6173 CITERES à laquelle appartiennent les enseignants-chercheurs du département aménagement.

Le travail de recherche, dont l'objectif de base est d'acquérir une compétence méthodologique en matière de recherche, doit répondre à l'un des deux grands objectifs :

- Développer toute ou partie d'une méthode ou d'un outil nouveau permettant le traitement innovant d'un problème d'aménagement
- Approfondir les connaissances de base pour mieux affronter une question complexe en matière d'aménagement.

Afin de valoriser ce travail de recherche nous avons décidé de mettre en ligne sur la base du Système Universitaire de Documentation (SUDOC) les mémoires à partir de la mention bien.

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INTRODUCTION

Tree encroachment in grasslands is a worldwide issue. The consequences of the phenomenon are numerous, but the most visible is the loss of agricultural lands (Archer, 1995). The matter, linked to the issue of feeding a growing world population, has led to many scientific studies. However, while the research focuses on the impact of tree encroachment on soil properties, there is a lack of knowledge concerning its effect on the nematode communities (Klass et al., 2012).

Nematodes are the most numerous species in the soil mesofauna (Biederman et Boutton, 2009). Their importance in the soil is not to prove. However, they are often known as pests for culture (Fourie et al., 2015). They are thus the subject of many researches to eradicate them (especially in sugarcane cultures in South Africa) (Berry, Spaull et Cadet, 2009). Some species that are more damaging to cultures are more studied, because they lower the productivity. But other species are in fact useful and have positive effects on cultures : they are non-pathogenic and do ecosystemic favors (Berry et al., 2009; Malherbe et Marais, 2015; Tabarant et al., 2011; Yeates, 1999). These species also limit pathogenic nematodes by improving soil decomposition (Berry et al., 2009; Bridge, 1996). Nematodes are good indicators as to the assessment of a changing ecosystem because of their sensitivity to variations (Bongers et Ferris, 1999; Nandjui et al., 2008; Tom, 1990). They are moreover a major part of the soil fauna, they intervene at several trophic levels and also play a role in mineralization process of C and denitrification (Beyrem et Aissa, 2000; Biederman et Boutton, 2009).

Research focuses also on the effects of vegetation on nematode communities, used as an ecological indicator for soil quality (Tom, 1990; Zhao et al., 2015). Studies have been conducted in cultivated lands, rarely in a tree encroachment context. But tree encroachment is affecting soil properties, hence nematode communities and thus the soil resistance. However, consequences of an ecosystem disturbance on nematode communities is not the same in every case, depending on the place and the nature of the modification (Grellier et al., 2013; Klass et al., 2012). In the case of a vegetal encroachment of an exotic plant, the effect on nematodes depends also of a time factor and is not constant (Chen et al., 2007; Li et al., 2007). If the vegetation affects nematodes, the other way round is also true. Nematode activity modifies the vegetation type (De Deyn et Van der Putten, 2005) by, in some cases, accelerating microbial organisms' decomposition and N mineralization (Biederman et Boutton, 2009).

Studies have been conducted in some regions of the world. In the plains of the Rio Grande, in Texas (USA), the research has enlightened changings in the soil microbial constitution following tree encroachment. C and N pools became more important due to modifications in the litter (its quantity and quality) (Liao et Boutton, 2008). Also, pools changed depending on soil, topography and vegetation structure (Hibbard et al., 2001). In China, the test was to grow tea trees in a grassland and in a woodland in parallel. The study showed a long term raise in the quantity in biomass, N and P, and more nematode species in the tea plantation (Li et al., 2007).

In South Africa, KwaZulu-Natal's semi-humid natural grasslands are encroached by *Acacia Sieberiana,* and the tree density increase transforms the ecosystem into a savanna. The transformation has been witnessed for about 30 years (Grellier et al., 2012). Research has been set up in this country, and the process has been widely studied. However, no link between tree encroachment and nematode communities has been searched.

The aim of this study is to understand how can tree encroachment influence nematode populations so we can use them as bioindicators as of the state of the semi-humid natural grasslands.

It was proven that *Acacia Sieberiana* has an impact on soil and sub-canopy vegetation. Indeed, tree encroachment in natural grasslands increases the C and N sequestration in the soil, and the *Acacia Sieberiana*, a legume plant, is especially known for enriching the soil in N (Frouz et al., 2013; Jackson et al., 2002; Liao et Boutton, 2008; Sitters, Edwards et Olde Venterink, 2012). The tree's leaves and ground vegetation might play a role in the accumulation process (Grellier et al., 2013). Tree encroachment therefore significantly modifies soil chemical composition, and it has consequences on all of the ecosystem (Sitters, Edwards et Olde Venterink, 2012), especially on nematode populations taking part in mineralization and denitrification. In fact, even if the total density of nematodes seems to stay stable, there would be a modification in the trophic groups level : root-parasitic nematodes would decrease, while bacterivore nematodes would increase under trees (Biederman et Boutton, 2009; 2010). Also, impacts caused by the *Acacia Sieberiana* on sub-canopy vegetation extends depending on the age and size of the tree, and a decline of the ground vegetation is observed under medium-size trees (Grellier et al., 2013). Yet, nematode populations are particularly influenced by the identity and specific diversity of vegetable communities (De Deyn et Van der Putten, 2005; Kostenko et al., 2015). Cattle also plays a role in the control of vegetable populations and tree encroachment by grazing and stamping the ground, but an action that is too intense could stimulate tree encroachment (Grellier et al., 2013; Macias, Mazía et Jacobo, 2014; Scholes et Archer, 1997; Van Auken, 2009). Moreover, the effects of tree encroachment on sub-canopy vegetation varies between an arid climate and a humid one (Scholes et Archer, 1997). Also, C and N storage rates depend on the climate and the abundance of precipitation (Frouz et al., 2013; Jackson et al., 2002). Seasons have to be taken into account, because of their strong effect on biogeochemical processes in soils. To conclude on this part, the effect of trees can be direct - because nematodes are influenced by the vegetation (Biederman et Boutton, 2009) - or indirect – because trees influence sub-canopy vegetation and some soil properties, like N. These changes can influence nematode communities.

At the study site, water presence depends on the season and the clay soil humidity at different deeps on the grassland. Different slopes on the catchment between the top, middle and bottom modify water drainage. Also, it was already proven in a grassland that nematode abundance increases when soil gets wet (Song et al., 2016). In Israel, the amount of nematodes is higher during the rainy season (Fitoussi, Pen-Mouratov et Steinberger, 2016). Moreover, the pasture of Potshini presents a gully erosion phenomenon. This ground erosion is emphasized by the presence of encroaching trees that change the water flow in the ground (Grellier et al., 2012). But few studies exist concerning the effect of gullies on soil fauna, and particularly on nematode populations (Baxter et al., 2013).

With all this information, we hypothesize that :

- Nematode communities are larger during humid season, when there are heavy rains, because of their affinity with water;
- Nematodes will fluctuate due to direct and indirect effects of tree encroachment;
- The study of nematodes with different diets and their alimentation (roots for example) will bring changing results regarding the type of nematodes.

MATERIALS AND METHODS

Most of the information of this paragraph is taken from the article (Grellier et al., 2013). The team that wrote it also did all of the experiment on the field.

STUDY SITE

The study is set up in South Africa, in the province of KwaZulu-Natal, in Potshini (8km south east of Bergville) (Figures 1-A and 1-B). The biome there is Northern KwaZulu-Natal moist grassland. Vegetation is dominated by *Hyparrhenia hirta* and *Sporobolus africanus*.

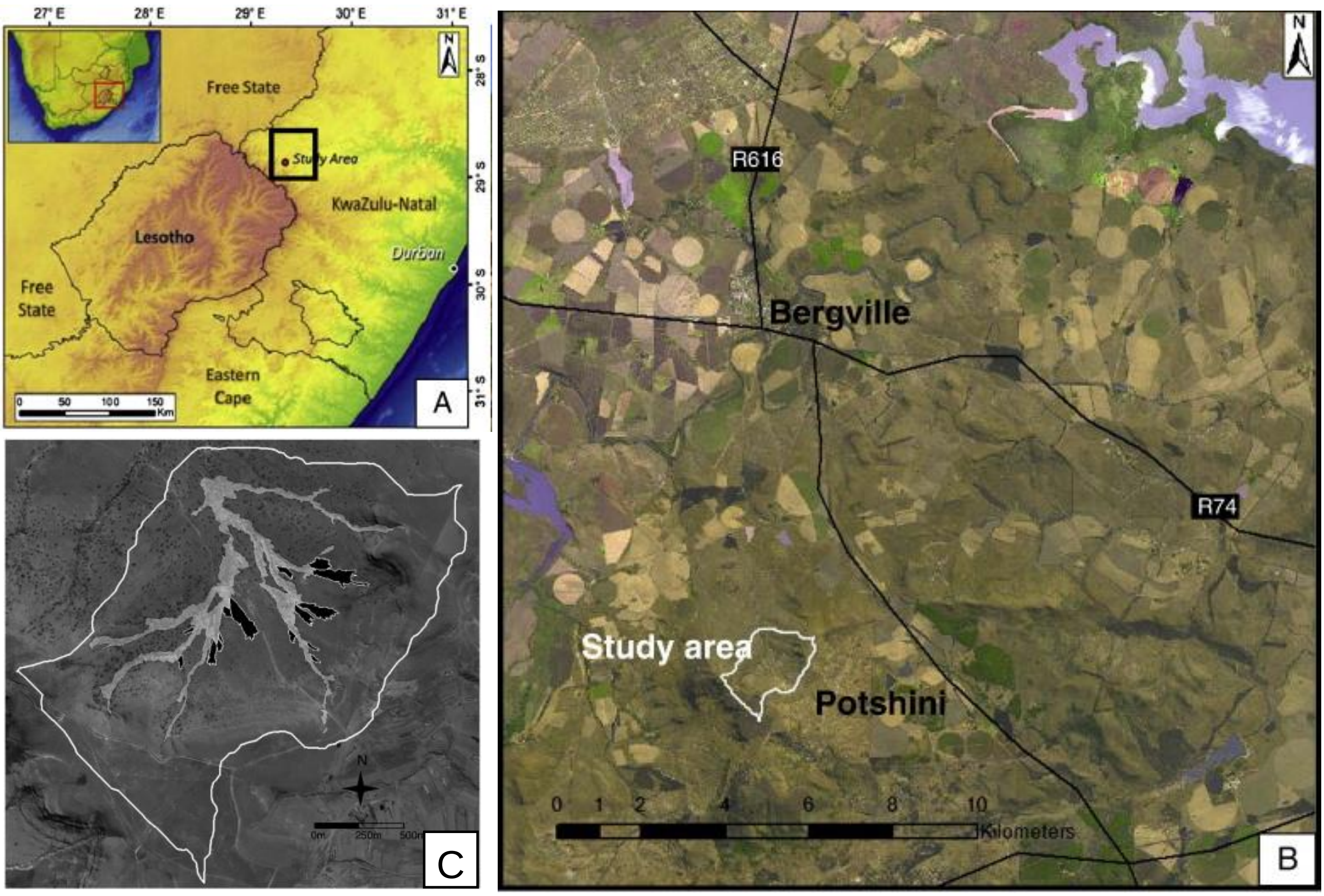


Figure 1 : Situation of the stuy site (A) with an aerial picture of 2008 (B) and an aerial view of the catchment with gullies in 2006 (C) (Grellier et al., 2012)

This place is also a pasture for cattle. Its management is commune to cattle owners. Animals are left to graze from November to June.

