### Joint French-South-East Asia Research and Training initiative

DYNAMIC OF LAND USE CHANGES AND SOIL ECOSYSTEM SERVICES (LUSES)





### Appendix of the report "How to monitor Soil Biodiversity"

# Appendix 1: result of evaluation

# Collective Training on Soil Biodiversity November 03<sup>rd</sup> -14<sup>th</sup> 2014

#### Chachoengsao Rubber Research Center, Kasetsart Univ. and LDD Bangkok Thailand

Please indicate your impressions of the items listed below.

		Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
	1. The training met my expectations.	3	8	1	0	0
	2. I will be able to apply the knowledge learned.	3	9	0	0	0
	3. The training objectives for each topic were identified and followed.	2	7	2	0	0
	4. The content was organized and easy to follow.	0	7	6	0	0
	5. The materials distributed were pertinent and useful.	4	8	3	0	0
	6. The trainer was knowledgeable.	2	10	0	0	0
	7. The quality of instruction was good.	2	9	0	0	0
	8. The trainer met the training objectives.	2	9	1	0	0
	9. Class participation and interaction were encouraged.	3	7	2	0	0
	10. Adequate time was provided for questions and discussion.	2	5	5	0	0
11	. How do you rate the training overall?					
	Excellent Good 1 9	Average 1		Poor	Very poo	or O

# Appendix 2: Lamina baits

<u>What is it ?:</u> the bait lamina test is a simple in-situ indicator of soil activity. The test consists in small bait portions (various substrate mixed with cellulose) fixed in holes pierced in 16 cm long PVC strips with 16 holes of 2 mm diameter that are then exposed to the biological decomposition activity of the soil. When combined with various substrates, the test can also provide indications of soil functional biodiversity (i.e. soil overall capacity to degrade various substrates; similar to a Biolog / Microresp test that would be performed in-situ) with considerable amount of biometric data that can easily be treated with statistical tests procedures (richness, diversity, evenness).

<u>Where I can find it?</u>: The method was developed by a german researcher (Werner Kraft). Commercial substrate (bran flakes < 500µm) and PVC strips can be purchased in Germany (<u>http://www.terra-protecta.de/englisch/ks-info-en.htm</u>).

#### Methodology

Bait portions are made of 70% of cellulose, 27% of substrate, 3% active coal (to facilitate the lecture of substrate degradation). Holes are filled as follow (Terra protecta):

- mix small quantities of filling substrate with tap water into a paste [NB: here Agar (0.5%) was added to water -about 1.5 ml for 1 g of mixt cellulose+substrate to insure increased adhesion of the paste into the holes]
- fill the paste in the holes of the test stripes by pulling the strips between the thumb and finger and filling the paste.
- dry the first test strip layer in the holes on a gauze (do not use the paste during the next days in order to avoid fungal growth)
- bring in the second layer of paste and dry again
- this filling procedure has to be repeated several times (4-5 layers) to fill the hole. This procedure is important to make sure that the bait substrate has good contact with the plastic material of the stripes. That the pasted layers of the baits are not removed during the insertion into the soil of the experimental plots.
- clean the test stripes from paste outside the holes with a wet tissue.
- before exposing the test strips in the field, make sure that the baits have no scraches and holes by using a light source. If this is the case, put on another layer of paste before use.
- fill the holes always some days before exposure
- don't store the filled test stripes on a heathing system or in the direct sun.

<u>*Tip 1:*</u> transport filled lamina baits in boxes filled with cottons (below and above strips) to avoid substrate losses (avoid frictions between strips; strips to be refilled if holes empty).

**Test is done in less disturbed and most representative areas** (here: between middle inter row and row, in between 2 trees).

<u>Strips insertion into the soil:</u> remove soil litter; using a knife, make a slit in the topsoil (15cm deep) and put the strips (vertical insertion, the first hole is just below the topsoil surface). Strips with different substrate are grouped (here 4 strips) with about 10cm length between strip groups. Close the slit with soil. Make sure that the baits have no damage just after introduce them in the soil (check for only one bait per substrate). Insure that strips are inserted about the same height. Put back soil litter after strips insertion.

