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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

KNEFATI Sarah

LENOIR Amélie

2015-2016

Directeur de recherche

GRELLIER Séraphine

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FORMATION PAR LA RECHERCHE ET PROJET DE FIN D'ETUDES EN GENIE DE L'AMENAGEMENT

La formation au génie de l'aménagement, assurée par le département aménagement de l'Ecole Polytechnique de l'Université de Tours, associe dans le champ de l'urbanisme et de l'aménagement, l'acquisition de connaissances fondamentales, l'acquisition de techniques et de savoir faire, la formation à la pratique professionnelle et la formation par la recherche. Cette dernière ne vise pas à former les seuls futurs élèves désireux de prolonger leur formation par les études doctorales, mais tout en ouvrant à cette voie, elle vise tout d'abord à favoriser la capacité des futurs ingénieurs à :

- 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 they 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 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 the tree encroachment is affecting soil properties, hence the nematode communities and thus the soil resistance. However, the 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. The 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 the changings in the soil microbial constitution following tree encroachment. The C and N pools became more important due to modifications in the litter (its quantity and quality) (Liao et Boutton, 2008). Also, the pools changed depending on the soil, the topography and the 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 the tree encroachment and the nematode community 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 the soil and the sub-canopy vegetation. Indeed, the 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 influences 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.

At the study site, water presence depends on the season and the clay soil humidity at different deeps on the grassland. The different slopes on the watershed between the top, middle and bottom of it also modifies the drainage. Moreover, the pasture of Potshini presents a gully erosion phenomenon. This ground erosion is emphasized by the presence of the encroaching trees that changes 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).

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). The biome there is Northern KwaZulu-Natal moist grassland. Vegetation is dominated by *Hyparrhenia hirta* and *Sporobolus africanus*.

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 watershed of Tugela Basin (30 000 km²). 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, Direction and Tree Size. The Season parameter takes two values : Dry and Humid. It determines the climate and the 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 watershed Zone parameter) can take three values : up (at the top of the watershed, highest altitude and steepest slope), mid (in the middle of the watershed) and down (at the bottom of the watershed, lowest altitude and smoothest slope). The orientation (Direction parameter) can be North or South. It influences sunshine conditions on the plot. 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
Direction	S, N	Exposition, North or South
TreeSize	Big, Med, Ctr	If there is a tree above the sample, and its size

Figure 1 : Table of the 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	%	Humid.5, H5
Humidity 25 cm	%	Humid.25, H25
Roots	Mg	
Grass	G	
Living leaves	G	Liveleaves, LL
Plant	G	

Figure 2 : Table of 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 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, orientation, watershed…). 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. Then we made biplots, that take previous results and displays all samples and one of the qualitative parameters. We made 6 biplots, each displaying a parameter : season, location, direction, exclos, tree size and zone in the watershed. 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 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-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 qualitative 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 PPN and once for FL. 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.

RESULTS

PCA

PCA is the first of the statistical tests performed. Its first result is a correlation circle of all variables (Figure 3). It shows a strong positive correlation between humidity in the soil (humid.5 and humid.25), nematode populations (Total, FL and PPN) and liveleaves of ground vegetation. Thus, the wetter the soil is and the more leaves there are in the ground vegetation, the more nematodes there are. These variables are also negatively correlated to bulk density (BD). Thus, the thicker the soil is, the fewer nematodes are living there. C and N concentrations in the soil are also positively correlated together. But there is no correlation between those two and the amount of nematodes, humidity in the soil or liveleaves. Bulk density is also negatively correlated with C, N and the grass.