This biome is slowly turning into a savanna because of an encroaching tree : the *Acacia Sieberiana*. This degradation, leading to a grassland loss, can be the result of frequent forest fires or increasing number of grazing cattle that stimulates the tree development (Grellier et al., 2012; Van Auken, 2009). For the community pasture of Potshini, it is mostly the anthropic pressure that encourages the monospecific encroachment of the *Acacia Sieberiana* (Grellier et al., 2012). This encroachment reduces the grazing space for cattle and is problematic for local traditional farmers.

The zone is situated on the north-sloping catchment of Tugela Basin (30 000 km²) (Figure 1-C). The study place is 2.5 km², between 1217 and 1452 m high.

The climate is defined as humid subtropical. It is characterized by hot and rainy summers (from October to April) and chilly dry winters (from May to September). Mean annual precipitations (measured over 65 years) are 750 ± 162 mm. In 2008, the mean annual temperature was 16.5°C, it was of 16.1°C in 2009.

SAMPLING METHODS

In total, 200 samples were taken off. They are in fact measures done in 100 plots, at each season.

Plots combined all parameters : Season, Exclos, Location, Zone, and Tree Size. The Season parameter takes two values : dry and humid. It determines the climate and precipitations of the sample. Enclosed zones were fenced so that cattle can't come to graze. It is the parameter Exclos, that has Fenced/not Fenced as values. The Location parameter (Gully/Intergully) locates the sample regarding gullies. The position in the catchment (Zone parameter) can take three values : up (at the top of the basin, highest altitude and steepest slope), mid (in the middle of the basin) and down (at the bottom of the basin, lowest altitude and smoothest slope). The last parameter, about the size of the tree, can take three values : Ctr (no tree), big (big tree on the plot, > 3 m high) and med (tree of a medium size, between 1 and 3 m high).

The table below sums up parameters and their value :

Parameter	*Values*	*Definition*
Season	Dry, Humid	Season
Exclos	Fenced, Not Fenced	Cattle can come to graze or not
Location	Gully, Intergully	The sample is taken in a gully or not
Zone	Up, Mid, Down	Zone in the watershed
TreeSize	Big, Med, Ctr	If there is a tree above the sample, and its size

Table 1 :Parameters of the study

At each sampling plot, vegetation was first cut to the ground. We separated herbs, living leaves and other plants. Three samples of soil were then taken. The first is for the assessment of mass of roots. It's a cylinder of soil that is 10 cm high and 15 cm large (diameter), taken in the first 10 cm of the ground. The two others are for soil properties : cylinders of 250 cm^3, one between 0 and 10 cm deep, the other between 20 and 30 cm deep.

SOIL PROPERTIES ANALYSIS

Samples for soil properties were weighted on site and then dried at 105°C for 24 hours. Bulk density and humidity rate were therefore calculated. Soil carbon and nitrogen were measured by the Leco CNS 2000 with the automated Dumas dry combustion method.

Samples for roots data were passed through a 2 mm sieve to keep only roots and soil. It was washed with water afterwards to separate roots, that were dried at 70°C for 48 h and then weighted. Other green elements (living leaves, herbs and other plants) were cut before the sampling and weighted on site.

The table below sums up soil properties, their unit and other words to call them :

Parameter	*Unit*	*Also referred as*
C	%	
N	%	
Bulk Density	g/cm^3	BD
Humidity 5cm	%	Humid5, H5
Humidity 25 cm	%	Humid25, H25
Roots	Mg	
Grass	G	
Living leaves	G	Liveleaves, LL
Plant	G	

Table 2 : Soil properties and their units

NEMATODE DATA ANALYSIS

Counting of nematodes and identification of the species was done with the same protocol followed in (Cadet et al., 2007).

Once the samples of soil were collected with a shovel, they were stored in plastic bags to be transported to the laboratory. There, they were extracted from the soil : free living on one side, and roots (on which are the plant parasitic ones) on the other. Roots and nematodes on them were then separated. The genera was identified using a microscope, and the nematodes were sent to the Plant Protection Research Institute of Pretoria for identification.

In our study, 12 species of plant parasitic nematodes (PPN) are considered : Pratylenchus, Helicotylenchus, Meloidogyne, Xiphinema, Paratrichdorus, Criconematids, Scutellonema, Rotylenchulus, Hemicycliophora, Tylenchorhynchus, Longidorus, Paratylenchus. Free living nematodes (FL) are also taken into account but we don't know the detail of species.

STATISTICAL ANALYSIS

We separated our data in two classes : soil properties (carbon and nitrogen, humidity rate, bulk density, roots, leaves, herbs, plants…) and sampling properties (gully, trees, exclos, catchment…). R software was used for the whole statistical tests. Three tests were run : a multivariate PCA (Principal Component Analysis), a multiple regression and an ANOVA (Analysis of variance).

PCA was made with all variables. It first resulted in a correlation circle, on which it is possible to evaluate the correlations between variables by the proximity on the circle. Then we made biplots, that take previous results and displays all samples and one of the qualitative parameters. We made five biplots, each displaying a parameter : season, location, exclos, tree size and zone in the catchment. All of the PCA was realized using the ADE4 package of R.

Multiple regression allows us to express a variable (total number of nematodes, number of free living and plant parasitic nematodes) in function of other variables that we selected after PCA (humid.5, liveleaves, C, N, grass and bulk density). We start by describing the variables and all of existing combinations between them. Then we run a multiple regression. At each iteration, we delete a term : the one with highest p-value and highest level of interaction. We run the test on the new variables, until all terms are significant ($p\text{-value}<0.05$). Then we can have an equation. To ensure that this model is correct, we create a graph of residuals and fitted values. If residuals increase with increasing fitted values, the model is not right. If so, the whole test is ran again after transforming the variable (number of nematodes) into its logarithm. We then have log(number) expressed with other variables.

We carry out an ANOVA to establish a relation between nematodes and explicative variables. We study here the qualitative variables of sampling properties and their effect on a quantitative variable (nematodes). ANOVA for soil properties was already tested for the same samples in another article (Grellier et al., 2013), so we test the effect of Season, Location, Exclos, TreeSize and Zone on the number of nematodes. The whole process is repeated 3 times : once for Total number, once for plant parasitic nematodes and once for free living ones. After making an ANOVA, we need to test the homogeneity of variances and the normality of the residuals. If those two tests are not successful, or if the ANOVA does not show any reliable result, we carry out a non-parametrical test (that is weaker and accept one parameter at a time). We did the ANOVA using nlme package in R.

All programming codes used in R are in annex 1 and diagrams summarizing ANOVA treatments are in annex 3.

RESULTS & DISCUSSIONS

RELATION BETWEEN NEMATODES AND SOIL PROPERTIES

We started by analyzing the relation between the number of nematodes and variables of soil properties. The PCA gave us a basic idea of it with a correlation circle (Figure 2) :

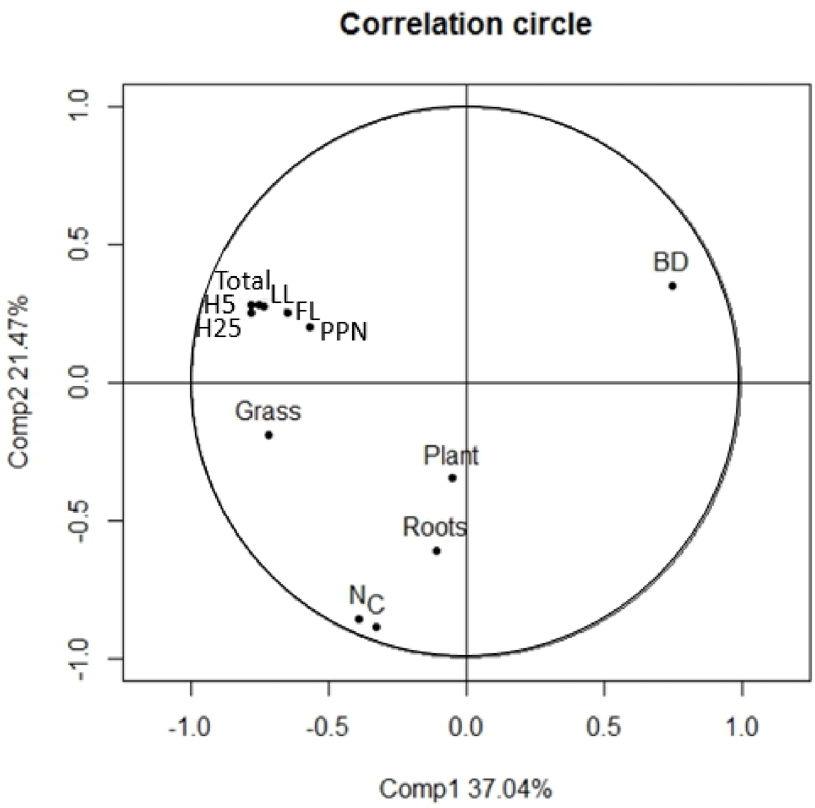


Figure 2 : Correlation circle of the PCA

Because of their proximity in the circle, we can say that there is a strong positive correlation between nematodes (Total, PPN and FL), humidity in the soil (humid 5 and humid 25), and living leaves of ground vegetation. Nematode communities are lightly correlated to grass amount, and negatively correlated to bulk density (BD). C and N concentrations in the soil are positively correlated together but it is difficult to assess anything as of their dependence towards nematodes, as they are on the same side on F1 axis and on a different side on F2 axis. The link between nematodes and plants and roots is not very representative, because both vegetation variables are too far from the line of the circle.

We also notice that the relations between the total number of nematodes and humidity and liveleaves is stronger than those between free living and plant parasitic nematodes and the two variables.

Using this correlation circle, we were able to select six variables for further analysis with a multiple regression : humidity 5, liveleaves, bulk density, grass, C and N. Humidity, grass and liveleaves were selected for their correlation to nematodes, bulk density for its negative correlation to them, and C and N concentrations to better study the link to nematodes, if it exists. All of this will help us describe more precisely the relation between nematodes and soil properties.