What happen to the lamina baits in the field? Soil invertebrates and soil microorganisms (mainly bacteria, fungi, micro and mesofauna) progressively degrade the bait placed in the

soil substrate. It is assumed that the disappearance of the bait material is directly associated to the feeding activity of soil invertebrates. After 7 to 14 days (according to climate and soil type, see control) of field incubation, the bait lamina are carefully collected.

How to monitor the substrate degradation in-situ? 0 if all holes are full; 0.5 if partially degraded; 1 if fully degraded (empty hole); NA if soil is covering the hole and notation cannot be done without prior cleaning.

<u>Lab analysis (with scan:</u> baits are then transported to lab (protect the bait during the transport from the field to the laboratory). The soil present on the plastic of the stripes is removed carefully with a wet tissue Scan all the bait using a scanner whatever the resolution (from 200 to 600 dpi) but avoid overlapping of bait lamina. Use the program to calculate the decomposition rate.

*Tip2: soil removing- let the lamina baits air-dried before to remove gently the soil Tip3: to get better result, use a scanner with double source of light: above and below the bait* 

Costs: (Terra protecta)

PVC strips: 2.5 euros/strip (price decrease if > 1,000) Kraft original substrate (bran flakes): 200 euros/60g



# Appendix 3. Soil fauna sampling and identification

Soil macro-invertebrate (i.e. all invertebrate with body-size > 2mm) were collected using a combination of "quantitative" and "qualitative" methods.

#### Quantitative sampling (3 blocks x 3 rep/block)

<u>TSBF (Tropical Soil Biology and Fertility) conventional method (Anderson and Ingram, 1993)</u>: Three blocs of soil (25 x 25 cm surface and 20 cm depth) were dug out in each plantation and divided in two layers (litter and 0-15 cm) to allow the production of quantitative community data (i.e. density or biomass of invertebrates per surface unit). Each layer was then hand sorted for the whole macro-invertebrates community.



#### Qualitative sampling (1 per block)

A qualitative approach was thus used to complete the results for earthworms, which represent the main detritivorous group in terms of biomass. These results will be used to evaluate total richness (with data from TSBF in cumulative curves).

This consisted in searching specimens during a fixed period of time (15 mms for the group but it could be 1h for one person) in all the microhabitats present on the area of the plantation (i.e. mainly soil and litter).



Storage of soil Fauna

All specimens will be fixed and conserved in pure ethanol (100%), then put in freezer as soon as possible.

*Tip: labelling is done on calque paper writing with paper pencil (non sensitive to ethanol)* 

#### Soil fauna identification

- In the lab, invertebrates were assigned, using binocular, to broad taxonomic units (to family or species level following key of soil fauna identification see documents attached)
- They were counted and weight to calculate species density and biomass at each sampling point.
- Tissue samples were further collected on a selection of invertebrates in prevision of further DNA analyses (using Cytochrome c oxidase 1 (CO1) as universal barcode for animal kingdom).

*The Tip: Do not put too many invertebrates together in pills (reduce ethanol efficiency, degrade fauna DNA)* 

The aim is to describe the topsoil layers encountered in the field in order to achieve a morphofunctional diagnosis or 'litter diagnosis' *[mainly for forest and/or perennial crops diagnosis.* 

*NB: for a complete description of humus form see the document attached "Terrestrial humus forms: ecological relevance and classification" by JF Ponge and coll.* 

### Methodology

Similar sampling (3 blocks, 3 rep/block).

- 1. Choose a location that is representative: avoid local accumulations; unusual perturbation (here: sampling between row and middle of inter-row).
- 2. Describe in sufficient detail the site conditions (exposure, vegetation, topography or micro-topography) as well as any human intervention.
- 3. Put the frame (25 x 25 cm) on the selected area [a transparent frame was used to better see sample representativeness as well as any possible litter movements during cutting].
- 4. Cut around the square and then clear the ground around the frame.
- 5. Remove the frame then gently manually harvest the different layers one after another and put it in a plastic bag.