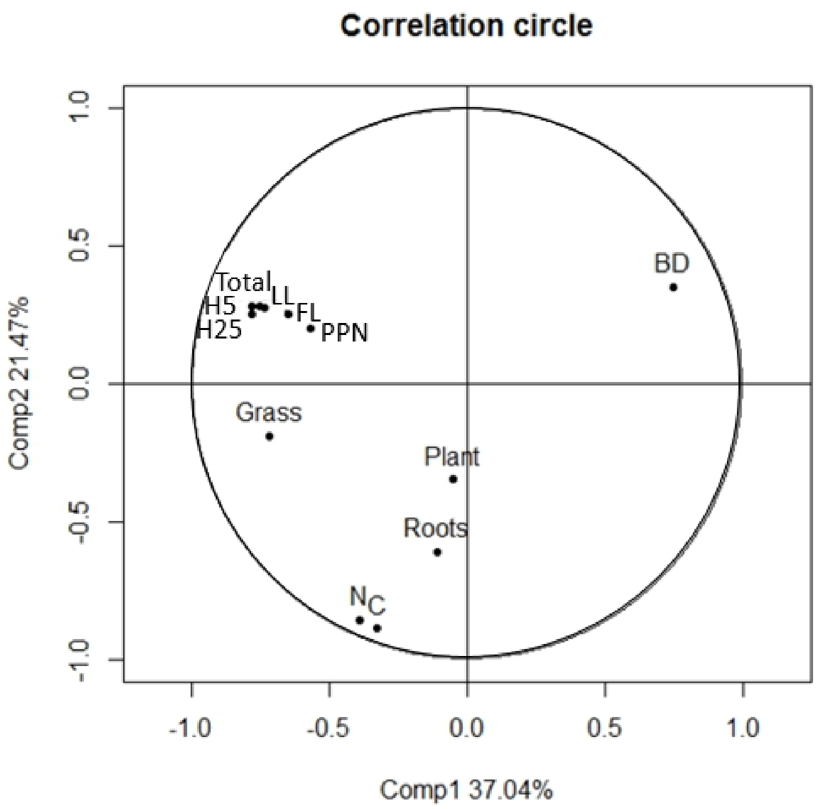

Figure 3: Correlation circle of the PCA. (H5 : Humidity 5cm, H25 : Humidity 25cm, LL : Liveleaves)

We can also have the contribution of each variable in each axis (Figure 4) :

	Comp 1	*Comp 2*	*Comp 3*
FL	9.57	2.52	8.71
PPN	7.35	1.61	26.69
Total	12.83	3.09	25.69
C	2.44	30.59	0.01
N	3.45	28.47	0
BD	12.47	4.77	1.90
Humid 5	13.77	3.02	12.22
Humid 25	13.80	2.43	14.34
Root	0.26	14.46	4.11
Grass	11.76	1.41	0.26
Plant	0.07	4.72	0.03
Liveleaves	12.22	2.92	6.04

Figure 4 : Contribution of variables on the axis of PCA

F1 axis is described at 27% by humidity in the soil and at 12% each by total number of nematodes, bulk density and liveleaves. The F2 axis is reprensented at about 30% each by C and N concentrations in the soil.

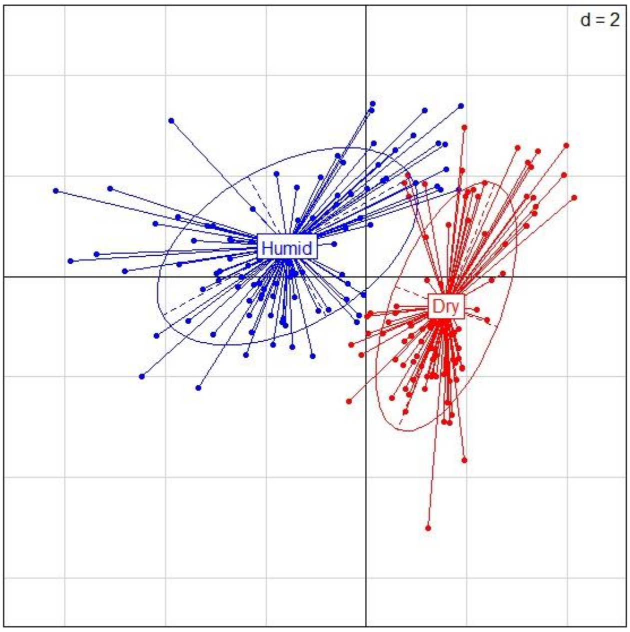

Figure 5 : Biplot of the samples - Qualitative variable : Season

For studied variables, PCA biplot (Figure 5) shows that their behavior changes between humid and dry seasons. Indeed, humidity in the soil, total number of nematodes and quantity of liveleaves are higher in humid season, and bulk density is lower.

