


centre d'étude de la biodiversité amazonienne

6th International Barcode of Life Conference August 18-21, 2015

Dissecting earthworm biodiversity patterns in tropical rainforests through the use of DNA barcoding

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Embrapa

Centre d'Écologie Fonctionnelle & Évolutive

cnrs

Rationale

Soil invertebrate functions

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The values of soil invertebrates

- Ecological importance: regulators of soil processes
- Economic importance: maintenance of soil fertility
- Strategic importance: highly diverse communities
- Strategic importance: invasive species
- Practical importance: used as bioindicators of soil quality or pollution

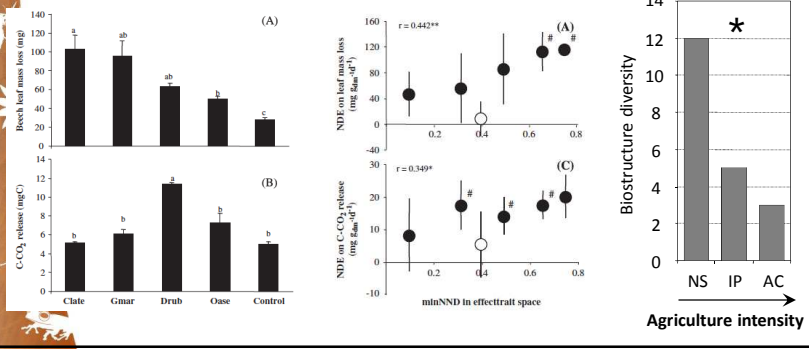
Decaëns et al. (2006) *Eur J Soil Biol*

Rationale

Soil invertebrate functions

Diversity matters

- Different species perform differently in ecosystems
- Invertebrate functional diversity impacts ecological functions
- Global change can affect soil biodiversity and the production of related ecosystem services

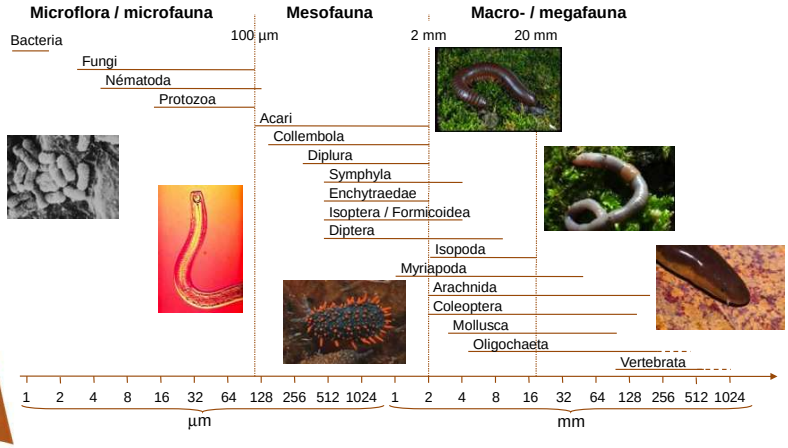


Hedde et al (2007) *Appl Soil Ecol*
Hedde et al (2010) *Basic Appl Ecol*
Decaëns et al. (2002) *Eur J Soil Biol*