Because of a non homogeneity in the residuals while doing a multiple regression on the total of nematodes, we had to transform it into its logarithm and do the test again. The following table displays the results of it :

	Estimate	*Std. Error*	*t value*	*Pr(>|t|)*	
(Intercept)	5.886e+00	2.698e-01	21.813	<2e-16	***
Liveleaves	1.181e+00	4.119e-01	2.866	0.004653	**
N	3.422e+01	6.672e+00	5.129	7.55e-07	***
N^2	-6.818e+01	1.682e+01	-4.053	7.54e-05	***
Liveleaves:BD	-7.799e-01	2.860e-01	-2.727	0.007040	**
Liveleaves:Humid5	-5.915e-02	2.098e-02	-2.819	0.005357	**
N:BD	-1.231e+01	3.975e+00	-3.097	0.002271	**
Liveleaves:N	-1.040e+01	3.403e+00	-3.055	0.002593	**
Humid5:Grass	5.083e-04	1.488e-04	3.416	0.000786	***
Liveleaves:BD:Humid5	3.964e-02	1.498e-02	2.646	0.008879	**
Liveleaves:N:BD	7.019e+00	2.407e+00	2.916	0.004004	**
Liveleaves:N:Humid5	4.467e-01	1.549e-01	2.885	0.004399	**
Humid5:Grass:C	-1.288e-03	3.610e-04	-3.568	0.000463	***
Liveleaves:Grass:C	-1.278e-03	3.683e-04	-3.470	0.000653	***
Liveleaves:N:BD:Humid5	-2.989e-01	1.121e-01	-2.666	0.008394	**
Liveleaves:Humid5:Grass:C	8.868e-05	2.086e-05	4.252	3.41e-05	***
N:Humid5:Grass:C	6.341e-03	1.677e-03	3.781	0.000213	***
Liveleaves:N:Grass:C	7.940e-03	2.167e-03	3.664	0.000328	***
Liveleaves:N:Humid5:Grass:C	-5.418e-04	1.192e-04	-4.544	1.01e-05	***
Signif. codes :	0 '***' 0,001 '**' 0,01 '*' 0,05 '°' 0,1 ' ' 1				
Residual standard error	0,4149				
Multiple R-squared	0,4757				
Adjusted R-squared	0,4227				

Table 3 : Multiple regression of log(total number of nematodes)

We can see that N influences the most nematode communities, and this even independently of other variables. Density in the soil and liveleaves are also playing a role, but their effect is linked to N concentration. R^2 in this model is almost 0.48, which means that 48% of the variation of log(total of nematodes) is explained by the regressors (the six variables and their interactions) in the model. This R^2 is a bit weak, it does not represent a real situation. Some other factors that we did not take into account in this study must play a role in nematode populations. But we still decide to accept this result (and do the same for free living and plant parasitic nematodes with small R^2) for the rest of the work.

We did the same work for free living and plant parasitic nematodes. The corresponding tables are in annex 2.

We observe for free living nematodes that C and N have a major effect, followed then by humidity and bulk density that have a lighter effect. Moreover, humidity impact is linked to N, as bulk density is liked to C concentration.

For plant parasitic nematodes, N concentration (combined to liveleaves) influences the size of the population. Bulk density, mostly associated to N concentration, also has an impact.

To conclude on those results, we saw that :

- Humidity and liveleaves have a strong positive effect on nematode communities;
- Grass is positively correlated to nematodes;
- Bulk density is negatively correlated to nematodes;
- C and N concentrations have an effect on nematodes, but we do not know its nature.

With those results, we are able to confirm that nematodes are comfortable living in a wetter soil. The link with humidity is direct : the more water in the soil, the more nematodes there are. This effect is verified by two studies (Fitoussi, Pen-Mouratov et Steinberger, 2016; Song et al., 2016).

We can also see that nematodes live more in lighten soils, because bulk density is negatively correlated to them. This means that the thicker the soil is, the fewer nematodes there are in it.

Moreover, we confirm the importance of vegetation for nematodes : the more vegetation there is, the more nematodes are present. This is obvious with living leaves, but grass is also quite well correlated. It is a logical cause for the effect of bulk density. Vegetation tends to stop flowing water and keep it around. When there are no plants, there is less water, and then soil is thicker. With plants, there is water, and their roots also help lightening the soil, thus decreasing its density.

We show in our study that there is an effect of C concentration on nematode communities. It is proven in a study that the impact is positive (Ito et al., 2015). However, we have no clue concerning the exact effect in our case, so we can not infer anything here.

According to our study, the number of nematodes is affected by N soil concentration. We do not know however if nematode population increases as N increases or decreases. Even scientific studies show sometimes that N has a positive impact on nematodes (Song et al., 2015) and sometimes that its effect is negative (Ruan et al., 2013; Zhao et al., 2014). These differences are due to geographical location and soil structure. Also, it seems that, in Potshini's pasture, *Acacia Sieberiana* does not play a role in N concentration, given that N pool is not larger under big trees (Grellier et al., 2013), even if it does not go along with several proofs that *Acacia Sieberiana* stores more N in the soil (Sitters, Edwards et Olde Venterink, 2012; Sitters et al., 2015).

In another hand, it was proven that *Acacia* modifies Phosphorus flow (Sitters et al., 2015) and that P has more impacts on nematodes than N (Zhao et al., 2014). Taking P into account in a study like ours could possibly show a link between nematodes and tree encroachment.

RELATION BETWEEN NEMATODES AND SAMPLING PARAMETERS

We study here the effects of the season, location, tree size, exclos and zone in the catchment basin on nematode communities.

Season

It is the parameter that plays the most important role. We find its effects in the ANOVA and PCA. In the ANOVA, it was the first parameter we tested, and its effect was undeniable on total number of nematodes, free living and plant parasitic nematodes. The graphic below shows the difference in the number of nematodes depending on the season :

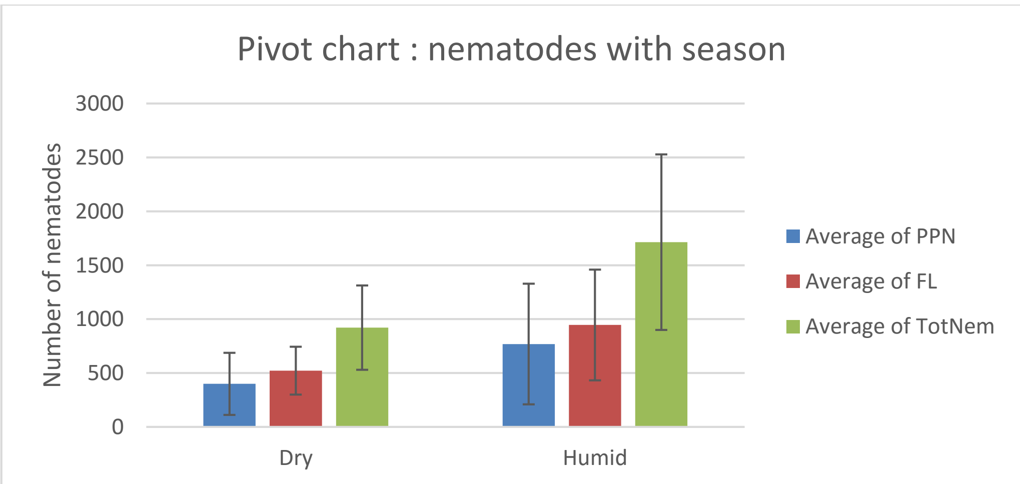


Figure 3 : Graph of the number of nematodes plotted with seasons

Nematode communities are larger in humid season. This is because humidity in the soil and the amount of liveleaves are higher and bulk density is lower in humid season (by superimposition of the correlation circle and the biplot) (see figure 4).

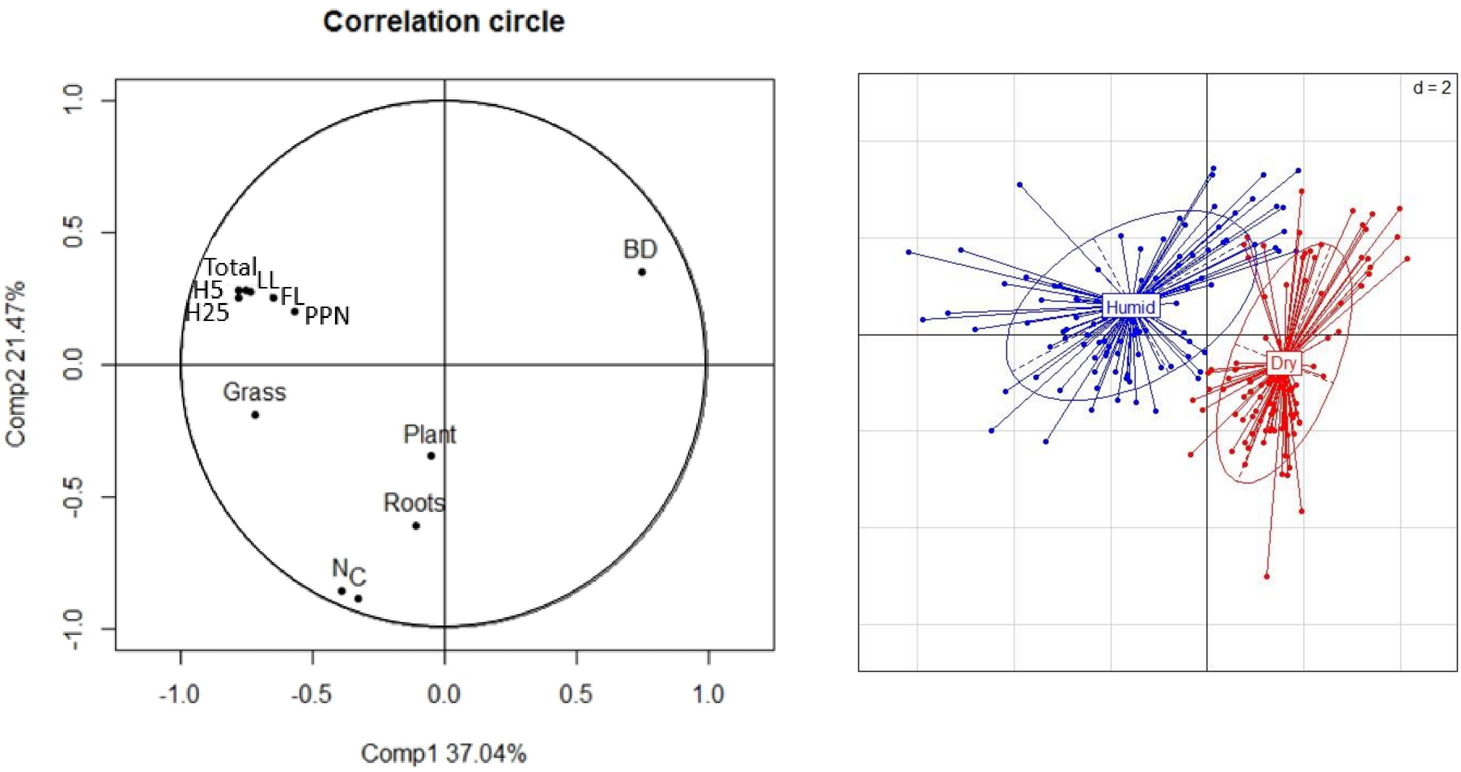


Figure 4 : Correlation circle of PCA and biplot displaying seasons

Soil density is higher in dry season because of the lack of water. And humidity in humid season is logical because of heavy rains. Other studies show an effect of the season on nematode communities (Fitoussi, Pen-Mouratov et Steinberger, 2016; Song et al., 2016), and ours is confirming them.

