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Study of the effect and after-effect of legumes onsoilbiologyusingabio-indicator:Nematodes.

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Abstract

Knowing and valorizing the effect of agricultural practices on soil biology is a promising way to progress towards more sustainable cropping systems. This study was conducted to gain a better understanding of the effect of legumes on soil biology. Its aim is to determine whether the presence of legumes has a specific effect on soil biology, by assessing this effect using a bio- indicator: the soil nematofauna.

To achieve this objective, we tested four plots, including three with legumes as a preceding effect and after-effect (TNLt1C, TLt0PC, TLT0V) and one control plot (TT) with a non-leguminous preceding. Soil samples were taken from these plots for physico-chemical analysis. The resultsthus obtained revealed significant differences in soil biological activity between leguminous and non-leguminoussoils,withthecontrol(TT)showingapopulationof389.74NN/100ml,followedby the TLT0V field with a population of 265.81 NN/100ml, TNLt1C with 205.13 NN/ml and TLT0PCwith 185.47 NN/100ml. The fact that we obtained an average result for the effect of legumes on soil biology (through a more or less low abundance of nematodes) in no way proves that they do not have this power;

Keywords:leguminous,effectandafter-effect,nematodes,bioindicator,soilbiology.

Introduction

In the high plains of the Sétif region, agriculture is predominantly rain-fed, dominated by the "cereal/fallow" cropping system combined with sheep farming. The soil is worked conventionally, using deep ploughing. Mechanized tillage has led to soil crumbling, compaction, erosion and organic matter depletion (Mrabet., 2001). In addition to the structural degradation of agricultural soils, conventional production techniques applied to cereal crops in our country generate threats to biodiversity, fertility and soil quality through erosion (Benniou and Aubry, 2012). Deterioration in structuralconditionaffectsallsoilmacrobiologicalcommunities.Indeed,allorganismsaresensitive to the presence of compact structures that hinder their movements and limit the volume of usable soil from both a "trophic resource" and a "place of residence" point of view (Girard et al, 2005).

Plants have a strong influence on the biological functioning of the soil: they represent the mainroute by which organic matter enters the soil in the form of litter and rhizodeposits (De Deyn and Van der Putten, 2005), in addition to organic fertilizers based on livestockeffluents and other waste materials.Theythushaveadirectinfluenceontheflowofmineralelementsandthestructureofsoil animal and microbial communities. However, the effect of plants on these soil components is likely to vary according to the type of cover. Anderson and Domsch (1989) recorded Cbiomass/Ctotal ratios of 2.32% for monocultures, 2.37% for cereal-only rotations and 3.02% for rotations with mixed crops.

Thanks to their ability to fix atmospheric nitrogen, legumes represent an interesting source of nitrogen nutrition for plants, as an alternative or complement to inputs. This is why species fromthis botanical family are mobilized as intermediate crops or in rotations when the aim is to develop innovative low-input or more environmentally-friendly cropping systems (Drinkwater et al., 1998; Schneider and Huyghe., 2015). This property of improving soil fertility is of major interest in agriculture, and the chemical dimension is certainly not the only one at stake. Little is known about the effects of legumes on the biological functioning of soils (Villenave et al., 2018). In addition to their role in the nitrogen cycle, legume production interacts with other biogeochemical cycles such as those relating to phosphorus or xenobiotics. The presence of legumes in agricultural production systems helps to increase the functional diversity of agroecosystems, which is favorable to the biodiversity of agricultural landscapes and territories. In this way, they contribute to the balance of agro-ecological systems in several ways (Schneider and Huyghe., 2015).

By avoiding monoculture, legumes help control root diseases and nematodes in cereals (Puckridge andFrench,1983;Bahhadyetal.,1997);theyalsohelpcombaterosionandimprovesoilstructure;

and they improve dry matter and protein production in biennial cereal-fodder legume rotations, as shown by various ICARDA studies (SHIPLEY et al., 1992, cited by ABBAS et al., 2006).

The question therefore arises: does the introduction of legumes into crop rotations have an impact on soil biology and the sustainability of cropping systems?