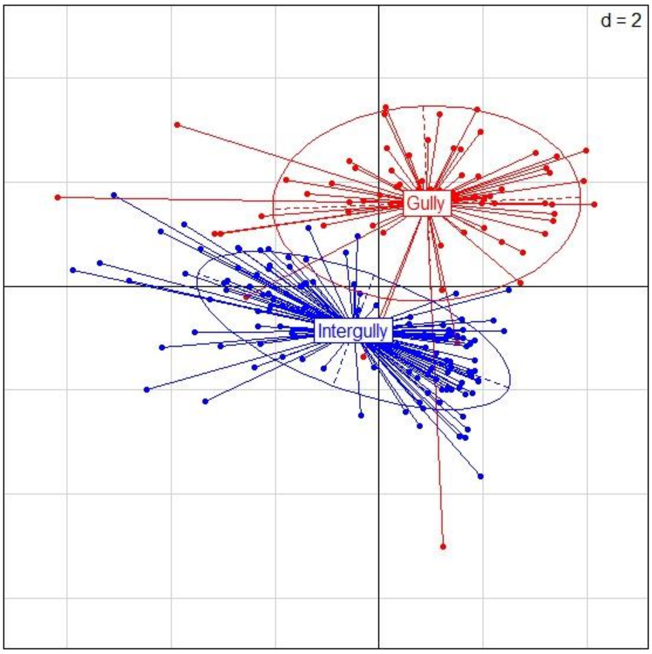

Figure 6 : Biplot of the samples - Qualitative variable : Location regarding gullies

Also, variables change regarding the position of the sample (in a gully or out of it) (Figure 6). The soil seems to be less rich of C and N in gullies. Moreover, humidity and the amount of liveleaves and nematodes is higher in intergullies, where the ground is also less thick.

Following biplots are not significant enough, but we can infer interpretation of them :

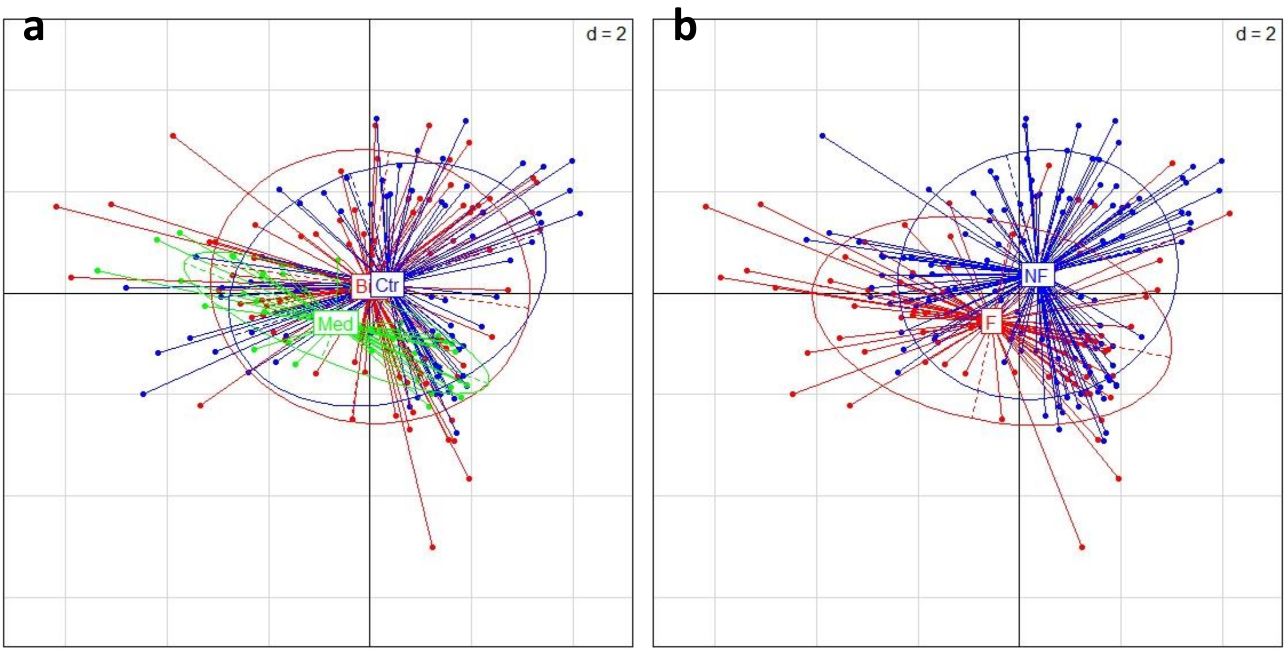

Figure 7 : Biplots of the samples - Qualitative variables : Tree Size (a) and Exclos (b)

Figure 7a : We note here that variables have a similar behavior under big trees and in the pasture (no tree). Soil humidity, total number of nematodes, bulk density and C and N concentrations are not influenced by big trees or pasture. However, this changes under medium-size trees where the soil is thicker and the other variables are higher.