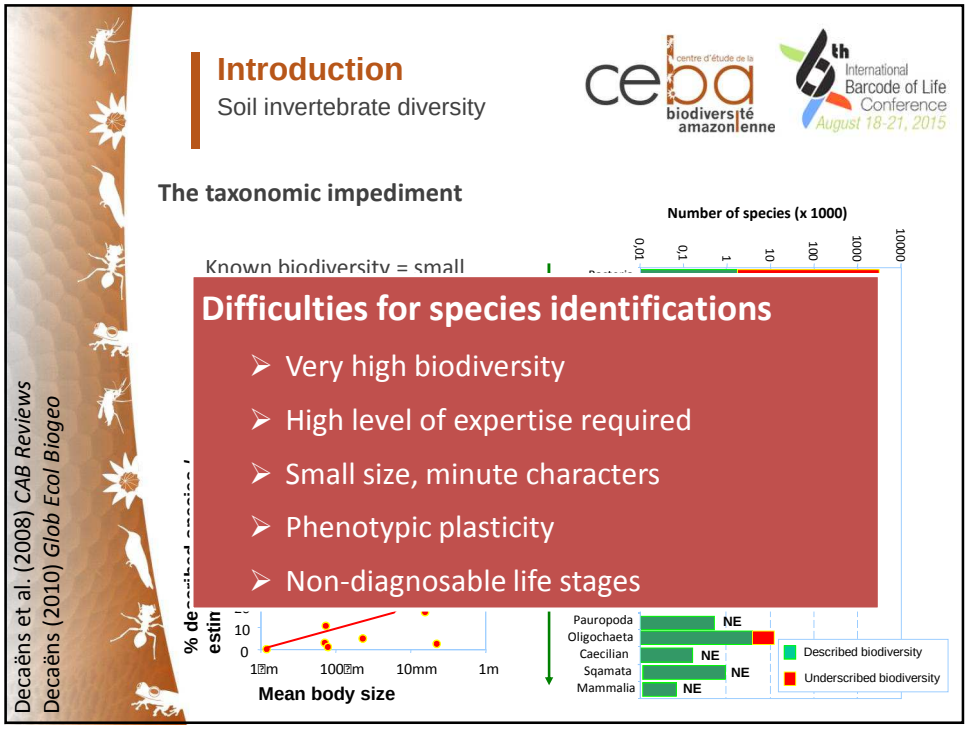
Introduction

Soil invertebrate diversity

Global soil biodiversity



Swift et al. (1979), Decaëns et al (2006), Brussaard (1997), Behan-Pelletier (1999), André (2001), Wall, André (2002), Decaëns (2010)


Introduction
Soil invertebrate diversity
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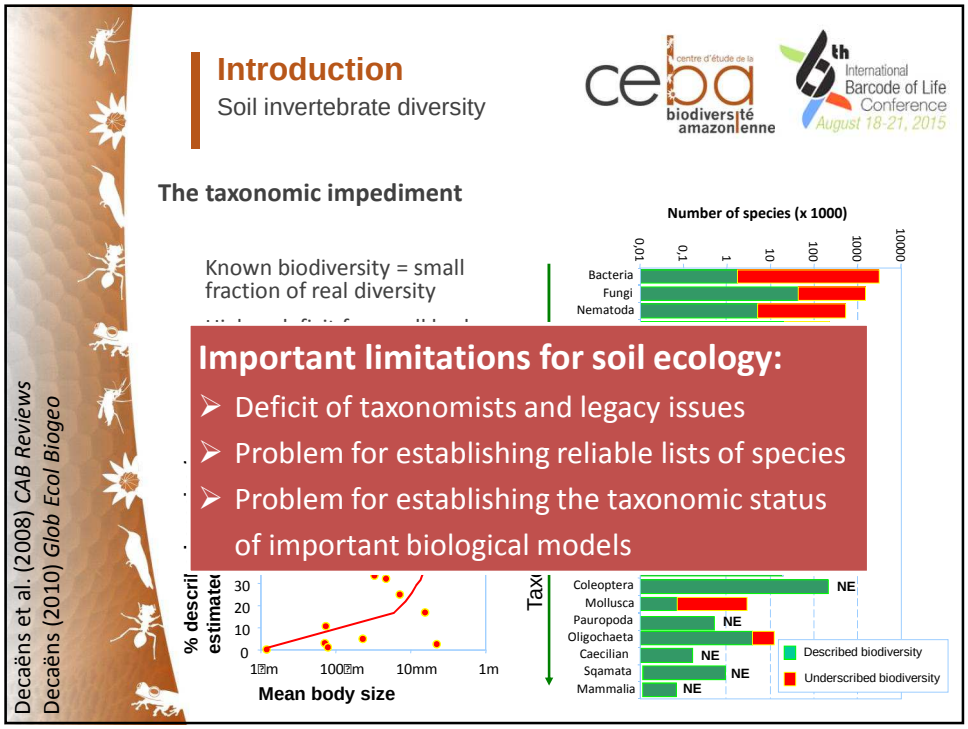
6th International Barcode of Life Conference August 18-21, 2015
The taxonomic impediment
Number of species (x 1000)
0,01
0,1
1
10
100
1000
10000
Known biodiversity = small
Difficulties for species identifications
Very high biodiversity
High level of expertise required
Small size, minute characters
Phenotypic plasticity
Non-diagnosable life stages
% described species / estimated species
30
20
10
0
1m
100mm
10mm
1m
Mean body size
Pauropoda
Oligochaeta
Caecilian
Sqamata
Mammalia
NE
NE
NE
Described biodiversity
Underscribed biodiversity
Decaëns et al. (2008) CAB Reviews
Decaëns (2010) Glob Ecol Biogeo