Toanswerthis question,this studywas carriedout to betterunderstand theeffect oflegumes on soil biology. Its aim is to determine whether the presence of legumes has a specific effect on the biological functioning of soils, by assessing this through a bio-indicator: communities of soil nematodesknownasthe"nematofauna".Nematodesarethemostabundantmulti-cellularorganisms (metazoans)on earth,and areusedin awiderangeofagricultural activities. Theyhavebeen usedas bioindicators of soil biological status since the 1990s (Bongers, 1990; Neher, 2001) to characterize the impact of an agricultural practice or cropping system on soil biological activity and functioning (Djigal et al., 2012; Coll et al., 2012; Salomé et al., 2016). Nematodes are microscopic vermiform organisms (around1 mm longwith adiameterof 20 µm)that areoften knownin agricultural circles for their parasitic activity and the damage they can cause to crops. In reality, they form acommunity of organisms with very varied feeding habits: bacterivorous, fungivorous, omnivorous, predatory, phytophagous, or entomatopathogenic. Nematodes thus actively participate in varioussoil processes, notably through their influence on the biomass, composition and activity of soil microbial communities, as well as on the mineralization of organic matter (Bongers and Ferris, 1999; Trap et al., 2016). Nematode communities thus act as indicators of the overall structure and functioning of soil food webs (Villenave et al., 2018).

Materialsandmethods

Presentationoftheexperimentalsite:

The experiment took place at the experimental site of the Technical Institute of arable cropping of Sétif (ITGC), at a place called R'MADA, administratively linked to the commune of Mezloug,Daïra of Ain Arnat, south-west of the capital of the wilaya of Sétif.

Our study consisted in researching and measuring the effect and after-effect of legumes on soil biology, using a bio-indicator: the soil nematofauna. To achieve this objective, we set up several cultivatedspecies,extractedthenematodespresentandcarriedoutphysico-chemicalanalysesofthe soil.

Plantmaterial:

Ourworkinvolvedtestingtwocerealspecies,durumwheatandbarley,andtwolegumespecies, vetch and chickpea.

Methodology:

The experiment began with the establishment of the different crops. The four species were sown in four 30/10 m strips as follows:

- Plot01: planted withdurum wheat(*Triticumdurum*, Defs),preceded byfodderpea.

- Plot02: barley(*Hordeum vulgare*), preceded byfallowland.

- Plot03:cultivated withchickpea(*Cicerarietinum*),preceded byfallow land.

- Plot04: vetch (*Vicia sativa*),preceded byfallowland.

Tocarry outourstudy,we tooksoilsamplesfromthefourplots,eachbearingadifferentcropas shown in Table 1:

Table1:Technicalcharacteristicsofthecropsstudied

Plot	Abbreviation	Currentcrop	Previous	Typeofrotation
01	TNLt0C	Durumwheat	Fodderpea	Legumes/cereals/fallow
02	TT	Barley	Fallow	Cereals/fallow
03	TLt1PC	Chickpea	Fallow	Legumes/cereals/fallow
04	TLt1V	Vetch	Fallow	Legumes/cereals/fallow

Theexperimentwascarriedoutinseveralstages:

- collectionofsoil samplesinthefield,

- Extractionofnematodesfromthesoil.

- Nematodeenumerationundermicroscope.

- Soilphysico-chemicalanalysis.

a-Sampling:

Soilsampleswereta kenatrandomfromthevariousplotsstudied.Eachtwo-kgsoilsampleconsisted of six samples taken in a zigzag pattern to a depth of 20 cm, then mixed and referenced.

Samplesarestored at5-8°Ctopreventnematodedeterioration(Villenaveetal.,2009).

Itshouldbepointedoutthatsamplingwascarriedoutatdifferentstagesofdevelopmentofthe crops tested, as shown in Table 2:

Table2:Samplingperiodsandcorrespondingvegetativestages.

Treatment	1st sampling	ativestage	2nd sampling	Vegetativestage	3rd sampling	Vegetativestage
		tillering				
TLt0V	/2021	gence	/2021	owering	/2021	aturity
Lt0PC		gence				
Lt1C		tillering				

b- Nematodeextraction:

Extraction is carried out using the modified Baermann method which is classically used for mobile nematode extractions from plant shreds, seeds, organic potting soils or small quantities of soil (our case). It takes many steps as follows:

- Sieving:asieveisusedto removestones, plantdebrisand clodsof soil.

- Thesievedsoilis mixedon “newspaper” and a 100ml volumeofsoilismeasuredina beaker.