Layers were separated as follow:

- Living (fresh) vegetation/plant
- Wood (OL) [were also included fruits and barks but these latters could also be separated into specific categories]
- Entire leaves (OL, organic litter): more than ½ of the leaf is entire [including the ones artificially cut during square sampling]; can be free (ie isolated) or compacted (stuck with others) leaves, with or without presence of bleach (white fungal pigmentation); in lab, after drying (24H, 65°C) entire leaves will be separated and weighted both into free vs compact compartment and entire vs skeletonized (if > 50% skeleton) compartments
- Fragmented leaves (OF) (<1/2 leaf); later on (in lab) separated and weight into 2 compartments (free vs compact)
- Humified (OH) if the leaf fragment is mixed with more than 70% of fine organic matter (FOM)
- Earthworm cast (either on 25x25cm surface if the number of cats islimited, or on a 10x 10 cm sub-sample if abundant)

A sub-sample of 10x10x5 cm (0-5 cm depth) was taken for lab analysis

- Soil humidity % (sieving 2mm, fresh weight of sub-sample then dry weight after 24h in oven at 105°C)
- Root weight (dry weight after 48h at 65°C)

Additional plot characterization was performed based on the following criteria:

- 1) Light penetration (as indicator of canopy closure): from 0 (no light) to 3 (high penetration)
- 2) Living plant cover at soil surface (0-3)
- 3) Wood at soil surface (0-3)
- 4) Slope (0-3) (not used here)

5) Others (e.g. related to plot/land management, water fluxes, neighboring plots etc.) <u>Humus classification (8 types):</u>

- Mull (bio macro-structures A horizon) 4 types according to % of OL and OF: eu, meso, oligo and dysmull

- Moder (juxtaposition of mineral and organic compartments; "pepper and salt" structure); 3 types: hemi, eu, dys

- Mor: no organo-mineral layer (specific acid soils)

[Not done: The thickness of the layers can be measured. Make a small trench (15cm depth) near the sampling point with a spade at least to the base of organo-mineral horizons (A) recognized by their darker color (presence of organic matter). This trench will allow the measurement of the thickness of the horizons. Refresh the trench carefully with a knife, especially at the holorganic horizons that the blade must cut cleanly. Take pictures of all layers]



#### Example of humus forms description

	N°sample				
	Date				
	Operator				
	Time				
	Environment				
		null (<	-3%)		
			,		
	Slope		<u>3 à 10%)</u>		
			nediate (10 à 259	/0)	
		high (2	>25%)		
	Exposition (N/E/S/O)				
lol	organic layer – Wood.				
	Woods materials in holorganic layers				
Dr.	Descriptors		Units	Values	Remarks
	Entire little twigs (diameter $< 2 \text{ mm}$ )		mg	Ì	
	Entire large twigs (diameter $> 2 \text{ mm}$ )		mg		
	Little twigs (diameter < 2 mm) decayed and tunne	lled by fauna			
	Large twigs (diameter $> 2$ mm) decayed by fauna		mg		
	Intact petioles		mg		
	Petioles decayed and browsed by fauna		mg		
	Intact wood fragments		mg		
8	Bark fragments				
			mg		
∠ay	Remarks ver OL. Intact litter (<10% de FON	M, fragm	entation > 1		
L <b>ay</b> Dr	Remarks ver OL. Intact litter (<10% de FON Descriptors	∕I, fragm	entation > 1	half of leaves, fr	ree or compacted) Remarks
L <b>ay</b> Dr 9	Remarks <b>ver OL</b> . Intact litter (<10% de FON <i>Descriptors</i> Woody species (seedlings or saplings)		entation > 1 Units Number		
2 <b>ay</b> Dr 9 10	Remarks ver OL. Intact litter (<10% de FON <i>Descriptors</i> Woody species (seedlings or saplings) Vegetation (bryophytes, lichen, pteridophytes, etc.		entation > 1		
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Dr 9 10 11 11a 11b 11c 11d 11e	Remarks ver OL. Intact litter (<10% de FON Descriptors Woody species (seedlings or saplings) Vegetation (bryophytes, lichen, pteridophytes, etc. Intact litter Total mass Entire green leaves Entire brown leaves Entire bleaches leaves Entire skeletonzied leaves		entation > 1 Units Number Number mg mg mg		
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Dr 9 10 11 11a 11b 11c 11d 11c 11d 11c 12a 12b 12c 12d 12c 12d 13 14 15	Remarks Ver OL. Intact litter (<10% de FON Descriptors Woody species (seedlings or saplings) Vegetation (bryophytes, lichen, pteridophytes, etc. Intact litter Total mass Entire green leaves Entire brown leaves Entire bleaches leaves Entire skeletonzied leaves Fragmented litter (> 50% of leaves) Total mass Brown leaf fragments skeletonized by mesoj Brown leaf fragments cut out by macrofaun Brown leaf fragments both M & m Bleached leaf fragments Fine roots (diameter < 2 mm) Roots of intermediate diameter (6 mm > diameter	) fauna (m) a (M)	entation > 1 Units Units Number Mumber mg		
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Lag           Dr           9           10           11           11a           11b           11c           11d           12a           12b           12c           12d           12d           12d           12d           12d           13           14           15           16           17           18           19           20	Remarks         ver OL. Intact litter (<10% de FON	) fauna (m) a (M)	entation > 1 Units Number Number mg mg mg mg mg mg mg mg mg mg		
Jay           Dr           9           10           11           11a           11b           11c           11d           12d           12d           12d           12d           12d           12d           13           14           15           16           17           18           19           20           21	Remarks         /er OL. Intact litter (<10% de FON	) fauna (m) a (M)	entation > 1 Units Units Number Number mg		