Introduction
Soil invertebrate diversity
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6th International Barcode of Life Conference August 18-21, 2015
The taxonomic impediment
Number of species (x 1000)
0,01
0,1
1
10
100
1000
10000
Known biodiversity = small fraction of real diversity
Bacteria
Fungi
Nematoda
Important limitations for soil ecology:
Deficit of taxonomists and legacy issues
Problem for establishing reliable lists of species
Problem for establishing the taxonomic status of important biological models
% described species / estimated species
30
20
10
0
1m
100mm
10mm
1m
Mean body size
Coleoptera
Mollusca
Pauropoda
Oligochaeta
Caecilian
Sqamata
Mammalia
NE
NE
NE
NE
Described biodiversity
Underscribed biodiversity
Decaëns et al. (2008) CAB Reviews
Decaëns (2010) Glob Ecol Biogeo

Rationale

Earthworms





Earthworm global patterns

- Are present in most soils of the world
- Represent the dominant component of soil faunal biomass in many ecosystems
- 5500 species described to date

DNA barcoding

- Rapid identifications, species discrimination
- Juveniles identification
- Cryptic diversity

➜ **Molecular operational taxonomic units (MOTUs)**

➜ Community scale barcoding to describe diversity patterns in regions where taxonomy is mostly unresolved

➜ **Useful tool in soil ecology, macroecology, biogeography…**

Lavelle & Lapied 2003, Rougerie et al. (2009), James et al. (2010), Richard et al. (2010), Novo et al. (2011), Decaëns et al. (2013)

Rationale

Earthworm diversity patterns in tropical rainforests

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Context

- Earthworm communities are poorly studied when compared to other groups
- We still know very few about earthworm diversity and distribution in tropical rainforests
- For example: only 22 sp listed from French Guiana (Pavlicek & Csuzdi, 2012)

The WormBank project:

➜ To build a library of DNA barcodes (COI) for earthworms of French Guiana

➜ To use the data to explore community patterns at different spatial scales

Funding : CEBA, CNRS (APEGE, Nouragues), ANR, iBOL

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International BARCODE OF LIFE

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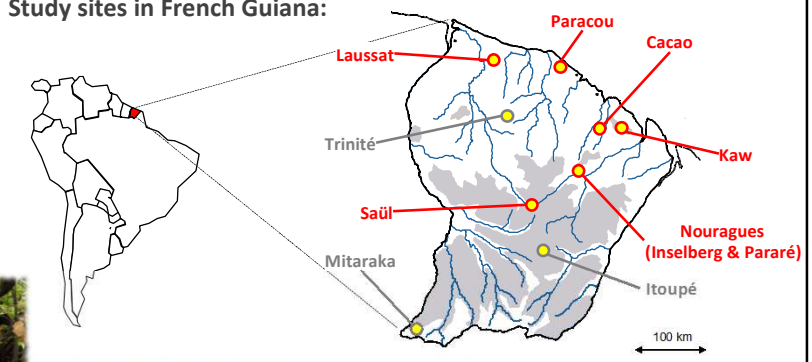
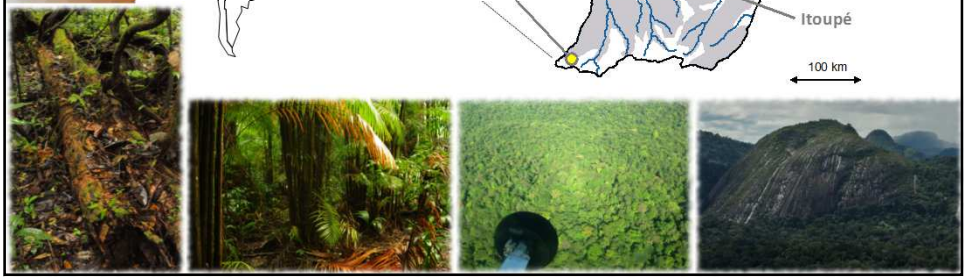
Material and methods