- Filtration of a 100 ml volume of soil, wetted with a volume of water that neither submerges the sample nor leaves it dry, while avoiding soiling of the extraction solution.

- After24h,adjust thewater level.

- During 48 hours, in the dark, the nematodes will migrate from the volume of soil to the water inthe plates, passing through the filter paper.

- After the 48 hours, thefilter paper is drained and the volume ofextraction solution in each plate is collected in plastic cups for settling for a few hours.

c- Nematodecounting:

Thesteps below illustratethemethod usedto count nematodes:

- Using the medical syringe, 1ml of nematode solution is drawn off and poured into the counting cell; here, we used a self-designed counting cell: a rigid, transparent polystyrene lid, rectangular in shape and hollow (to prevent the solution under examination from spilling over). At the bottom of the lid, a grid is drawn with a fine metal point. The grid is 60mm long and 40mm wide, andcontains 1040 small squares (cells) measuring 2.25mm².

- The cell containing the 1ml of nematode solution is placed under the Diascopic microscope. An X40 magnification and a light at the bottom of the cell are ideal for good observation of the nematodes.

- Countingisdone byobservingthe nematodesbathingineachsquare of the grid(Figure1),

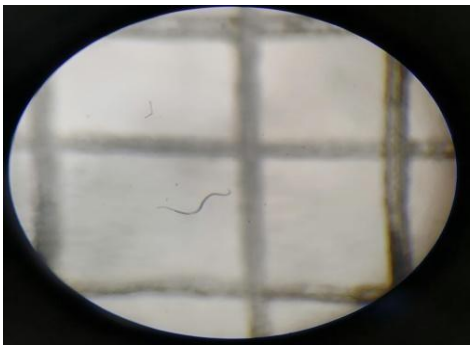

Figure1:nematodeundermicroscope(X40magnification).

- For each volume recovered from each plate, the observation is repeated 13 times (13ml observed for each solution) to obtain maximum precision.

NB: Forthenematofaunal study, we extractedonlyfree-living nematodes and thevarious nematode species extracted were not all determined for lack of identification keys.

d-Physico-chemicalsoil analysis:

Soil physico-chemical analyses were carried out by the "Siddra" laboratories, a subsidiary of the "FERTIAL, les fertilisants d'Algérie" group. Table 3 shows the parameters analyzed:

Table3:physico-chemicalparametersanalyzed

Parameter	abbreviation	unitofmeasurement
pH	Ph	-
ElectricalConductivity	EC	mS/cm
OrganicMatter	MO	%
Carbone/Azote	C/N	-
Carbonates	CaCO3	%
Totalnitrogen	N	%
TotalPhosphore	P	Ppm
Exchangeablepotassium	K	meq/100gr
Exchangeablemagnesium	Mg	meq/100gr
Exchangeablecalcium	Ca	meq/100gr
Exchangeablesodium	Na	meq/100gr

Statisticalanalysis

The data collected during the experiment were subjected to an analysis of variance using EXCELSTAT software. The Newman-Keuls test was used to establish homogeneous groups at the 5% threshold. Relationships between the different variables studied were examined using the correlation matrix.

Resultsandinterpretation

All the data generated by this experiment, i.e. the number of nematodes collected and the results of the physico-chemical analysis of the soil were statistically processed using a factorial approach (two-factor ANOVA):

-studyof theimpact ofthecrops tested(treatments) and

-vegetativestagesonnematodesnumberandsoilparameters. Four

H0 hypotheses will be tested:

a-nodifferencebetweentreatmentsinnematodesnumber b-

no difference between treatments in soil parameters

c-nodifferencebetweenvegetativestagesinnematodesnumber d-

no difference between vegetative stages on soil parameters

1- Studyofthevarianceforthenumberofnematodesinrelationtothetwofactorsstudied (treatments and vegetative stages)

Onlyone of the two factors studied (treatments) revealed a significant effect on nematode numbers, but their interaction was also highlysignificant as showed on table 4, hence the rejection of the first hypothesis H0 (a) and the acceptance of the third one (c), which leads us to conclude that there are differences between treatments but not between vegetative stages

Table4:resultsoftheanalysisofvarianceforthenumberofnematodesin relationtothetwo factorsstudied

Sourcedevariation	Ddl	SCE	CM	F	P
Stade	2	469359	234679	2,5275	0,080980
Traitement	3	0	991510	10,6784	0,000001
Stade*Traitement		1	1427215	15,3709	0,000000
Erreur	456	42340513	92852		
Totale	467	92			

d.d.l=degreeoffreedom,ns=non-significanteffect,***=significantat5%threshold.