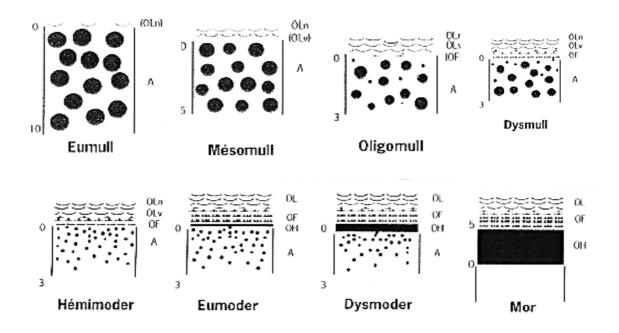
r	Descriptors	Units	Values	Remarks
36	Fragmented litter with more than 70% of FOM		•	
36a	Total mass	mg		
36b	Brown leaf fragments both M & m	mg		
36c	Bleached leaf fragments	mg		
37	Fine roots (diameter < 2 mm)	mg		
38	Roots of intermediate diameter ( $6 \text{ mm} > \text{diameter} > 2 \text{ mm}$ )	mg		
39	Big roots (<6 mm)			
40	Rhizomorphs	mg		
41	Mycorhizas	mg		
42	Reproductive organs (fruits, flowers, seeds)	mg		
43	Millipede faeces	mg		
44	Organic epigeic earthworm faeces	mg		
45	Earthworm casts (organo-mineral aggregates)	mg		
	Organic epigeic earthworm faeces	mg		
47	Others (shell of snails, cuticles, carpophore, etc.)	mg		
	Remarks			
Jav	yer OH. Humification litter (FOM >70%)			
	Descriptors	Units	Values	Remarks
	Fragmented litter with more than 70% of FOM	Onus	values	Пеншикз
36a	-	ma		
36b		mg		
		mg		
36c		mg		
	Fine roots (diameter < 2 mm)	mg		
	Roots of intermediate diameter ( $6 \text{ mm} > \text{diameter} > 2 \text{ mm}$ )	mg		
	Rhizomorphs	mg		
	Mycorhizas	mg		
	Reproductive organs (fruits, flowers, seeds)	mg		
	Millipede faeces	mg		
	Organic epigeic earthworm faeces	mg		
	Earthworm casts (organo-mineral aggregates)	mg		
	Organic epigeic earthworm faeces	mg		
46	Others (shell of snails, cuticles, carpophore, etc.)	mg		
	Remarks			
A la	ayer (organo-mineral). Mixture of organi	ic materials an	d minerals.	
	Descripteurs	Unités	Valeurs	Remarques
	Thickness	ст		1
-		$Diffuse > 8 \ cm$		
40		Gradual 4 à 8 cm	!	
49	Transition with mineral horizon	Distinct 2 à 4cm		
		Net <2cm		
		%blocs (>20cm)		
		%pebbles(2-20cm	1)	
		%gravels(0,2-2cm		
		Sandy	·	
		Sand-loam		
51	Texture	Loamy		İ
		Loam-clay		l l
		Clay		
52	Coloration	Munsell		l l
	Rhizomorphs	mg		l l
	Mycorhizas	mg		
	Fine roots (diameter < 2 mm)	mg		
	Roots of intermediate diameter (6 mm > diameter > 2 mm)	mg		İ
	Big roots (<6 mm)	mg		İ
	Hcl effect	0 or 1		
20				