Location

Location regarding to gullies is also a very important parameter, that we can observe with ANOVA and PCA. The PCA result is more obvious, but the ANOVA test shows a reliable correlation between total number of nematodes and gullies in dry season. As we can see on the graph below, there is a small effect on free living and plant parasitic nematodes too in dry season. It also confirms clearly that there is no effect of gullies in humid season, because there is a lot of water in the soil everywhere, so there is no searching for it. In dry seasons, gullies have less water in the soil because there is less vegetation to retain it. So there are less nematodes.

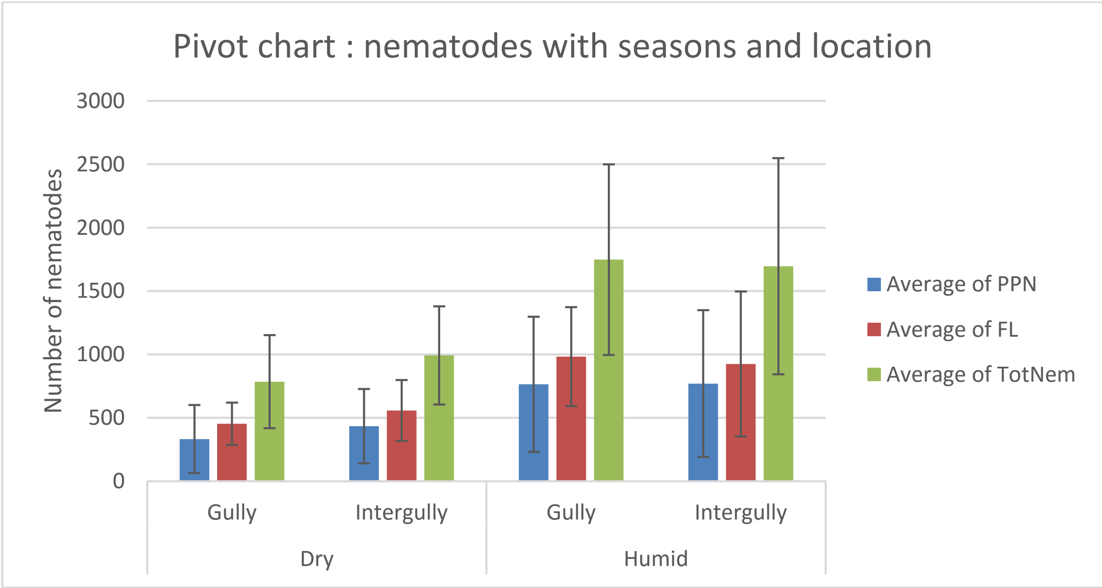
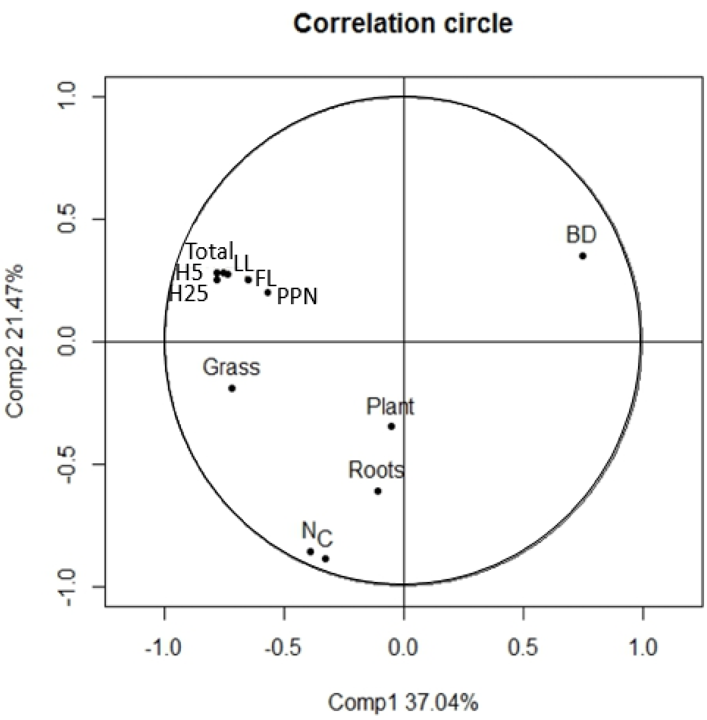


Figure 5 : Graph of the number of nematodes plotted with season and location regarding gullies

The biplot obtained with PCA shows that gullies tend to have a higher bulk density. This is another reason for the least amount of nematodes in gullies : the ground is thicker, nematodes can not live comfortably there. It also confirms that there is more humidity, liveleaves, grass and nematodes in intergullies.



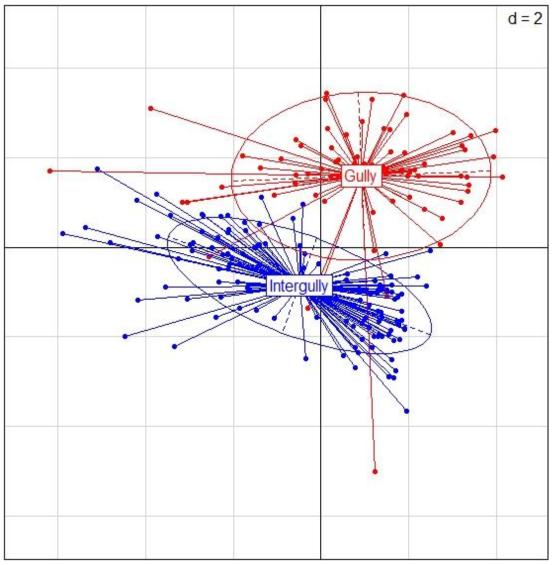


Figure 6 : Correlation circle of PCA and biplot displaying location regarding gullies

It is not possible to confront these results to other articles because of the lack of literature about it (Baxter et al., 2013).

Tree size

Effects of trees and their size is light on nematode communities. We can see on the graph below (figure 7) that there are generally more nematodes in under trees in gullies, except for plant parasitic ones during humid season. In intergullies, samples were taken under medium-size trees. In dry season, free living and total number of nematodes increase progressively and slightly as trees appear and grow. Plant parasitic nematodes, in this case, are more numerous under medium-size trees and then decrease under big trees. In humid season, all nematodes increase under medium trees and then decrease as the tree grows. However, the size of the population in the end is the same as without trees for free living and total number of nematodes, and the population is bigger than before for plant parasitic nematodes.

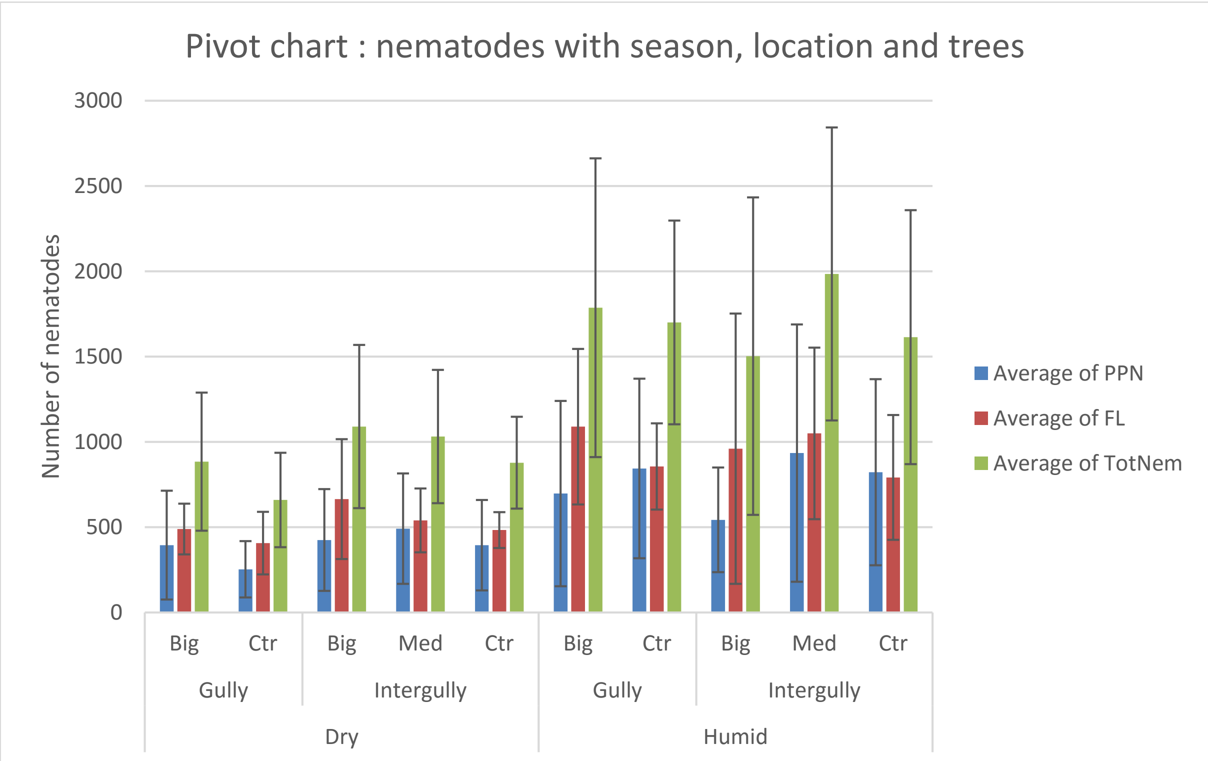


Figure 7 : Graph of the number of nematodes plotted with season, location regarding gullies and tree presence and size

All of these effects are slight, and ANOVA test shows only one correlation : in dry season, in intergullies, there is an effect of trees on free living nematode populations when transforming the variable in its sinus. Here is the table with this result :

	numDF	*denDF*	*F-value*	*p-value*
(Intercept)	1	61	16.126424	0.0002
TreeSize	2	61	4.447661	0.0157

Table 4 : Results of an ANOVA - sin(number of free living nematodes) with tree size in dry season in intergullies

Biplot following shows with more details the difference between size of trees (figure 8) :