The ANOVA was followed by the Newman & Keuls test the results of which revealed that the control treatment (TT) yielded the highest number of nematodes (389.74 NN/100ml). It should be noted that treatments containing a legume as previous effect (TLt0V with 265.81) or after effect (TNLt1C with 205.13), recorded lower nematode populations than TT, which contains no legume. It appears that the effect of legumes on soil biology, and in particular on nematofauna, has been moderatelydemonstrated in our case (table 5 and Figure 2).

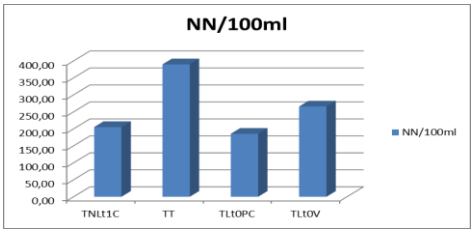

Figure2: Moyennedunombredenématodesenfonctiondescultures

Table5:Averageeffectofcropstestedonnematodesnumber Ppds = smallest significant difference

Traitement	NN/100ml	Groupestatistique
TNLt1C	205,13	Bc
TT	389,74	A
TLt0PC	185,47	C
TLt0V	265,81	B
Ppds5(%)	78,29	

Effectofcropsxvegetativestagesinteractiononnematodesnumber:

With regard to the study of tested crops x vegetative stages interactions, it was shown (table 6) that the highest number of nematodes was recorded for the "Flowering x TT" interaction with anaverage of 612.82 NN/100ml followed by the "Maturity x TNLt1C" interaction with 492.3 NN/100ml. the lowest nematode population was observed in the "Flowering x TNLt1C" interaction with a value of 25.64 NN/100ml (table 6, Figure 3). We note that for the same treatment (TNLt1C), the number of nematodes was higher in interaction with the maturity stage than with the flowering stage, the difference between them being so great that we have to ask why?

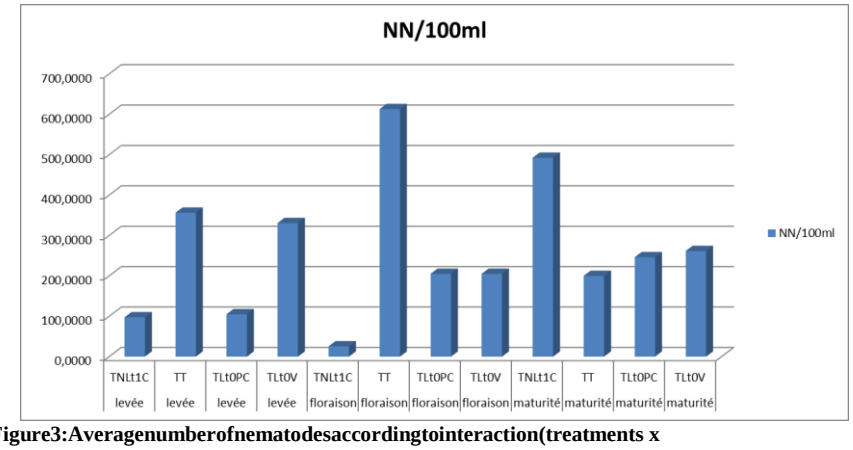

Figure3:Averagenumberofnematodesaccordingtointeraction(treatments x vegetative stages)

Table6: Averageeffectofvegetativestagesxtreatmentsinteractionon nematodes number

vegetativestages	Treatment	NN/100ml
Emergence	TNLt1C	97,4359
Emergence	TT	**356,4103**
Emergence	TLt0PC	105,1282
Emergence	TLt0V	**330,7692**
Flowering	TNLt1C	25,6410
Flowering	TT	**612,8205**
Flowering	TLt0PC	205,1282
Flowering	TLt0V	205,1282
Maturity	TNLt1C	**492,3077**
Maturity	TT	200,0000
Maturity	TLt0PC	246,1538
Maturity	TLt0V	261,5385

2- Analysisof varianceof soilphysico-chemical parametersforthetwofactorsstudied:

We accept the H0 hypothesis for the vegetative stage factor, as it onlyhad a significant effect at 9% (i.e. a single parameter, pH, out of the eleven studied). This leads us to conclude that there is virtually no distinction between the effects of the different vegetative stages on physico-chemical parameters.