#### Theory link to the humus form

The Humus form, i.e. the part of the soil which is influenced by organic matter (Brêthes et al., 1995), has been recognized for a long time as the seat of most biological and physico-chemical processes essential to soil development and terrestrial ecosystem functioning. This concept applies to every kind of soil the upper part of which (the topsoil) is not permanently disturbed by human activity, i.e. to all non-tilled soils.

Ponge (2003) considered Mull, Moder and Mor (Fig. 1) as three strategies of terrestrial ecosystems. Mull is characterized by an intense mixing of organic matter with mineral matter (i.e. the result of earthworm activity), stemming in a crumby and nutrient-rich organo-mineral horizon, Moder by a less rapid transformation of litter by litter-dwelling animals and fungi, resulting in the accumulation of organic humus, Mor by the slow transformation and accumulation of undecayed plant debris, with a sharp transition to the mineral soil. Mull, Moder then Mor correspond to a scale of decreasing nutrient availability and colder conditions, stemming in decreasing biological diversity and activity on siliceous substrates. Animals, microbes and plants are involved in positive (building forces) and negative (stabilizing forces) feed-back relationships most of them taking place in the humus profile. Look at the example of a forest mull: if the parent rock is rich in easy weathering minerals and the climate is mesic (not too cold, not too dry), then plant growth is rapid, including trees (site quality and productivity is high) and more exacting plants are allowed to grow (i.e. flower plants, with nutrientrich and lignin-poor foliage, renewed annually). In turn litter (trees + forest vegetation) is nutrient-rich and will favoured more exacting microbes (bacteria) and animals (earthworms) the activity of which will contribute to favour tree growth and a diverse vegetation, which is typical of multi-layered forests. The same ring of causes and consequences explains why Mor, on the reverse side, is poorer in microbial, faunal and plant species and characterizes less productive but more conservative ecosystems: in familiar words, mull is a waster (the cicada of the fable), while Mor is a hoarder (the ant of the fable), but each of them allows a safe use of resources offered by geology and climate. Hence, the indicator value of humus forms.

Based on the knowledge accumulated on the relationships between morphological, biological and physico-chemical features of humus forms, several attempts have been made to classify them on the base of characters discernable to the naked eye directly on the field, and to derive from them properties at the ecosystem level (site quality assessment).



Fauna		Fast litter disappearance	OM and minerals mixture	Litter fragmentation	"A" structuration	Argilo-humic complexes formation	Brown pigment degradation	Accumulation of faeces (OH)
	Anecic	YES	YES YES		YES	YES	YES	NO
Earthworms	Epigeic	NO	NO	YES	NO	NO	?	YES
	Endogeic	NO	YES	NO	YES	YES	?	NO
Enchy	rtreids	NO	NO	SLIGHTLY	NO	NO	NO	YES
Micro-ar	thropods	NO	NO	YES	NO	NO	NO except isopoda	YES
Larvae of diptera		YES	NO	YES	NO	NO	NO	YES
Fu	ngi	YES	NO	NO	NO	NO	YES	NO

# Appendix 5: Percentage of litter cover

Lane point method: a rope whose length is known (here a 4.5m-long rope) is put on soil surface (here perpendicular to rubber rows to cover both row and inter-row). Along this rope, all the points without litter (bare soil visible) are measured (cm) Percentage of bare soil is known using the following formula: sum of the points without litter

(cm) : rope length (cm) x 100.

3 replicates per plot.