Sampling design

Study sites in French Guiana:

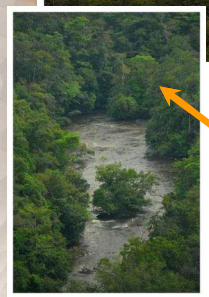




Material and methods

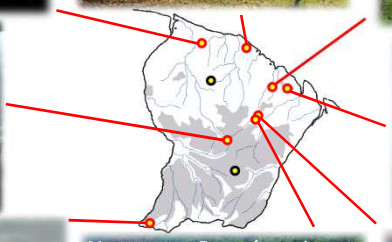
Sampling design

- A range of selected habitats in each site
- At least 3 replicates / habitats
- In each replicate:
  - ➜ Hand sorting of 3 soil blocks of 25x25cm
  - ➜ 2 hours (2 people) of qualitative sampling on a 1ha area
  - ➜ Soil, litter, decaying trunks, epiphytic soils…



Material and methods

Sampling design



Material and methods

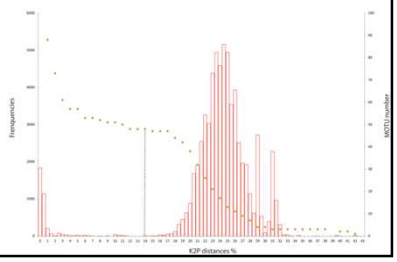
Sample processing

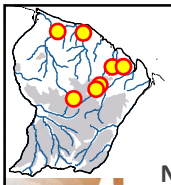
DNA barcoding

- Specimens fixed in 100% ethanol, separated into morphospecies
- Up to 5 specimens / morpho sp / sample for individual-based barcoding
- COI sequencing using Sanger (iBOL funding) or MiSeq technology (APEGE funding)

Barcode gap identification

- Identification of the barcode gap at 14% (Nouragues data)
- MOTU ➔ species richness and community structure





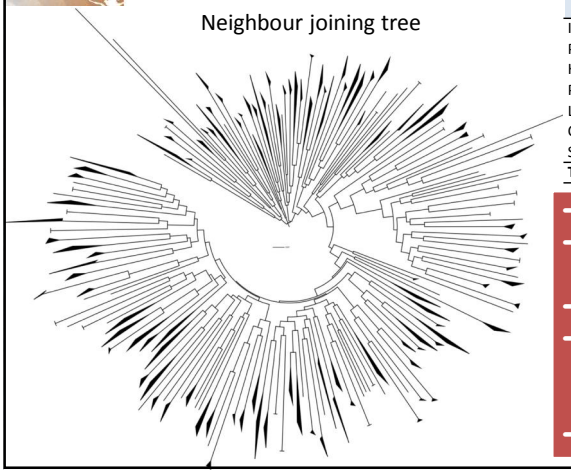
Results

Regional diversity

New results for seven study sites

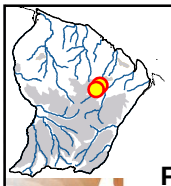
- 2561 specimens barcoded in seven different locations

Neighbour joining tree



	Year	# spcimens	# of MOTUs
Inselberg	2010	440	38
Pararé	2010	213	27
Kaw	2013	227	29
Paracou	2013	389	45
Laussat	2014	97	11
Cacao	2011-13	793	36
Saul	2013-14	402	36
Total		**2561**	**148**

- ➜ Total of 148 MOTUs
- ➜ Good match with morphology (Nouragues data)
- ➜ Most are sp new for science
- ➜ Dramatic increase compared to the 22 species checklist (Pavlíček & Csuzdi, 2012)
- ➜ Highlight a strong deficit

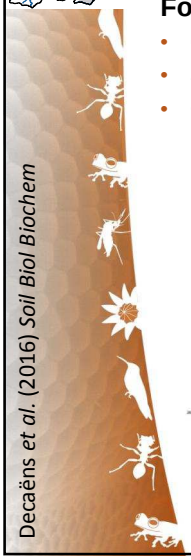


Results

Regional diversity

Focus on Nouragues' communities

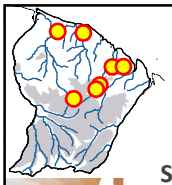
- 650 specimens barcoded in two different locations (Pararé and Inselberg)
- 5 families, 12 genera, 48 species level MOTUs
- Good match with morphology, except in a few cases