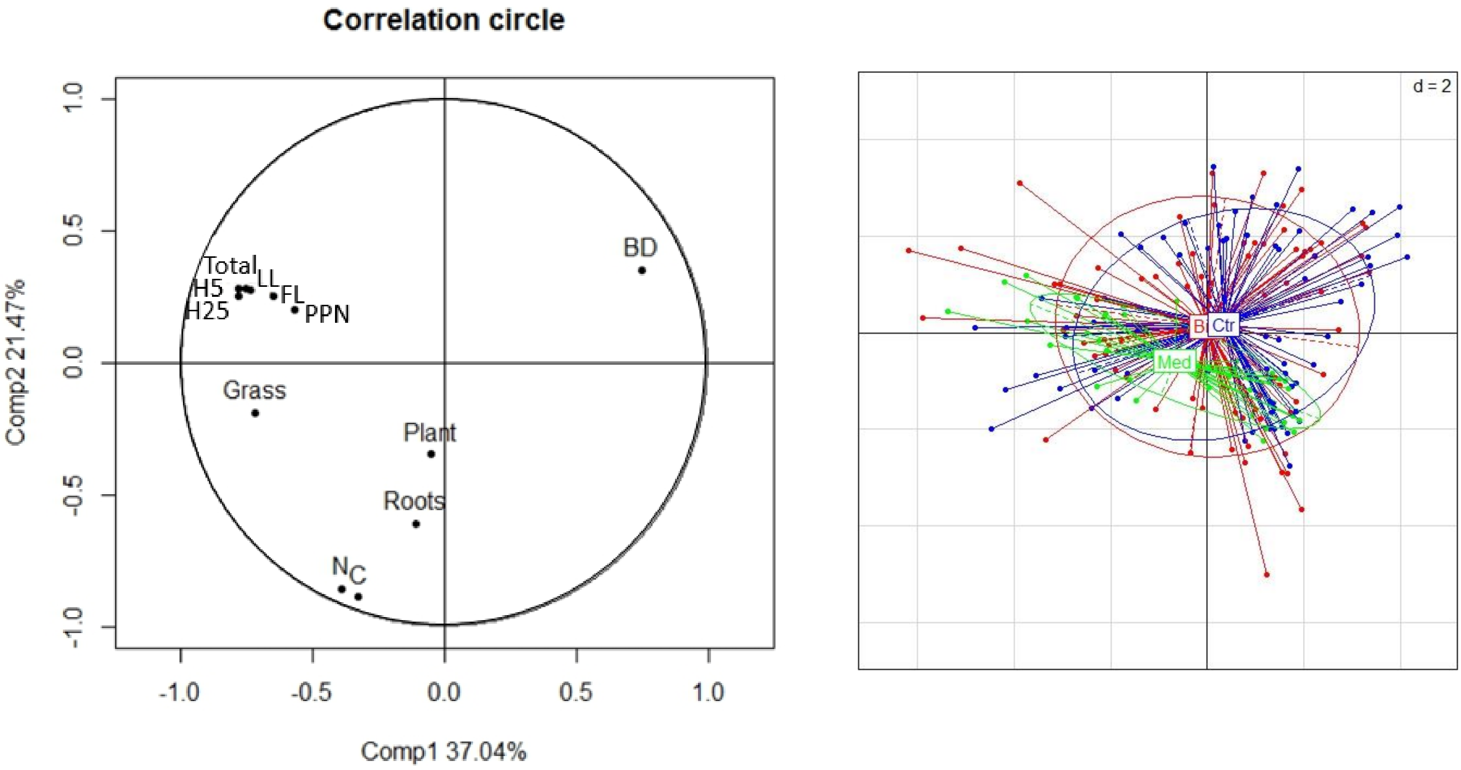


Figure 8 : Correlation circle of PCA and biplot displaying presence and size of trees

We can see there that the dispersion for places with big trees or without trees is quite the same. Although, there is a difference for medium trees : here seem to be more grass under them. Soil is thicker under big trees or without trees than with medium-size trees, but it is difficult to infer anything about other variables : nematodes, liveleaves, humidity, N and C.

The results here remind of the article about nematodes in a tee plantation in China (Li et al., 2007). It was shown there that there is an age/size effect of the trees on nematode communities. There is a disturbance at the beginning of tree encroachment, and it seems like there is an adaptation time after which things go back to normal. But the effect in our case is much smaller.

The fact that we have few results regarding the impact of trees on nematode populations is in contradiction with a study that showed that there were less root parasitic nematodes under woodland in a Texan pasture (Biederman et Boutton, 2009). In our case, plant parasitic nematode communities are smaller under big trees only during humid season. This confirms the importance of the role played by the season in the behavior of nematodes. Since climate changes a lot regarding the study place, results can change notably.

We can confirm here that woodland has an impact on trophic structure (Keith et al., 2006). We saw some changes with trees regarding the diet of nematodes (free living, and plant parasitic, eating roots).

Exclos

The ANOVA shows an effect of the exclos on total number of nematodes and free living nematodes in dry seasons in gullies. The graph below confirms it, and also displays not very significant differences in other cases :

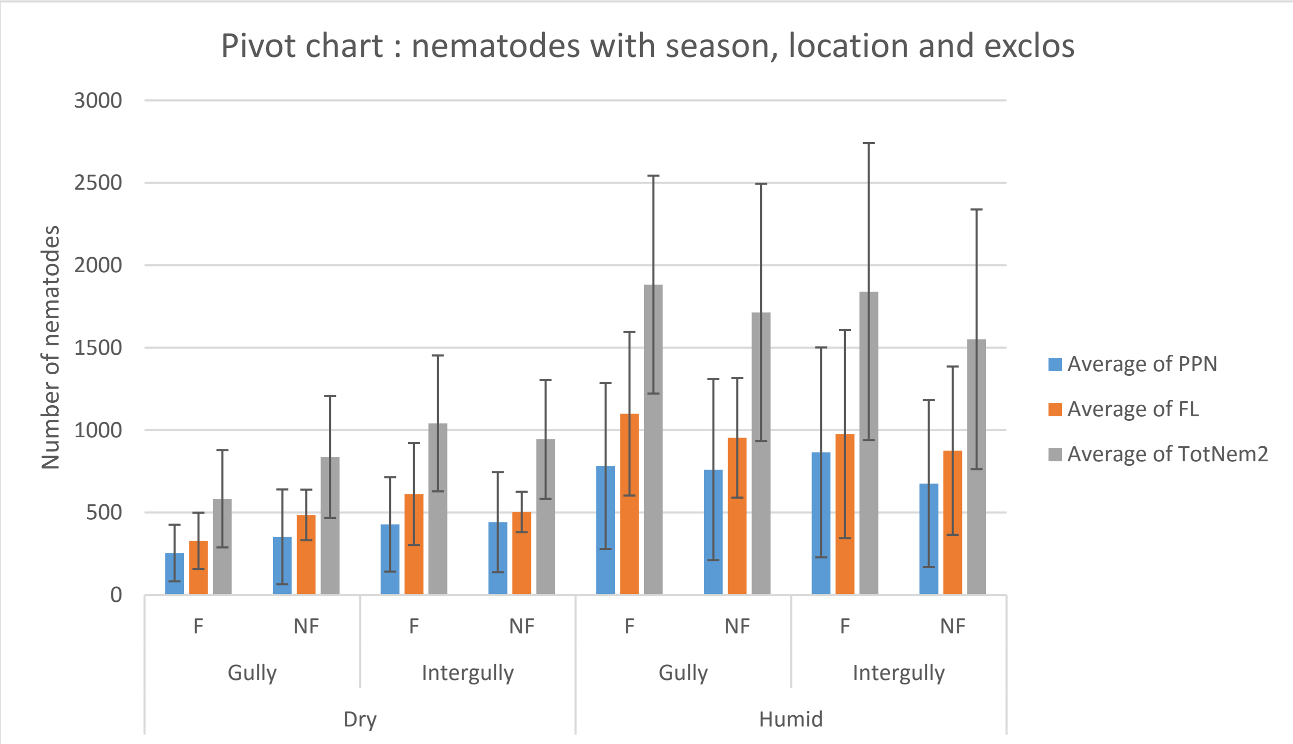


Figure 9 : Graph of the number of nematodes plotted with season, location regarding gullies and grazing spaces

Even if not significant, there is a small difference as nematode communities are a bit larger in fenced zones during humid season.

Here are results tables from the ANOVA :

- In dry season in gullies, there is an effect of the exclos on total number of nematodes. We had to transform the variable in its logarithm :

	numDF	*denDF*	*F-value*	*p-value*
(Intercept)	1	30	7746.901	<0.0001
TreeSize	1	30	3.281	0.0801
Exclos	1	30	4.418	0.0441
TreeSize:Exclos	1	30	1.149	0.2924

Table 5 : Results of an ANOVA - log(total number of nematodes) with tree size and exclos in dry season in gullies

- In dry season in gullies, there is an effect of the exclos on the number of free living nematodes.

	numDF	*denDF*	*F-value*	*p-value*
(Intercept)	1	32	283.13140	<0.0001
Exclos	1	32	5.53437	0.025

Table 6 : Results of an ANOVA – number of free living nematodes with exclos in dry season in gullies

We see here that cattle plays a role on nematode communities, especially on free living ones.

It is shown that cattle has an impact on vegetation and C and N concentration in the soil (Grellier et al., 2013). C and N increase with cattle because of its dejections on the soil. And ground vegetation is weaker as cattle stamps it.

Grazing reduces the amount of plants on the ground, and hence creates a less favorable soil for nematodes by decreasing humidity rate. Bulk density is also higher because of less vegetation and stamping cattle.

Zone

We studied the effect of the zone in the catchment of the sample on nematodes. Our results of ANOVA and PCA (with a biplot) show no correlations.

Finally, a comparison between free living and plant parasitic nematodes is hard to make. We proved with the ANOVA that plant parasitic ones are only sensitive to the season while free living nematodes change with the season but also with tree size (in dry season in intergully) and cattle (in dry season in gullies). Moreover, the parameters affect differently the total number of nematodes. The multiple regression showed that free living nematodes are more influenced by the bulk density and the liveleaves, whereas plant parasitic nematodes are changing with humidity. The total number of nematodes is sensitive to the quantity of liveleaves. Those two studies show that plant parasitic nematodes seem to be less affected by external factors.

CONCLUSION

Our study, located in KwaZulu-Natal, in South Africa, interrogates the link between a tree encroachment and nematodes. In parallel of *Acacia Sieberiana* encroachment, there is a phenomenon of gully erosion in the grasslands. Our goal was to evaluate the effect of trees and gullies on nematode communities.

After statistical analysis (PCA, multiple regression and ANOVA), we can infer some results.

Humidity rate in the soil seems to be one of the main parameters to describe the number of nematodes. Thus, nematode populations change a lot from a season to another because of precipitations. Even if there is no direct correlation between the number of nematodes and the presence of the *Acacia Sieberiana*, presence of living leaves in ground vegetation is an indicator as of the amount of nematodes. Indeed, the more leaves there are, the more nematodes are in the ground. Also, number of nematodes fall in gullies in dry season and it shows that gully erosion phenomenon has an impact on nematodes, even if it is not prevailing. This is because there is less vegetation in gullies, then less water and the soil is thicker. Bulk density is also correlated to nematodes : as it increases, nematode populations decrease. Cattle emphasizes the negative impact of gullies when it grazes there in dry season, and tend generally to make nematode populations smaller by stamping (that lessens vegetation and increases soil density). Finally, we showed that C and N soil concentrations have an effect of nematodes, but we are not quite sure of the exact nature of the relation.

We can also validate our third hypothesis : the study on different trophic levels of nematodes shows a difference. They were found at every test, but were very small changings, questioning us on the validity of this assessment.

We have quite numerous results, but we can not forget that tests were sometimes not realistic enough. That poses a limit to our work. Maybe there are other factors to take into account to have even better results depicting the reality of the dependences. We saw for example that phosphorus is known to change with tree encroachment. We can therefore expect an impact of it on nematode communities.

To open the subject, we should question nematodes as bioindicators. The effect of nematodes and how they can be used as indicators of the state of a grassland is something that should be tested.