For the second factor (treatments), two parameters were significant (i.e. 18%): the C/N ratio and phosphorus P content (Table 7).

- ConcerningC/N ratio, theresult can beexplained bythe fact that among thefourtreatments tested we have TNLt1C, TLt0PC and TLt0V which contain a legume either in effect (preceding), or in after effect (the second preceding), which as we know, are the most efficient plants in terms of external nitrogen supply.

- For phosphorus, we believe that this result is fortuitous, because the treatments cannot have a direct impact on the mineral composition of the soil, especiallyas the crops tested received external inputs as part of their fertilization.

All other parameters proved insignificant (82%), confirming our reasoning on the point studied above (phosphorus levels).

Table7:Analysisofvarianceforsoilphysico-chemicalparameters

SV	ddl	NN/100ml	CEmS/cm	pH	C/N	CaCO3%	MO%	Ntotal%	Pppm	Kéch%	Mgéch%	Caéch%	Naéch%
stade	2	0,080980ns	0,74955ns	0,021961*	0,522602ns	0,090617ns	0,479403ns	0,418349ns	0,933306ns	0,221528ns	0,488740ns	0,127334ns	0,572217ns
traitement	3	0,000001***	0,476093ns	0,526820ns	0,014815*	0,329329ns	0,248454ns	0,217852ns	0,000876**	0,666218ns	0,204986ns	0,479015ns	0,260171ns
CMstade		234679,487	0,000108	0,0114	0,0271	117,208	0,12	0,000833	1,013	0,030833	0,28583	38,57	0,0175
Fstade		2,52745749	0,2977	6	0,7	3,17262	0,7987	0,9615	0,0696	1,7903	0,776	2,614	0,59434
CMtraitement		991510	0,000297	0,0031	0,0957	95,347	0,20483	0,001289	38,196	0,01222	0,54222	17,77	0,037778
Ftraitement		10,6784	0,915	0,8	6,6	1,33569	1,676	1,8413	16,464	0,5432	1,9194	0,9077	1,61905

pH was the only parameter on which the vegetative stages had a significant effect, namely emergence and flowering, according to Newman & Keuls test, with an average of 8.35 compared with maturity (8.26). We note that pH tends to fall at maturity, which may be linked to plant nutrition and morepreciselyto themigration ofreserves for fruit filling that takes placeat this stage (table 8 and Figure 4).

Table8:Comparisonofmeansforthephysicochemicalparameters in relation to the two factors studied

Facteur		Physico-chimicalparameters		
		pH	C/N	P
Vegetativestages	Emergence	8.35a		
	Flowering	8.35a	/	/
	Maturity	8.26b		
Treatments	TNLt1C		7.51b	14,93b
	TT		7.85a	23.40a
	TLt0PC		7.45b	20.73a
	TLt0V	/	7.52b	20.67a

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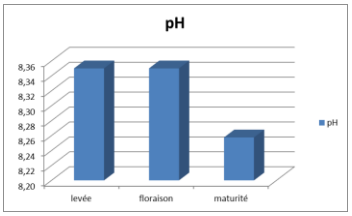

Figure4:AveragepHvaluesaccordingtovegetativestage

Furthermore, according to the work of Hinsinger et al, (2002) and Jaillard et al, (2003), which corroborates our results, a change in pH depends on several factors linked to the plant, plant species, physiological stage and symbiotic status, or to its environment. Indeed, these authors explain that a plant that takes up more cations than anions, releases H+ protons and therefore tends to acidify its rhizosphere.Conversely,aplantthattakesupmoreanionsthancationsreleaseshydroxyls(OH-),and therefore tends to alkalinize its rhizosphere.

The fallow/barley (TT) control treatment shows the best average C/N parameter in relation to the "crops tested" factor, with a value of 7.85 (Table 8), meaning that the higher this ratio, the higher the total carbon content (Figure 5).