Decaëns *et al.* (2016) *Soil Biol Biochem*

- ➜ Highest local richness ever recorded worldwide
- ➜ Coexistence of most South-American families at a local scale (museum hypothesis)
- ➜ Coexistence of clusters of closely related species (cradle hypothesis)

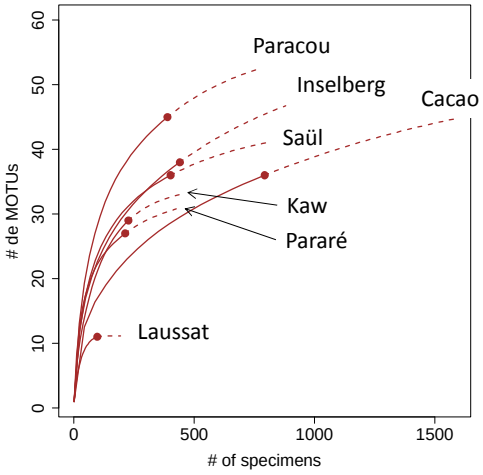
Family / genus	# of MOTUs
Rhinodrilidae	
Rhinodrilus	2
Pontoscolex	3
Urobenus	1
Glossoscolecidae	
Atatina	1
Glossodrilus	4
Martiodrilus	8
Nouraguesia	1
Righiodrilus	6
Acanthodrilidae (Benhaminae)	
Dichogaster	3
Neogaster	7
Wegeneriona	6
Haplotaxidae	
Haplotaxis	1
Ocnerodrilidae	
Ocnerodrilidae	3
Not identified	
Not identified	2
Total	**48**



Results
Regional diversity

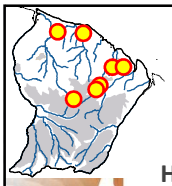
Sampling completion

Rarefaction curves by localities



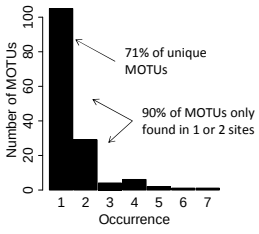
	Obs richness	*% singletons*	*Chao 1*
Inselberg	38	26	56
Pararé	27	22	33
Kaw	29	26	34
Paracou	45	27	57
Laussat	11	9	11
Cacao	36	33	54
Saul	36	22	44
Total	**148**	**26**	-

- ➜ % of singletons usually > 20%
- ➜ Local richness estimates range from 11 to 57 species
- ➜ Very high local richness compared with other tropical sites

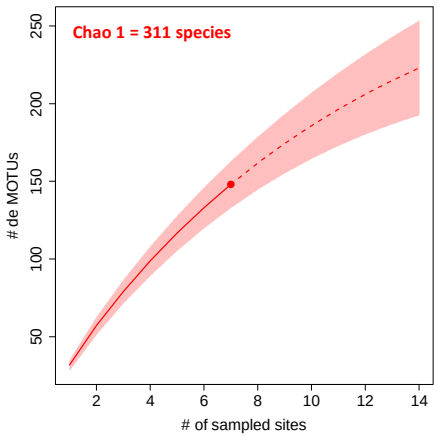


Results
Regional diversity

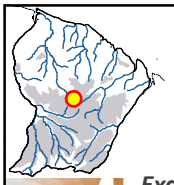
How many species in French Guiana?



Rarefaction curve at regional scale



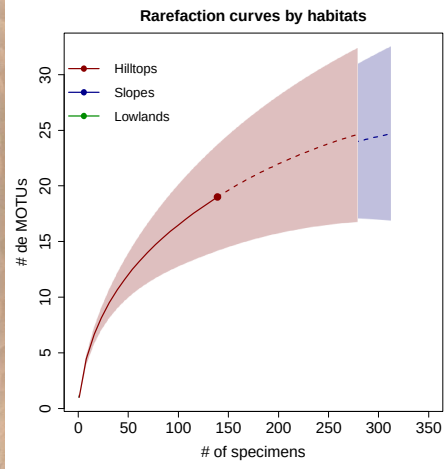
- ➜ 134 MOTUs out of 148 (i.e. 90%) were found in less than two localities
- ➜ Mean pairwise Sorensen's dissimilarity = 81%
- ➜ % of shared MOTUs in pairwise comparisons < 20% except in one case