Figure 7b : This biplot shows that grazed spaces' (Not Fenced) bulk density is higher than fenced ones. Those last have more C, N, liveleaves and nematodes, and the humidity is higher.

MULTIPLE REGRESSION

Total

With PCA, we were able to select 3 significant variables to perform a multiple regression. Those are : bulk density, liveleaves and humid.5.

We started with the total number of nematodes for the first multiple regression. We defined variables and all possible interactions between them this way :

- Bd2<-BD²
- H2<-Humid.5²
- Ll2<-Liveleaves²
- Bh<-BD * Humid.5
- Bll<-BD * Liveleaves
- Hll<-Humid.5 * Liveleaves
- Bhll<-BD * Humid.5 * Liveleaves

This is the first table we obtained :

	Estimate	*Std. Error*	*t value*	*Pr (>|t|)*	
(Intercept)	7013.3625	9457.4065	0.742	0.4593	
BD	-8274.1427	12579.2517	-0.658	0.5115	
Humid.5	190.0407	196.5089	0.967	0.3348	
Liveleaves	109.1399	159.7584	0.683	0.4954	
Bd2	2757.5264	4182.7024	0.659	0.5105	
H2	-0.4133	1.7611	-0.235	0.8147	
Ll2	-1.0.774	0.5695	-1.892	0.0601	°
Bh	-119.0028	108.5668	-1.096	0.2744	
Bll	-66.5727	112.5735	-0.591	0.5550	
Hll	-15.5170	9.0609	-1.713	0.0885	°
Bhll	12.6401	6.6642	1.897	0.0594	°
Signif. Codes :	0 ‘ *** ’ 0.001 ‘ ** ’ 0.01 ‘ * ‘ 0.05 ‘ ° ‘ 0.1 ‘ ‘ 1				
Residual Standard error	619.9				
Multiple R^2	0.3556				
Adjusted R^2	0.3209				

Figure 8 : Table at the beginning of multiple regression - Total number of nematodes

After deleting all non-significant terms, we have this table :

	Estimate	*Std. Error*	*t value*	*Pr (>|t|)*	
(Intercept)	910.1309	111.1706	8.187	3.74 e-14	***
Humid.5	215.6262	59.9310	3.598	0.000409	***
Ll2	-0.6990	0.3475	-2.011	0.045687	*
Bh	-143.3420	39.8642	-3.596	0.000412	***
Hll	-12.0389	3.1212	-3.857	0.000157	***
Bhll	10.2828	2.1850	4.706	4.84 e-06	***
Signif. Codes :	0 ‘ *** ’ 0.001 ‘ ** ’ 0.01 ‘ * ‘ 0.05 ‘ ° ‘ 0.1 ‘ ‘ 1				
Residual Standard error	617				
Multiple R^2	0.3444				
Adjusted R^2	0.3272				

Figure 9 : Table at the end of multiple regression - Total number of nematodes

We are now able to express the total number of nematodes with 3 variables and some combinations :

Total = 910,1309 + (215.6262 * Humid.5) – (0,6990 * Liveleaves²) – (143,3420 * BD * Humid.5) – (12,0389 * Humid.5 * Liveleaves) + (10,2828 * BD * Humid.5 * Liveleaves) + (619 : residual standard error)

We created a graph to study generated residuals of our model :

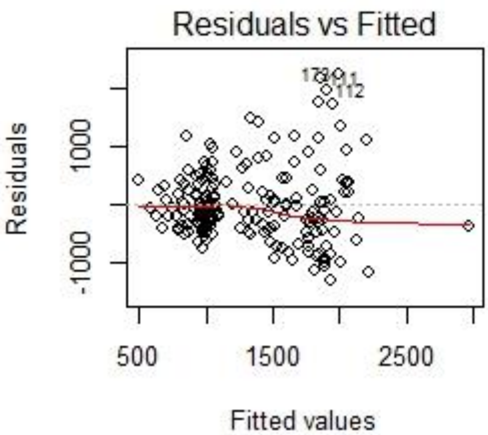

Figure 10 : Graph of residuals of the model in function of fitted values - Total number of nematodes

The first graph, that shows increasing residuals with increasing fitted values, is not representative of a good multiple regression. The equation we obtained is not reliable. We can't consider the total of nematodes as a linear function. That's why we decided to perform again a multiple regression after transforming total of nematodes in its logarithm.