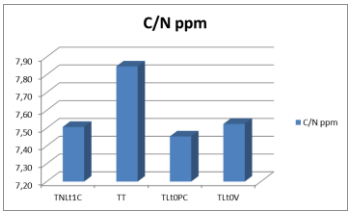

Figure5:averageC/Nratiovaluesaccording to treatments

Given that the other three treatments contain a legume as an effect or after-effect, vetch for (TLt0V) with 7.52, field pea for (TNLt1C) with 7.51 and chickpea for (TLt0PC) with 7.45, it's normal that the latter record higher nitrogen levels than the (TT). While noting that the total carbon level remains the highest

In terms of phosphorus P values, the three treatments TT, TLt0PC and TLt0V performed best, withno statistical difference (23.40; 20.73 and 20.67 respectively) as shown on Figure 6. We can suggest that these treatments have in common a fallow as a previous crop (effect) compared to the TNLt1C treatment (14.93) which has a leguminous previous crop. However, this can be explained by the fact that the crops tested received external inputs as part of their fertilization as already mentioned.

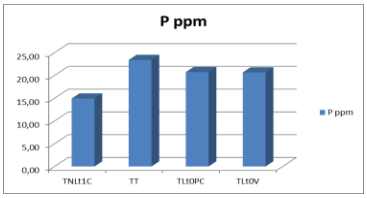

Figure6:averagePcontentvalues(ppm)according to treatments

3- Studyofcorrelations:

For our work, what is most important are the correlations between the number of nematodes (NN/100ml) and the various physico-chemical parameters studied. In fact, the number of nematodes was found to correlate significantly with only four of the eleven parameters studied, as shown in Table 9.

We note that the higher the electrical conductivity EC (r= -0.6851) and calcium carbonates CaCO3 (r= -0.6787), the lower the number of nematodes, which determines an inversely proportional relationship between them. This situation may reveal that saline soils do not seem to be favorable for good nematode proliferation.

However, the number of nematodes seems to have a proportional relationship with potassium K (r=0.5341) and the C/N ratio (r=0.5646), which implies that these two elements contribute to providing a favorable environment for nematode outbreaks. Furthermore, according to Justes et al (2009) in France, the quantities of nitrogen supplied by a legume to subsequent crops (in effect or after effect) depend on several factors, including thequantityofnitrogen present in theresidues ofthelegumecropand theirC/N ratio.

Table9:correlationmatrixbetweenthestudiedvariables

VariablesNN/10	NN/100ml	CE(mS/cm)	pH	C/N	CaCO3%	MO%	Ntotal%	Pppm	Kmeq/100grMgmeq/100grCameq/100grNameq/100gr			
0ml	1											
CE(mS/cm)	**-0,6851**	1										
pH	-0,0981	-0,2069	1									
C/N	**0,5646**	-0,0899	-0,3657	1								
CaCO3%	**-0,6787**	0,3641	**0,7133**	**-0,6537**	1							
MO%	0,0527	0,3122	-0,0842	0,0563	0,1174	1						
Ntotal%	0,0014	0,3247	-0,0549	-0,0308	0,1736	**0,9962**	1					
Pppm	0,2030	-0,2073	0,0743	0,4261	-0,2112	-0,4841	-0,5249	1				
Kéchmeq/100gr	**0,5341**	-0,5029	-0,4428	0,5261	**-0,7392**	-0,0673	-0,1178	0,1539	1			
Mgéchmeq/100gr	0,0136	0,2461	0,0212	-0,0104	0,0597	**0,6576**	**0,6656**	-0,4929	-0,1562	1		
Caéchmeq/100gr	0,4457	-0,5565	-0,2725	0,3766	-0,5661	-0,4271	-0,4674	0,3482	**0,8155**	**-0,6823**	1	
Naéchmeq/100gr	-0,2818	0,3708	0,0274	-0,2645	0,2440	0,3241	0,3565	-0,5380	-0,5099	**0,7964**	**-0,8392**	1

Discussion

The aim of this study was to understand the effect and after-effect of legumes on soil biology, using nematofauna as an indicator. Analysis of the data obtained showed that legumes did not particularly promote nematode outbreaks compared with the control plot (TT), which does not contain legumes in its rotation. These results corroborate those obtained by Zhao et al, (2014) and Crotty et al, (2015). According to these authors, this can be explained by the specific pedoclimatic context (soil pH in particular) and time scale of these two studies, which focused on trees or four-year-old crops. On theotherhand, otherresearchhasshown thatleguminousplantsdopromotethedevelopmentoftheseworms, particularly the Bacterivore group, which represents the best indicator of environmental enrichmentwithin the trophic chain groups (Trap et al., 2016), as stipulated by Villenave et al. in their 2018 work. This work proved that the crops studied influence soil biology differently by varying nematodeabundance from one crop to another. However, the result is probablylinked to the qualityof legume litter and rhizode deposits, which are particularly rich in nitrogen and easily degradable elements (DuPont etal., 2009; Schneider and Huyghe, 2015).