REFERENCES

Baxter, C., J. S. Rowan, B. M. McKenzie et R. Neilson. 2013. « Understanding soil erosion impacts in temperate agroecosystems: bridging the gap between geomorphology and soil ecology using nematodes as a model organism ». *Biogeosciences,* vol. 10, n° 11, p. 7133.

Berry, S. D., P. Dana, V. W. Spaull et P. Cadet. 2009. « EFFECT OF INTERCROPPING ON NEMATODES IN TWO SMALL-SCALE SUGARCANE FARMING SYSTEMS IN SOUTH AFRICA ». *NEMATROPICA,* vol. 39, n° 1, p. 11-34.

Berry, S. D., V. W. Spaull et P. Cadet. 2009. « Field assessment of biologically-based control products against nematodes on sugarcane in South Africa ». *African Plant Protection*, p. 1.

Beyrem, Hamouda, et Patricia Aissa. 2000. « Les nématodes libres, organismes-sentinelles de l'évolution des concentrations d'hydrocarbures dans la baie de Bizerte (Tunisie) (French) ». *Free-living nematodes, bio-indicators of the evolution of hydrocarbon concentrations in the bay of Bizerta (Tunisia) (English),* vol. 41, n° 3, p. 329-342.

Biederman, Lori A., et Thomas W. Boutton. 2009. « Biodiversity and trophic structure of soil nematode communities are altered following woody plant invasion of grassland ». *Soil Biology and Biochemistry,* vol. 41, n° 9, p. 1943-1950.

Biederman, Lori A., et Thomas W. Boutton. 2010. « Spatial variation in biodiversity and trophic structure of soil nematode communities in a subtropical savanna parkland: Responses to woody plant encroachment ». *Applied Soil Ecology,* vol. 46, n° 2, p. 168-176.

Bongers, Tom, et Howard Ferris. 1999. « Nematode community structure as a bioindicator in environmental monitoring ». *Trends in Ecology & Evolution,* vol. 14, n° 6, p. 224-228.

Bridge, J. 1996. « Nematode management in sustainable and subsistence agriculture ». *Annual Review of Phytopathology,* vol. 34, p. 201-225.

Cadet, P., S. D. Berry, G. W. Leslie et V. W. Spaull. 2007. « Management of nematodes and a stalk borer by increasing within-field sugarcane cultivar diversity ». *Plant Pathology,* vol. 56, n° 3, p. 526-535.

Chen, Huili, Bo Li, Changming Fang, Jiakuan Chen et Jihua Wu. 2007. « Exotic plant influences soil nematode communities through litter input ». *Soil Biology and Biochemistry,* vol. 39, n° 7, p. 1782-1793.

De Deyn, Gerlinde B., et Wim H. Van der Putten. 2005. « Linking aboveground and belowground diversity ». *Trends in Ecology & Evolution,* vol. 20, n° 11, p. 625-633.

Fitoussi, Nathalia, Stanislav Pen-Mouratov et Yosef Steinberger. 2016. « Soil free-living nematodes as bio-indicators for assaying the invasive effect of the alien plant Heterotheca subaxillaris in a coastal dune ecosystem ». *Applied Soil Ecology,* vol. 102, p. 1-9.

Fourie, Hendrika, Dirk De Waele, Alexander H. Mc Donald, Charlotte Mienie, Mariette Marais et Annelie De Beer. 2015. « Nematode pests threatening soybean production in South Africa, with reference to Meloidogyne : research article ». *South African Journal of Science*, n° 9, p. 125.

Frouz, Jan, Veronika Jílková, Tomáš Cajthaml, Václav Pižl, Karel Tajovský, Ladislav Háněl, Andrea Burešová, Hana Šimáčková, Kateřina Kolaříková, Jennifer Franklin, Jack Nawrot, John W. Groninger et Peter D. Stahl. 2013. « Soil biota in post-mining sites along a climatic gradient in the USA: Simple communities in shortgrass prairie recover faster than complex communities in tallgrass prairie and forest ». *Soil Biology and Biochemistry,* vol. 67, p. 212-225.

Grellier, Séraphine, Jaco Kemp, Jean-Louis Janeau, Nicolas Florsch, David Ward, Sébastien Barot, Pascal Podwojewski, Simon Lorentz et Christian Valentin. 2012. « The indirect impact of encroaching trees on gully extension: A 64 year study in a sub-humid grassland of South Africa ». *CATENA,* vol. 98, p. 110-119.

Grellier, Séraphine, David Ward, Jean-Louis Janeau, Pascal Podwojewski, Simon Lorentz, Luc Abbadie, Christian Valentin et Sébastien Barot. 2013. « Positive versus negative environmental impacts of tree encroachment in South Africa ». *Acta Oecologica,* vol. 53, p. 1-10.

Hibbard, KA, S Archer, DS Schimel et DW Valentine. 2001. « Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna ». *Ecology,* vol. 82, n° 7, p. 1999-2011.

Ito, Takahiro, Masaaki Araki, Tatsuya Higashi, Masakazu Komatsuzaki, Nobuhiro Kaneko et Hiroyuki Ohta. 2015. « Responses of soil nematode community structure to soil carbon changes due to different tillage and cover crop management practices over a nine-year period in Kanto, Japan ». *Applied Soil Ecology,* vol. 89, p. 50-58.

Jackson, Robert B, Jay L Banner, Esteban G Jobbágy, William T Pockman et Diana H Wall. 2002. « Ecosystem carbon loss with woody plant invasion of grasslands ». *Nature,* vol. 418, n° 6898, p. 623-626.

Keith, Aidan M, Rene Van der Wal, Rob W Brooker, Graham HR Osler, Stephen J Chapman et David FRP Burslem. 2006. « Birch invasion of heather moorland increases nematode diversity and trophic complexity ». *Soil Biology and Biochemistry,* vol. 38, n° 12, p. 3421-3430.

Klass, Jeremy R., Debra P. C. Peters, Jacqueline M. Trojan et Stephen H. Thomas. 2012. « Nematodes as an indicator of plant–soil interactions associated with desertification ». *Applied Soil Ecology,* vol. 58, p. 66-77.

Kostenko, Olga, Henk Duyts, Saskia Grootemaat, Gerlinde B. De Deyn et T. Martijn Bezemer. 2015. « Plant diversity and identity effects on predatory nematodes and their prey ». *Ecology and Evolution,* vol. 5, nº 4, p. 836-847.

Li, Yujuan, Jingyuan Feng, Jiakuan Chen et Jihua Wu. 2007. « Original vegetation type affects soil nematode communities ». *Applied Soil Ecology,* vol. 35, nº 1, p. 68-78.

Liao, J. D., et T. W. Boutton. 2008. « Soil microbial biomass response to woody plant invasion of grassland ». *Soil Biology and Biochemistry,* vol. 40, nº 5, p. 1207-1216.

Macias, Daniel, Noemí Mazía et Elizabeth Jacobo. 2014. « Grazing and neighborhood interactions limit woody encroachment in wet subtropical savannas ». *Basic and Applied Ecology,* vol. 15, nº 8, p. 661-668.

Malherbe, Stephanus, et Diana Marais. 2015. « Nematode community profiling as a soil biology monitoring tool in support of sustainable tomato production: A case study from South Africa ». *Applied Soil Ecology,* vol. 93, p. 19-27.

Nandjui, J, PG Gnonhouri, JE Tondoh et Y Tano. 2008. « Réponse des nématodes à la perturbation des forêts dans la région d\'Oumé, Côte d\'Ivoire ». *Sciences & Nature,* vol. 4, nº 2, p. 189-196.

Ruan, Wei-Bin, Tao Ren, Qing Chen, Xiang Zhu et Jing-Guo Wang. 2013. « Effects of conventional and reduced N inputs on nematode communities and plant yield under intensive vegetable production ». *Applied Soil Ecology,* vol. 66, p. 48-55.

Scholes, R. J., et S. R. Archer. 1997. « Tree-Grass Interactions in Savannas ». *Annual Review of Ecology and Systematics,* vol. 28, p. 517-544.

Sitters, Judith, Peter J. Edwards et Harry Olde Venterink. 2012. « Increases of Soil C, N, and P Pools Along an Acacia Tree Density Gradient and Their Effects on Trees and Grasses ». *Ecosystems,* vol. 16, nº 2, p. 347-357.

Sitters, Judith, Peter Edwards, Werner Suter et Harry Olde Venterink. 2015. « Acacia tree density strongly affects N and P fluxes in savanna ». *Biogeochemistry,* vol. 123, nº 1/2, p. 285-297.

Song, Min, Shuishui Jing, Yaqiong Zhou, Yan Hui, Lili Zhu, Feng Wang, Dafeng Hui, Lin Jiang et Shiqiang Wan. 2015. « Dynamics of soil nematode communities in wheat fields under different nitrogen management in Northern China Plain ». *European Journal of Soil Biology,* vol. 71, p. 13-20.

Song, Min, Xiaoming Li, Shuishui Jing, Lingjie Lei, Jiali Wang et Shiqiang Wan. 2016. « Responses of soil nematodes to water and nitrogen additions in an old-field grassland ». *Applied Soil Ecology,* vol. 102, p. 53-60.

Tabarant, Perrine, Cécile Villenave, Jean-Michel Risede, Jean Roger-Estrade, Laurent Thuries et Marc Dorel. 2011. « Effects of four organic amendments on banana parasitic nematodes and soil nematode communities ». *Applied Soil Ecology,* vol. 49, p. 59-67.

Veuillez sélectionner un type de document autre que « Generic » afin de faire afficher la référence bibliographique.

Van Auken, O. W. 2009. « Causes and consequences of woody plant encroachment into western North American grasslands ». *Journal of Environmental Management,* vol. 90, n° 10, p. 2931-2942.

Yeates, G. W. 1999. « Effects of plants on nematode community structure ». *Annual Review of Phytopathology,* vol. 37, p. 127-149.

Zhao, Jie, Faming Wang, Jian Li, Bi Zou, Xiaoli Wang, Zhian Li et Shenglei Fu. 2014. « Effects of experimental nitrogen and/or phosphorus additions on soil nematode communities in a secondary tropical forest ». *Soil Biology and Biochemistry,* vol. 75, p. 1-10.

Zhao, Jie, Rui Xun, Xunyang He, Wei Zhang, Wei Fu et Kelin Wang. 2015. « Size spectra of soil nematode assemblages under different land use types ». *Soil Biology and Biochemistry,* vol. 85, p. 130-136.