This is the final result :

	Estimate	*Std. Error*	*t value*	*Pr (>|t|)*	
(Intercept)	8.6942236	0.6019056	14.444	< 2 e-16	***
BD	-1.3482966	0.4136663	-3.259	0.00132	**
Liveleaves	0.0290074	0.0121564	2.386	0.01800	*
Ll2	-0.0010645	0.0002738	-3.887	0.00014	***
Hll	-0.0040132	0.0018461	-2.174	0.03095	*
Bhll	0.0037393	0.0015302	2.444	0.01545	*
Signif. Codes :	0 ' *** ' 0.001 ' ** ' 0.01 ' * ' 0.05 ' ° ' 0.1 ' ' 1				
Residual Standard error	0.4389				
Multiple R^2	0.3704				
Adjusted R^2	0.354				

Figure 11 : Table at the end of multiple regression - log(Total number of nematodes)

We get the equation :

Log(Total) = 8,6942 - (1,3483 * BD) + (0,0290 * Liveleaves) – (0,0011 * Liveleaves²) – (0,0040 * Humid.5 * Liveleaves) + (0,0037 * BD * Humid.5 * Liveleaves) + (0,4389 : residual standard error)

And the graphs about residuals :

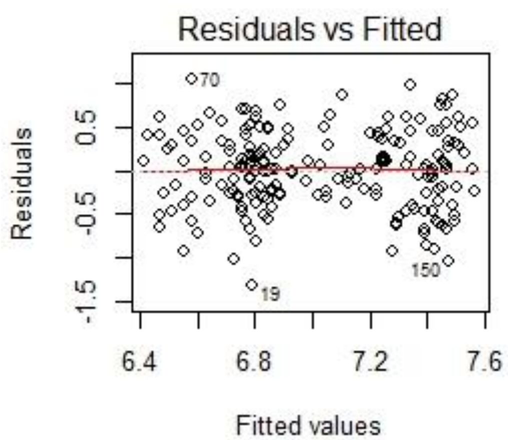

Figure 12 : Graph of residuals of the model in function of fitted values - log(Total number of nematodes)

Here the residuals are more dispersed and are not increasing according to fitted values. This is a more reliable result to keep.

We can see in our model that Liveleaves is a recurrent variable, it plays quite an important role. Our R^2 is of 0.3704, it means that 37.04% of the variation in log(Total) is explained by the regressors in the model. This measure is quite low, so our model isn't representative enough to well describe a reality, but we can keep some of its results for further analysis.

FL and PPN

We followed the same protocol to study free living and plant parasitic nematodes. In both cases, considering a linear model isn't the best option and the logarithm was chosen for the final result.

Here is the table obtained for log(FL) :

	Estimate	*Std. Error*	*t value*	*Pr (>|t|)*	
(Intercept)	8.2910348	0.86564486	9.578	< 2 e-16	***
BD	-1.7952164	0.5992544	-2.996	0.03097	**
Ll2	-0.0011982	0.0004637	-2.584	0.010505	*
Bll	0.0389630	0.0110732	3.519	0.000541	***
Signif. Codes :	0 ' *** ' 0.001 ' ** ' 0.01 ' * ' 0.05 ' ° ' 0.1 ' ' 1				
Residual Standard error	0.8637				
Multiple R^2	0.164				
Adjusted R^2	0.151				

Figure 13 : Table at the end of multiple regression - log(number of free living nematodes)

We then have the equation :

$$\text{Log(FL)} = 8{,}2910 - (1{,}7952 * \text{BD}) - (0.0012 * \text{Liveleaves}^2) + (0.0390 * \text{BD} * \text{Liveleaves}) + (0.8637 : \text{residual standard error})$$

We can see here that Liveleaves and Bulk Density are the only two variables to explain the number of FL nematodes. But here our R^2 is even lower than before, 16.4 % of the variations are explained by the variables. Our model isn't really reliable.