## Appendix 6: results obtained during the training

### 1- Lamina baits

	-		-	-	-	-	-								-	-	-	-	-	-
	А	В	С	D	E	F	G	H		J	K	L	M	N	0	P	Q	R	S	
1	Date of	insertion	Monday 3			BAIT LAMINA TEST														
2	Date of	sampling	Monday 10	nov 2014					DATI LAWIINA IESI											
3	Note the	number of "0"	' = FULL; "	1" = PAR	ГIAL	LY E	EMP1	ΓY; "	2" =	EM	РТҮ	; "3"	= SC	DIL A	TTA	CHI	ED. T	he to	otal =	16
4										]	BAIT	SU1	BSTE	RATE	2					
5			71.070			H (b	ean)			W (w	heat)			E (Euc	alypt)	)		A (Ac	ccacia)	)
6	BLOCK	PLANTATION	PLOTS	N° of bait	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
7				1																
8				2																
9				3																
10				4																
11			1	5																
12			1	6																
13				7																
14				8																
15				9																
16				10																
17				1																
18				2																
19				3																
20				4																
21			2	5																
22			-	6																
23				7																
24				8																
25 26				9																
				10																
27				1																
		• . · ·	·							_		-	-	-	_					

An example of Excel table for enter data from bait lamina

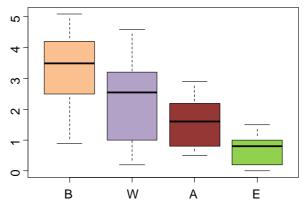
AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG
	Synthesis per plot										SYNTHESIS PER FIELD				
	Activity per plot Values number									Activity per field					
Depth	A	w	Е	Н	A	w	Е	Н	%loss		Depth	А	w	Е	Н
1	1	0	0	2	1	9	0	9	50		1	1	1	1	2
2	1	1	0	2	1	10	0	10			2	0	1	0	2
3	0	1	0	1	0	10	0	10			3	0	2	0	2
4	0	1	0	1	0	9	0	10			4	1	2	0	2
5	0	2	0	0	0	10	0	10			5	1	2	0	2
6	0	2	1	2	0	10	1	10			6	1	2	0	3
7	0	0	0	0	0	9	0	10			7	1	2	0	2
8	0	0	0	0	0	10	0	10			8	0	2	0	2
9	0	1	0	1	0	10	0	10			9	0	2	0	3
10	0	1	0	1	0	10	0	9			10	0	2	0	3
11	0	1	0	1	0	10	0	9			11	0	2	1	3
12	1	2	0	1	1	10	0	9			12	1	2	0	3
13	1	2	0	2	1	10	0	9			13	0	3	0	3
14	2	2	0	1	2	10	0	9			14	1	2	0	3
15	0	1	0	2	0	10	0	10			15	0	2	1	3
16	0	1	0	2	0	10	0	9			16	0	2	0	2
Weigthed mean	1,3	1,0	1,0	0,9							Mean	1,2	1,9	0,7	2,5
I OV 🖉 3 - IV KR 🖉 4	-II KR	/ 5 - IV	BT 🛛 6 -	II BT /	Feuil1 /	Table ra	w data 🟒	*			1 4				

An example of average calculation from bait lamina data

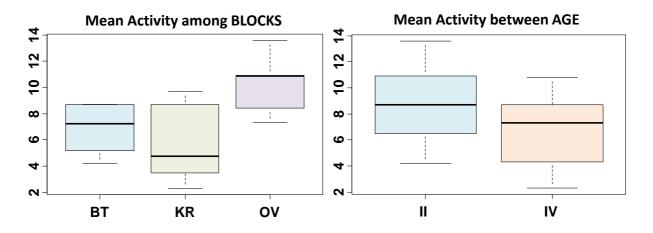
In order to take into account the number of holes that cannot be count, the mean of the activity has to be weighted by the number of values for each layer, or for each stick. The mean activity can be calculated from the sum or the average of individual activity.

#### 3. Results

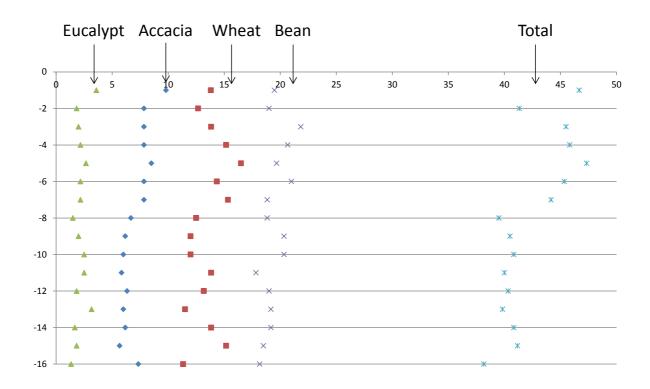
The soil activity of the rubber plantations decreased from Bean substrate to Eucalypt substrate. We observed a slight decrease of the Bait activity with plantation age, as well as a Block effect.