The fact that we obtained an average result for the effect of legumes on soil biology (through a low level ofnematodeabundance),innowayprovesthattheydonotactuallyhavethispower;wecanjustifythis

state of affairs by the absence of the resources needed to further our research into the identification of nematode trophic groups, of which the most appropriate for improving soil quality are the bacterivorous groups.

Conclusion

As an indicator of soil biological activity, soil nematodes are among the most representative. Indeed, this analysis revealed significant differences in soil biological activity between leguminous and non-leguminous plants. Variations in soil nematode abundance under different plants are the result of biotic interactions between roots, litter inputs, micro-organisms, and the direct and indirect effects of plants on the soil. According to many authors, leguminous plants have a particular impact on physico-chemical components, in particular through their ability to fix atmospheric nitrogen.

In our case, there is probably a specialization of soil trophic groups that could be observed byan increase in bacterivorous nematode populations in soils under the influence of legumes compared to soils underthe influence of non-leguminous plants. These nematodes indicate an enrichment of the environment.

To confirm these hypotheses, we recommend in perspective that this work be taken further, based on the following points:

- diversifyingthefactorstobe studied
- workingon alonger timescale
- increasingthe numberof physico-chemical andotherparametersto beanalyzed
- diversifyingthetreatments tobetested(differentlevels ofcroppinghistory).
- Last but not least, the use of nematode species identification keys and their mastery to better define the effect of legumes on soil biology, and why not deepen the study by using different species and types of legumes (seed, forage and tree).

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Figures

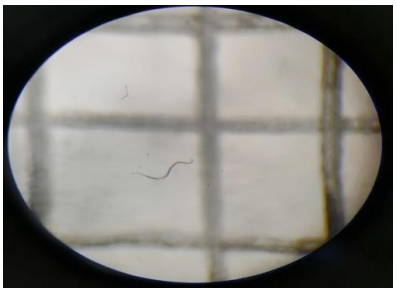

Figure1:nematodeundermicroscope(X40magnification).

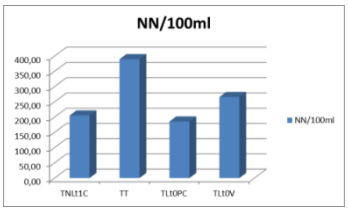

Figure2:Averagenumberofnematodesaccordingtotreatments

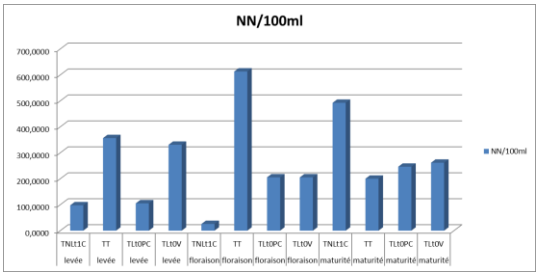

Figure3:Averagenumberofnematodesaccordingtointeraction (treatmentsxvegetativestages)

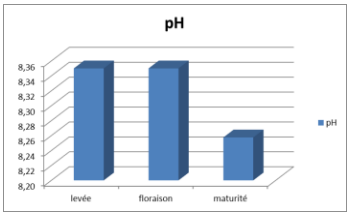

Figure 4: Average pH values according to vegetative stage

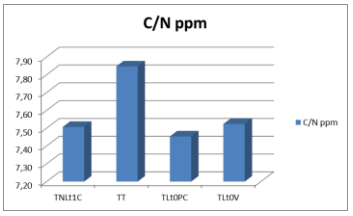

Figure5:averageC/Nratiovalu esaccording to treatments

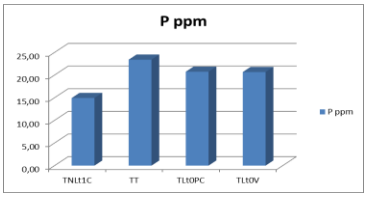

Figure6:averagePcontentvalues(ppm)acco rding to treatments