We have here the table for log(PPN) :

	Estimate	*Std. Error*	*t value*	*Pr (>|t|)*	
(Intercept)	5.076788	0.266938	19.019	< 2 e-16	***
Humid.5	0.460106	0.120307	3.824	0.00177	***
H2	-0.003645	0.001475	-2.471	0.014348	*
Bh	-0.234219	0.062464	-3.750	0.000235	***
Hll	-0.010949	0.004184	-2.617	0.009582	**
Bhll	0.008075	0.003051	2.647	0.008806	**
Signif. Codes :	0 ‘ *** ’ 0.001 ‘ ** ’ 0.01 ‘ * ‘ 0.05 ‘ ° ‘ 0.1 ‘ ‘ 1				
Residual Standard error	0.8625				
Multiple R^2	0.1748				
Adjusted R^2	0.1532				

Figure 14 : Table at the end of multiple regression - log(number of plant parasitic nematodes)

The equation is :

$$Log(PPN) = 5.0768 + (0.4601 * Humid.5) – (0.0036 * Humid.5^2) – (0.2342 * BD + Humid.5)$$
$$– (0.0109 * Humid.5 * Liveleaves) + (0.0081 * BD * Humid.5 * Liveleaves)$$
$$+ (0.8625 : \text{residual standard error})$$

We see that Humid.5 is the most recurrent term on this equation. It is thus quite important in the description of the number of PPN nematodes. But R^2 is too low for us to fully accept this model.

ANOVA

We performed 3 ANOVAs : one on the total number of nematodes, one on the number of free living nematodes and one on the number of plant-parasitic nematodes.

Total

We started by looking for effects of season and location. The ANOVA not leading to a result, we performed Kruskal tests on those variables. It resulted in a significant impact of the season, but not of the location. So we separated seasons for further analysis. For each, we tried to look for an effect of location. We found one on dry season. Given that there is no data about the zone in the watershed in intergullies, we have to separate both seasons according to the location. Then we finally run the last level of ANOVAs, consisting in 4 tests : one ANOVA for each location on each season. We test the variables exclos, size of the tree, and zone in the watershed when in intergullies.

We can sum up the results of all the process in this diagram :

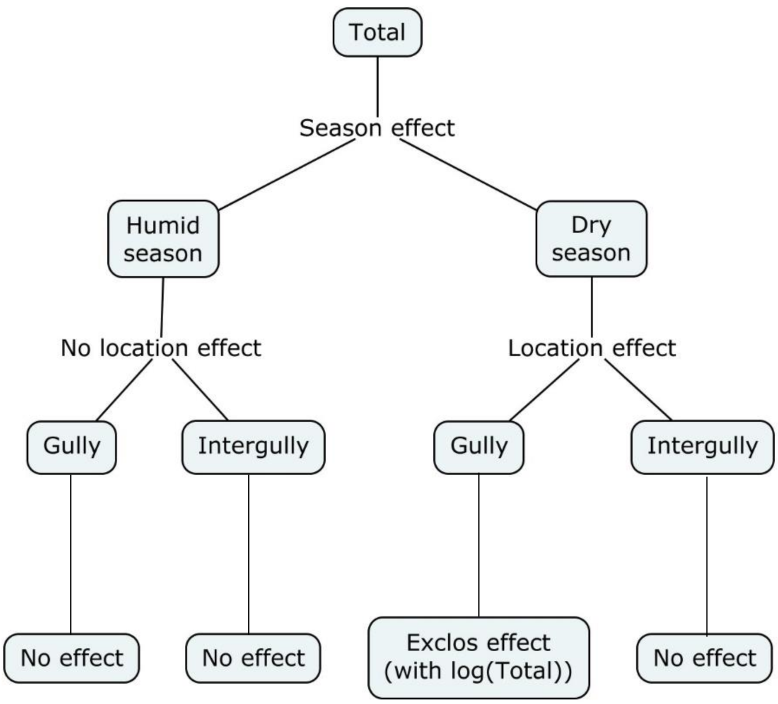

Figure 15 : Diagram of the ANOVA process - Total number of nematodes

The only functioning ANOVA is the one that shows an effect of the exclos in dry season in a gully. We had to transform the total in its logarithm for it. Here are the results :

	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

Figure 16 : Table of results of an ANOVA - log(Total number of nematodes) in function of TreeSize and Exclos, in dry season in a gully

We can only conclude on this process that the number of nematodes is influenced by season, location in dry season and exclos in dry season in gullies.