Overall range of activity according to substrate



The analysis can be done according to depth.

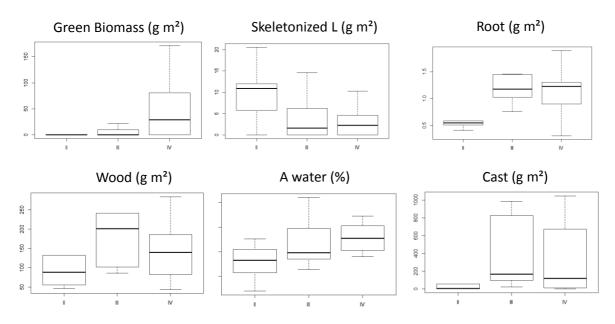


### 2- the humus form

Using a **Pivotable Table**, we can calculate the average and SD of each variable according to plantation age or blocks.

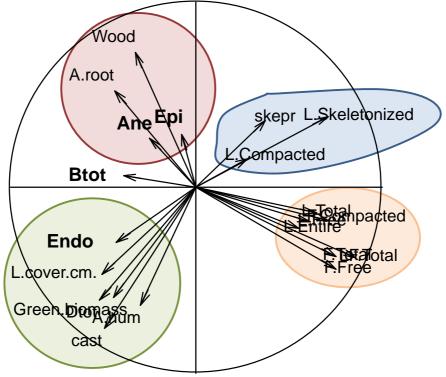
	ichie: Ascueil Ins	ertion Mise en page Formul	es Données	Révision	Affichage Compléme	nts Acrobat	
$\left( \right)$	17	🔜 📲 🕞 🔚		<u>ik</u> ٵ	) 불 📥 🗠	🜔 🚾 📠	1
Th	ICroiséDynamique Tableau	J Image Images Formes SmartArt	Capture Colonne	Ligne Secter	urs Barres Aires Nuage	Autres Courbes Histogramm	e Positii
	Tableaux	Illustrations		G	raphiques	Graphiques s	parkline
	A23 -	f <sub>x</sub>					
	A	В	C	;	D	E	
1							
2							
3	Étiquettes de lignes	Moyenne de Green-biomas	s Moyenne de	L-cover(cm)	Moyenne de L-Entire	Moyenne de L-Skeletonize	ed Sor
4	I		0	76,84	3,298888889	0,5653333	33
5		0,3488888	89	79,43444444	1,836666667	0,2588888	89
6	IV	3,2766666	67	84,46777778	2,663333333	0,3277777	78
7	Total général	1,2085185	19	80,24740741	2,59962963	0,3	84
8							
9	Étiquettes de lignes	Écartype de Green-biomass	Somme de L-	cover(cm)	Somme de L-Entire	Somme de L-Skeletonized	Sor
1	II I		0	230,52	9,896666667	1,6	96
1	L	0,6042932	82	238,3033333	5,51	0,7766666	67
1	2 IV	2,7702607	18	253,4033333	7,99	0,9833333	33
1	3 Total général	2,1068097	99	722,2266667	23,39666667	3,4	56
14	1						
1	5						
1	5 II	0,5653333	33				
1	7 111	0,2588888	89				
1	3 IV	0,3277777	78				

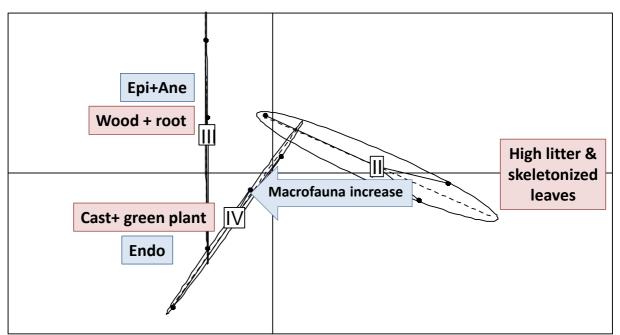
The analysis of the data showed that some morphological descriptors change with plantation age (skeletonized leaves, cast and root). We observed high green plant biomass in old plantations.