ANNEXES

ANNEX 1 : PROGRAMMING CODES FOR STATISTICAL ANALYSIS

PCA

```
library(ade4)
donnees<-read.table("Data.csv", sep=";", dec=",", header=T)
names(donnees)
acp<-dudi.pca(donnees[,7:18])
s.label(acp$li, xax=1, yax=2) #répondre 3

cc1<-acp$co[,1]
cc2<-acp$co[,2]
plot(cc1,cc2,type="n", main="Correlation circle", xlim=c(-1,1), ylim=c(-1,1), asp=1,
ylab= "Comp2 21.47%", xlab= "Comp1 37.04%")
abline(h=0,v=0)
text(cc1,cc2,row.names(acp$co), pos=3, cex=1)
points(cc1,cc2, pch=20)
symbols(0,0,circles=1,inches=FALSE,add=TRUE)

s.corcircle(acp$co, xax = 1, yax = 2)
symbols(0,0,circles=1,inches=FALSE,add=TRUE)
inertia.dudi(acp,col.inertia = T)$col.abs

# Données de lieu
lieu<-donnees$Location
s.class(dfxy = acp$li, fac = lieu, col = c("red","blue","green"), xax = 1, yax = 2)

# Données de direction
direction<-donnees$Direction
s.class(dfxy = acp$li, fac = direction, col = c("red","blue","green"), xax=1, yax=2)

# Données sur la position dans le bassin versant
bv<-donnees$Zone
s.class(dfxy = acp$li, fac = bv, col = c("red","blue","green","yellow"), xax=1, yax=2)

# Données sur la taille de l'arbre
arbre<-donnees$TreeSize
s.class(dfxy = acp$li, fac = arbre, col = c("red","blue", "green"), xax = 1, yax = 2)

# Données sur saison
saison<-donnees$Season
s.class(dfxy = acp$li, fac = saison, col = c("red","blue", "green"), xax=1, yax=2)

# Données sur le bétail
fence<-donnees$Exclos
s.class(dfxy = acp$li, fac = fence, col = c("red","blue", "green"), xax = 1, yax = 2)
```

Multiple regression

This code is the one for the total number of nematodes, after it was transformed into its logarithm. Codes for free living and plant parasitic nematodes are not here but follow the same method.

```
data<-read.csv("Data.csv", header=T, sep=";", dec=",")
pairs(data, panel=panel.smooth)
names(data)
attach(data)
library(mgcv)

BD2<-BD*BD
H2<-Humid5*Humid5
L2<-Liveleaves*Liveleaves
C2<-C*C
N2<-N*N
G2<-Grass*Grass

### Modèle de base
model2<lm(log(Total)~BD*Humid5*Liveleaves*C*N*Grass+BD2+H2+L2+C2+N2+L2+G2)
summary(model2)

### On supprime les interactions non significatives au fur et à mesure
model3<-update(model2,~.-G2)
summary(model3)

model4<-update(m33,~.-C:N)
summary(model4)

model5<-update(model4,~.-BD:Humid5:N)
summary(model5)

model6<-update(model5,~.-BD:Humid5:N:Grass)
summary(model6)

model7<-update(model6,~.-BD2)
summary(model7)

model8<-update(model7,~.-BD:Humid5:C:N)
summary(model8)

model9<-update(model8,~.-BD:Humid5:C)
summary(model9)

model10<-update(model9,~.-BD)
summary(model10)

model11<-update(model10,~.-C2)
summary(model11)

model12<-update(model11,~.-Liveleaves:C:N:BD)
summary(model12)

model13<-update(model12,~.-Humid5:Liveleaves:C:N:BD)
summary(model13)

model14<-update(model13,~.-Humid5:C)
summary(model14)

model15<-update(model14,~.-Liveleaves:N:Grass:BD)
summary(model15)

model16<-update(model15,~.-Liveleaves:N:Grass)
summary(model16)
```

```
model17<-update(model16,~.-C:BD)
summary(model17)

model18<-update(model17,~.-Humid5:C:N:Grass:BD)
summary(model18)

model19<-update(model18,~.-Humid5:Liveleaves:C:N:Grass:BD)
summary(model19)

model20<-update(model19,~.-C:Grass:BD)
summary(model20)

model21<-update(model20,~.-Liveleaves:C:N:Grass:BD)
summary(model21)

model22<-update(model21,~.-Liveleaves:C:N)
summary(model22)

model23<-update(model22,~.-Humid5:BD)
summary(model23)

model24<-update(model23,~.-Humid5)
summary(model24)

model25<-update(model24,~.-C:N:Grass:BD)
summary(model25)

model26<-update(model25,~.-L2)
summary(model26)

model27<-update(model26,~.-Liveleaves:N:Grass:BD:Humid5)
summary(model27)

model28<-update(model27,~.-Liveleaves:N:Grass:Humid5)
summary(model28)

model29<-update(model28,~.-Liveleaves:C:BD:Humid5)
summary(model29)

model30<-update(model29,~.-C:N:Humid5)
summary(model30)

model31<-update(model30,~.-N:Humid5)
summary(model31)

model32<-update(model31,~.-H2)
summary(model32)

model33<-update(model32,~.-C:Grass)
summary(model33)

model34<-update(model33,~.-C:N:Grass)
summary(model34)

model35<-update(model34,~.-C:N:BD)
summary(model35)

model36<-update(model35,~.-N:Grass:BD)
summary(model36)

model37<-update(model36,~.-Liveleaves:C:Humid5)
summary(model37)

model38<-update(model37,~.-Liveleaves:C:BD)
summary(model38)

model39<-update(model38,~.-Liveleaves:C:Grass:BD)
```

```
summary(model39)

model40<-update(model39,~.-Liveleaves:C:Grass:BD:Humid5)
summary(model40)

model41<-update(model40,~.-C)
summary(model41)

model42<-update(model41,~.-Grass:BD)
summary(model42)

model43<-update(model42,~.-Liveleaves:Grass:BD)
summary(model43)

model44<-update(model43,~.-Liveleaves:Grass:BD:Humid5)
summary(model44)

model45<-update(model44,~.-Liveleaves:C)
summary(model45)

model46<-update(model45,~.-Liveleaves:Grass)
summary(model46)

model47<-update(model46,~.-Grass)
summary(model47)

model48<-update(model47,~.-N:Grass)
summary(model48)

model49<-update(model48,~.-N:Humid5:Grass)
summary(model49)

model50<-update(model49,~.-BD:Humid5:Grass)
summary(model50)

model51<-update(model50,~.-BD:Humid5:Grass:C)
summary(model51)

model52<-update(model51,~.-Liveleaves:Humid5:Grass)
summary(model52)

model53<-update(model52,~.-Liveleaves:N:Humid5:C)
summary(model53)

### On a un modèle où tout est significatif, donc on fait les graphes pour voir si
les résidus sont bien dispersés
par(mfrow=c(2,2))
plot(model53)
```

ANOVA

```
####### ANOVA pour les FL
data<-read.csv("DataANOVA.csv", header=T, sep=";", dec=",")
str(data)
library(nlme)

attach(data)
lme1=lme(fixed=FL~Season*Location, random=~1|ID)
anova(lme1)
shapiro.test(residuals(lme1)) #Faux
# Tests non paramétriques
kruskal.test(PPN~Season) # Effet saison
kruskal.test(PPN~Location) # Pas d'effet de lieu
```

```
#### SAISON SECHE
dry<-subset(data, Season=="Dry")
dry
attach(dry)
lme2=lme(fixed=FL~Location, random=~1|ID)
anova(lme2)
shapiro.test(residuals(lme2)) # Faux
#Test non paramétrique
kruskal.test(FL~Location) # Pas d'effet de lieu

###RAVINE
gullydry<-subset(dry, Location=="Gully")
gullydry
attach(gullydry)
lme3=lme(fixed=FL~TreeSize*Exclos, random=~1|ID)
anova(lme3)
# On peut enlever l'intéraction car non significative
lme4 = update(lme3,~.-TreeSize:Exclos)
anova(lme4)
# On peut enlever TreeSize car non significatif
lme5 = update(lme4,~.-TreeSize)
anova(lme5)
shapiro.test(residuals(lme2m2)) #vérifié
bartlett.test(residuals(lme2m2),gullydry$Exclos) #vérifié

###INTERRAVINE
intergullydry<-subset(dry, Location=="Intergully")
intergullydry
attach(intergullydry)
lme6=lme(fixed=sin(FL)~Zone*TreeSize*Exclos, random=~1|ID)
anova(lme6)
lme7=update(lme6,~.-Zone:TreeSize:Exclos)
anova(lme7)
lme8=update(lme7,~.-Zone:Exclos)
anova(lme8)
lme9=update(lme8,~.-Zone)
anova(lme9)
lme10=update(lme9,~.-TreeSize:Zone)
anova(lme10)
lme11=update(lme10,~.-TreeSize:Exclos)
anova(lme11)
lme12=update(lme11,~.-Exclos)
anova(lme12)
shapiro.test(residuals(lme12)) #vérifié
bartlett.test(residuals(lme12),intergullydry$TreeSize) #vérifié


#### SAISON HUMIDE
humid<-subset(data, Season=="Humid")
attach(humid)
lme13=lme(fixed=FL~Location, random=~1|ID)
anova(lme13) # Pas d'effet de lieu

###RAVINE
gullyhumid<-subset(humid, Location=="Gully")
gullyhumid
attach(gullyhumid)
lme14=lme(fixed=FL~TreeSize*Exclos, random=~1|ID)
anova(lme14) # Aucun effet
kruskal.test(FL~Exclos) # Pas d'effet exclos
kruskal.test(FL~TreeSize) # Pas d'effet de taille des arbres

###INTERRAVINE
intergullyhumid<-subset(humid, Location=="Intergully")
intergullyhumid
attach(intergullyhumid)
lme15=lme(fixed=FL~Zone*TreeSize*Exclos, random=~1|ID)
anova(lme15)
```

```
lme16=update(lme15,~.-Zone:TreeSize:Exclos)
anova(lme16)
lme17=update(lme16,~.-Zone:TreeSize)
anova(lme17)
lme18=update(lme17,~.-Zone:Exclos)
anova(lme18)
lme19=update(lme18,~.-Zone)
anova(lme19)
shapiro.test(residuals(lme9)) # Vérifié
bartlett.test(residuals(lme9),intergullydry$TreeSize) # Vérifié
```

ANNEX 2 : TABLES OF MULTIPLE REGRESSION

Log (free living nematodes)