FL

We did the same process for free living nematodes.

Here is the same diagram that we did before :

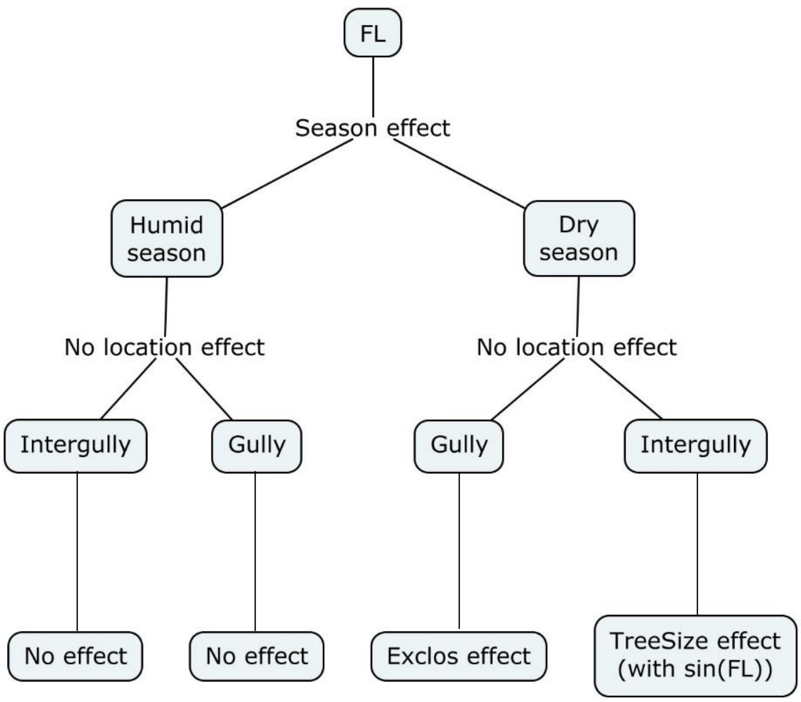

Figure 17: Diagram of the ANOVA process - Number of free living nematodes

We found 3 effects in this case :

- Seasonal effect on the number of nematodes, obtained with a Kruskal test.
- In dry season, there is an effect of exclos in gullies. We had this result with an ANOVA :

	numDF	*denDF*	*F-value*	*p-value*
(Intercept)	1	30	305.04202	<0.0001
TreeSize	1	30	2.51361	0.1234
Exclos	1	30	6.06417	0.0198
TreesSize:Exclos	1	30	1.86125	0.1826

Figure 18 : Table of results of an ANOVA – Number of free living nematodes) in function of TreeSize and Exclos, in dry season in a gully

- In dry season, there is an effect of the size of trees in intergullies. This result was obtained by transforming FL in its sinus for the ANOVA.

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

Figure 19 : Table of results of an ANOVA - sin(number of free living nematodes) in function of TreeSize in dry season in an intergully

PPN

We did the same thing a third time, but in this case there was just the season effect, obtained with the Kruskal test.