Patterns of some morphological descriptors along the chronosequence

We performed a Principal Component Analysis with humus data. The correlation circle showed that OL and OF descriptors exhibited high values in the young plantations while cast, Green biomass and L.cover exhibited high values in the old plantation. The PCA suggested that mesofaunal activity was high at the onset of the chronosequence while the macrofaunal activity, especially earthwoms, increased in old plantations.





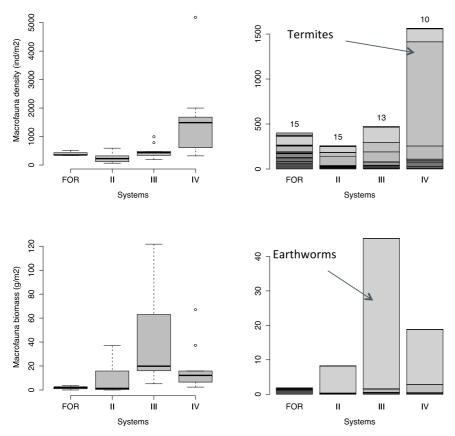
A Principal Component Analysis perfomed on Humus Forms data

### 3- Soil fauna sampling and identification

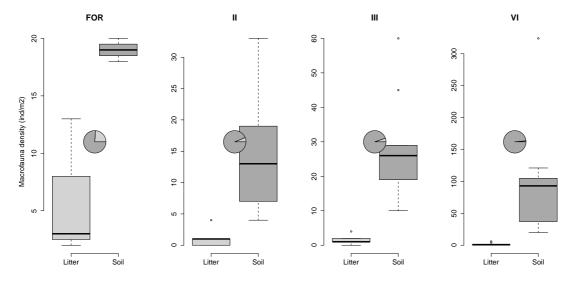
Variations in biomass and density for the whole macrofaunal communities are given in Figure 1. Total density tended to increase during the chronosequence, mainly because of sharp increase in termite populations in the older plantation. However, this result has to be considered with caution due to the huge spatial variability expected in the distribution of these social organisms, and to a relative inadequacy of the TSBF procedure for estimating their density. Total biomass also increased globally with plantation ageing, at least until age III, and then seemed to decrease in the older plantations. This was mainly due to variation in the global earthworm biomass, which was the higher, but also the more variable, in plantation age III. Taxonomic diversity measured as the cumulated number of broad taxonomic units observed in each system was high in the forest and young plantations and tended to decrease in the older plantations, maybe because of a strong dominance of communities by termites and/or earthworms in these systems.

The vertical distribution of macroinvertebrates was also strongly affected by land uses. In the forest, almost 25% of the invertebrates were collected in the litter layer, while in the plantations this proportion tended to decrease with the age of the system. Along the chronosequence, we also observed an increase in the variability of invertebrate density in the soil layer, while this variability in the litter was higher in the forest.

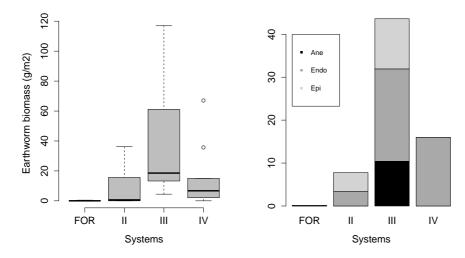
Regarding earthworm communities, biomass was very low in the forest, where the dominant groups were arthropods from the litter and superficial soil systems, and tend to increase in plantations, as previously mentioned. The relative importance of large pigmented species (anecic, epi-anecic and epigeic species) increased markedly in the older plantations, to the detriment of endogeics that were almost absent from the older systems. In plantations II and III, a significant proportion of endogeics were represented by the invasive *Pontoscolex corethrurus*, which is known to build high populations when introduced in disturbed tropical soils.



*Figure 1.* Soil macroinvertebrate density and biomass (illustrated using boxplots at the left and barplots at the right) in forest and rubber plantations.



*Figure 2.* Vertical distribution of soil macroinvertebrates in forests and rubber plantations (illustrated with density boxplots and pie-chart showing the proportion of litter versus soil dwelling invertebrates).



*Figure 3.* Earthworm biomass (illustrated using boxplots at the left and barplots at the right) in forest and rubber plantations.