	Estimate	*Std. Error*	*t value*	*Pr(>|t|)*	
(Intercept)	1.779e+01	2.508e+00	7.094	4.06e-11	***
BD	-7.387e+00	1.630e+00	-4.533	1.14e-05	***
Humid5	-1.411e+00	4.023e-01	-3.506	0.000590	***
Grass	-5.075e-01	1.863e-01	-2.724	0.007178	**
C2	6.238e-01	1.827e-01	3.415	0.000809	***
BD:Humid5	8.560e-01	2.619e-01	3.269	0.001325	**
Humid5:N	1.042e+01	2.855e+00	3.649	0.000357	***
N:Liveleaves	-5.580e+00	1.483e+00	-3.761	0.000237	***
N:C	-5.089e+01	1.361e+01	-3.739	0.000258	***
BD:Grass	3.129e-01	1.275e-01	2.454	0.015186	*
Humid5:Grass	3.754e-02	9.886e-03	3.797	0.000208	***
Grass:Liveleaves	4.199e-02	1.191e-02	3.525	0.000552	***
Grass:N	3.671e+00	1.364e+00	2.691	0.007883	**
BD:Humid5:N	-5.930e+00	1.841e+00	-3.221	0.001550	**
BD:N:Liveleaves	3.833e+00	1.047e+00	3.661	0.000342	***
BD:N:C	2.876e+01	9.004e+00	3.195	0.001688	**
Humid5:N:C	-5.020e-01	1.660e-01	-3.024	0.002908	**
BD:Humid5:Grass	-2.107e-02	6.438e-03	-3.273	0.001306	**
BD:Grass:Liveleaves	-2.423e-02	7.496e-03	-3.233	0.001492	**
Humid5:Grass:Liveleaves	-1.983e-03	5.931e-04	-3.343	0.001032	**
Humid5:Grass:C	-3.010e-03	6.640e-04	-4.533	1.14e-05	***
Grass:Liveleaves:C	-2.727e-02	8.507e-03	-3.206	0.001630	**
BD:Grass:N	-2.148e+00	9.126e-01	-2.354	0.019792	*
Humid5:Grass:N	-2.524e-01	7.435e-02	-3.394	0.000869	***
Grass:N:C	-1.243e-01	5.535e-02	-2.246	0.026084	*
BD:Humid5:Liveleaves:C	4.546e-03	1.326e-03	3.429	0.000772	***
Humid5:N:Liveleaves:C	1.369e-01	2.912e-02	4.700	5.59e-06	***
BD:Humid5:Grass:Liveleaves	1.034e-03	3.605e-04	2.869	0.004682	**
BD:Grass:Liveleaves:C	1.757e-02	5.523e-03	3.181	0.001766	**
Humid5:Grass:Liveleaves:C	1.294e-03	4.237e-04	3.055	0.002644	**
BD:Humid5:Grass:N	1.280e-01	4.634e-02	2.762	0.006430	**
BD:Grass:N:Liveleaves	-5.248e-02	1.430e-02	-3.670	0.000331	***
Humid5:Grass:N:C	3.108e-02	6.976e-03	4.455	1.57e-05	***
Grass:N:Liveleaves:C	2.961e-02	9.445e-03	3.135	0.002047	**
BD:Humid5:N:Liveleaves:C	-1.207e-01	2.552e-02	-4.732	4.89e-06	***
BD:Humid5:Grass:Liveleaves:C	-7.968e-04	2.771e-04	-2.875	0.004592	**
BD:Humid5:Grass:N:Liveleaves	3.594e-03	8.888e-04	4.043	8.19e-05	***
Humid5:Grass:N:Liveleaves:C	-2.013e-03	5.268e-04	-3.822	0.000190	***
Signif. codes :	0 '***' 0,001 '**' 0,01 '*' 0,05 '°' 0,1 ' ' 1				

Residual standard error	0,3785
Multiple R-squared	0,5658
Adjusted R-squared	0,4648

Table 7 : Multiple regression of log(number of free living nematodes)

Log(plant parasitic nematodes)

	Estimate	*Std. Error*	*t value*	*Pr(>|t|)*	
(Intercept)	4.131139	0.462618	8.930	4.34e-16	***
Liveleaves	1.764557	0.604658	2.918	0.003959	**
Liveleaves:BD	-1.043747	0.365650	-2.854	0.004805	**
Liveleaves:Humid5	-0.018358	0.006978	-2.631	0.009244	**
BD:N	14.803502	4.452838	3.325	0.001069	**
Liveleaves:N	-23.912880	8.479083	-2.820	0.005325	**
N:C	23.034388	6.541563	3.521	0.000542	***
Liveleaves:BD:N	14.477577	5.168249	2.801	0.005635	**
Liveleaves:Humid5:N	0.252665	0.094300	2.679	0.008045	**
BD:N:C	-20.375470	5.255524	-3.877	0.000147	***
Liveleaves:N:C	6.517060	2.736903	2.381	0.018278	*
Liveleaves:BD:N:C	-3.977055	1.666157	-2.387	0.018002	*
Liveleaves:Humid5:N:C	-0.072546	0.030384	-2.388	0.017971	*
Signif. codes :	0 '***' 0,001 '**' 0,01 '*' 0,05 '°' 0,1 ' ' 1				
Residual standard error	0,823				
Multiple R-squared	0,2763				
Adjusted R-squared	0,2291				

Table 8 : Multiple regression of log(number of plant parasitic nematodes)

ANNEX 3 : SUMMARIZING DIAGRAMS OF SEVERAL EFFECTS SHOWN BY ANOVA

Diagrams below represent the different effects of variables on nematodes. They were enlighten by ANOVA test.

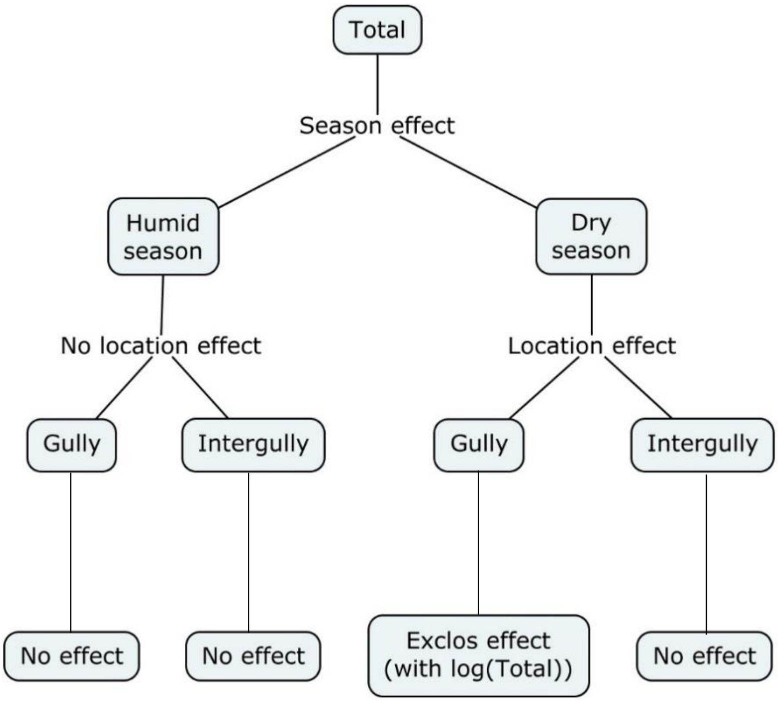


Figure 10 : Diagram of the ANOVA process - Total number of nematodes

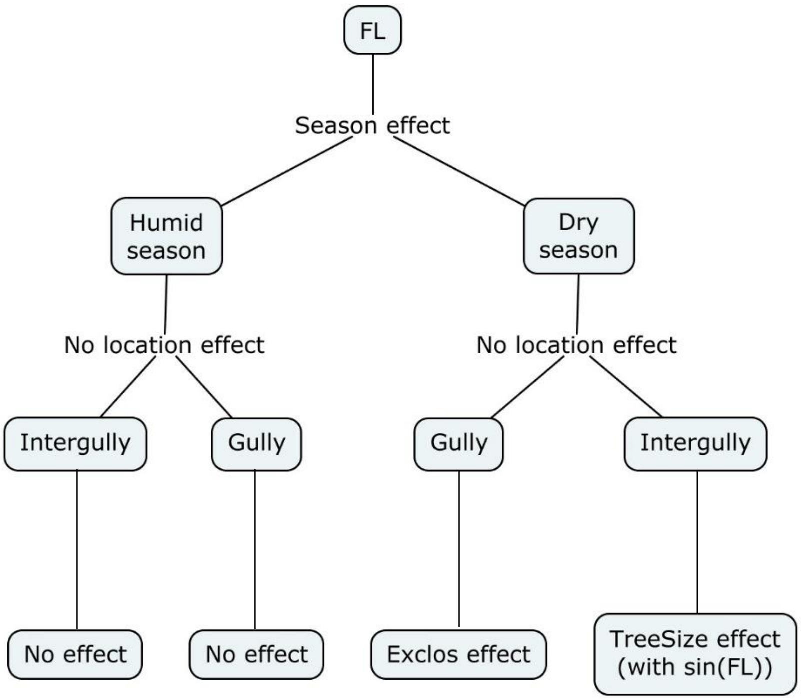


Figure 11: Diagram of the ANOVA process - Number of free living nematodes

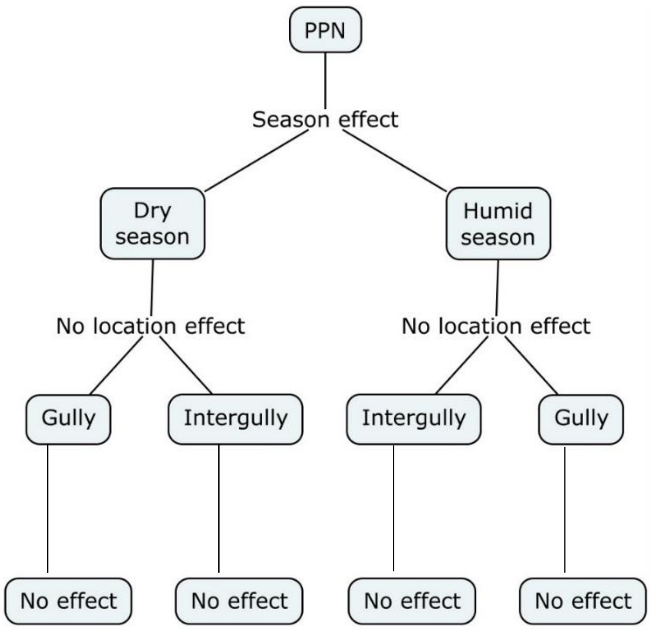


Figure 12 : Diagram of the ANOVA process - Number of plant parasitic nematodes

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2015-2016

Les nématodes, des bio-indicateurs en lien avec l'invasion ligneuse : cas de l'invasion de l'*Acacia Sieberiana* dans les pâturages d'Afrique du Sud

Résumé :

En Afrique du Sud, l'invasion d'*Acacia Sieberiana* transforme les prairies naturelles en savanes. L'enjeu principal est la perte de bons pâturages pour le bétail. En effet, les communautés locales reposent sur l'agriculture et l'élevage. Parallèlement à l'invasion ligneuse, une érosion en ravines est observée sur place. Des équipes scientifiques cherchent à bien comprendre les mécanismes en jeu : quelles sont les causes des ravines, quel est le lien avec l'invasion d'arbres, quelles sont les conséquences des deux phénomènes… Une approche, étudiée dans ce Projet de Fin d'Études, est de considérer les nématodes, partie importante de la faune du sol. Des recherches ont déjà démontré des liens entre les nématodes et les propriétés du sol, et l'effet d'une invasion d'arbres sur ces dernières est aussi connue. Notre travail vise à faire le lien entre les propriétés du sol, les nématodes, l'*Acacia Sieberiana* et les ravines. Pour ceci, nous avons des données qui caractérisent les populations de nématodes, le sol et le lieu. Après trois analyses statistiques (ACP, régression multiple et ANOVA), nous avons quelques résultats. Nous ne pouvons pas démontrer une causalité directe. Cependant, nous observons un effet de l'humidité dans le sol, de la saison et des feuilles encore vertes sur le sol. Aussi, nous notons que les ravines ont moins de végétation (favorable aux nématodes), ce qui explique leur effet négatif. Les arbres n'ont pas vraiment d'effet, mais la végétation basse est très positive pour les nématodes.

Mots Clés : nématodes, pâturages, invasion ligneuse, écologie