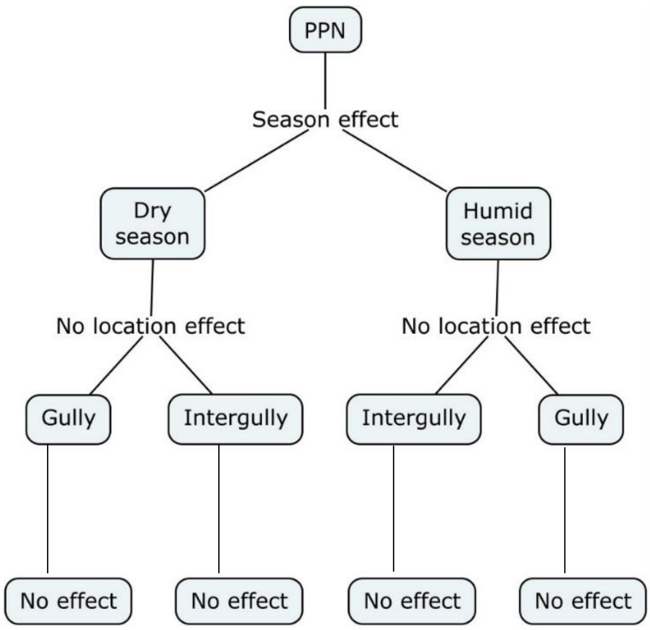

Figure 20 : Diagram of the ANOVA process - Number of plant parasitic nematodes

DISCUSSIONS

VEGETATION

Tree size effect is weak. The ANOVA showed it on free living nematodes during dry season and in intergullies. But the CPA and its biplot displayed that big trees and the absence of trees had a similar dispersion, that was not the same as medium-size trees. This reminds the article about a study of 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 so small that we could almost say that there is no effect of trees on the total number of nematodes, even when separating by free living or plant parasitic. However, this assessment 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). This confirms the importance of the role played by the season in the behavior of nematodes. They behave differently regarding the season, hence the geographical place.

By distinguishing nematodes regarding their diet, particularly within the free living group, we could observe a modification in nematode communities linked with the presence of *Acacia Sieberiana*. The fact that woodland has an impact on trophic structure (Keith et al., 2006) suggests it.

Regarding ground vegetation, we observe that grass amount is not correlated to the number of nematodes. However, the amount of living leaves is. Their presence is directly linked to humidity in the soil. Thus, their presence in surface is explained by the amount of water in the soil and gives an indication about the number of nematodes. This point is confirmed with the fact that nematodes communities are more affected by the presence of certain vegetal species than by specific diversity (Kostenko et al., 2015). A study like ours with another species of trees would possibly show if, in a case of tree encroachment, the effects on nematode communities are the same.

SAMPLING PARAMETERS AND SOIL PROPERTIES

We noticed with the PCA that nematodes are highly influenced by humidity in the soil. Bulk density also plays a role, but it is negatively correlated with nematodes and humidity. It means that the thicker a soil is, the fewer nematodes there are because it has less water in it. This result was verified with the PCA and the multiple regression (even with a weak R^2). Season must also play a role in the process because precipitations are not the same and hence modifies water flow during the year.

We observe with the ANOVA that gullies are a parameter influencing the number of nematodes in dry season, especially free living nematodes. It confirms the importance of humidity and season parameters. We see with PCA a higher number of nematodes outside gullies. This observation is quite difficult to interpret because of the limited literature existing (Baxter et al., 2013). We can suppose that the lack of vegetation in gullies is a cause for a small amount of water in the soil, hence a reason to a decreasing number of nematodes.

Cattle also plays a role on nematodes, especially on free living ones, during dry season and in gullies. It was partially shown by the PCA and then in part confirmed with the ANOVA (effect only in gullies in dry season). It is shown that cattle has an impact on vegetation and C concentration in the soil (Grellier et al., 2013). However, our study shows no link between C concentration and number of nematodes. Even if this result contradicts the fact that the number of nematodes increases with increasing C soil concentration (Ito et al., 2015). Nematodes are however highly correlated to living leaves, and weakly correlated to grass. The poor ground vegetation in gullies and cattle action might emphasize a fall in humidity rate and number of nematodes.

According to our PCA, the number of nematodes is independent of N soil concentration. It is in contradiction with a study that states that nematodes are affected by an increasing N concentration (Zhao et al., 2014). Also, it seems in Potshini's pasture that *Acacia Sieberiana* does not play a role in this, 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 Sieberiana* 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.

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

Humidity rate in the soil seems to be the main parameter to describe the number of nematodes. Thus, nematode populations change a lot from a season to another because of the precipitation. 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 to 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. Cattle emphasizes the negative impact of gullies when it grazes there in dry season. It could make the number of nematodes decrease.

We can however pose a limit to our study : effects are rarely significant in our tests. The goal is to find where there was a problem. Was it the sample that wasn't large enough? Were the statistical tests not strong enough? Maybe it was just that there were not a lot of effects to show here, possibly because nematodes are not a good indicator as of a tree encroachment. Research has to continue to determine what factor wasn't good, then solve the problem and run the study again.

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Ingénierie du Projet

d'Aménagement, Paysage,

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35 allée Ferdinand de Lesseps

BP 30553

37205 TOURS cedex 3

Directeur de recherche :
GRELLIER Séraphine

KNEFATI Sarah – LENOIR Amélie
Projet de Fin d'Etudes
DA5
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, 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 qu'il y a moins de nématodes en saison sèche en ravine, ce qui peut montrer que le ravinement n'est pas positif pour la faune du sol.

Mots Clés : nématodes, pâturages, invasion ligneuse, écologie