Results

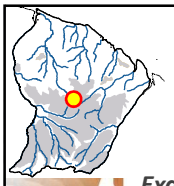
Local community structure

***Example*: community structure in three habitats at Saül**



	# spcimens	*Obs richness*	*Chao 1*
Lowland	82	18	22
Slopes	156	20	28
Plateaus	139	19	30
Total	**402**	**36**	**54**

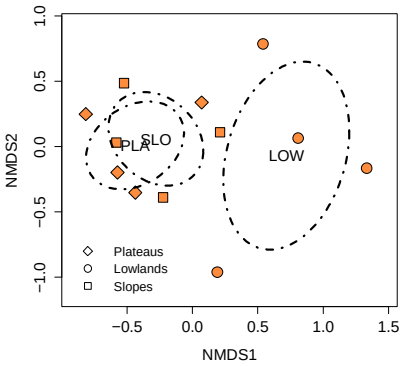
- ➜ **Species richness lower in lowland forests than in the other two habitats**
- ➜ **Alpha diversity patterns identical in slope and plateau forests**

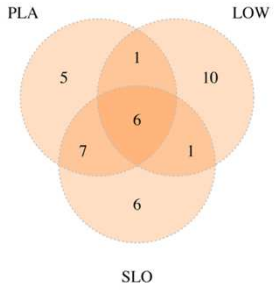


Results

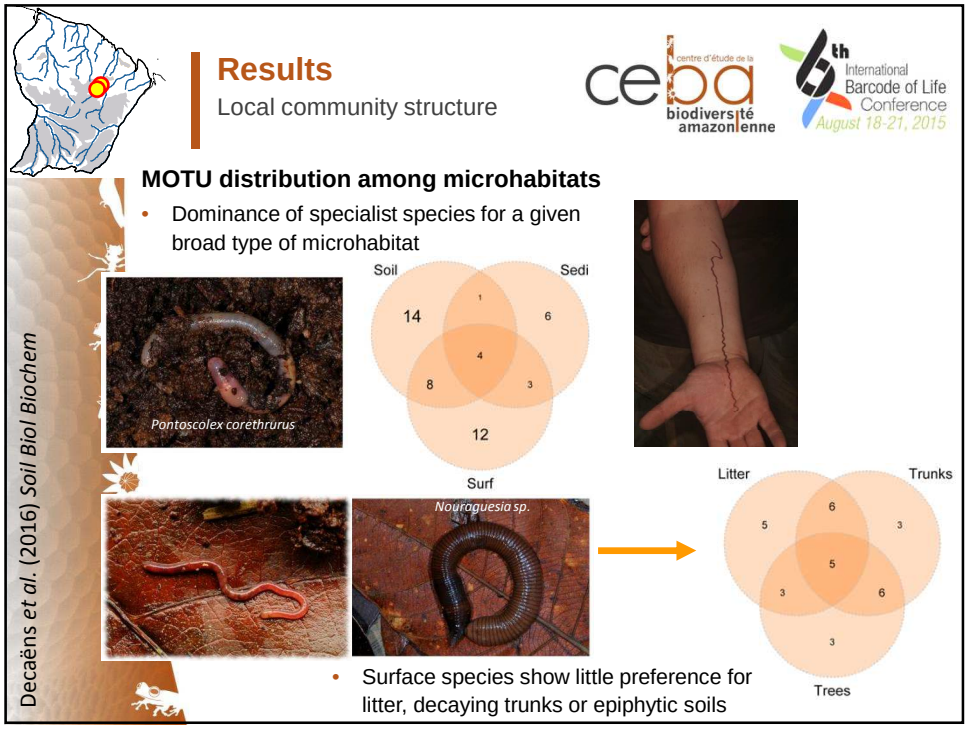
Local community structure

***Example*: community structure in three habitats at Saül**





- ➜ **Strong habitat signal**
- ➜ **Lowland forests differentiated from the other two habitats**
- ➜ **58% of MOTUs found in a single habitat**


Results
Local community structure
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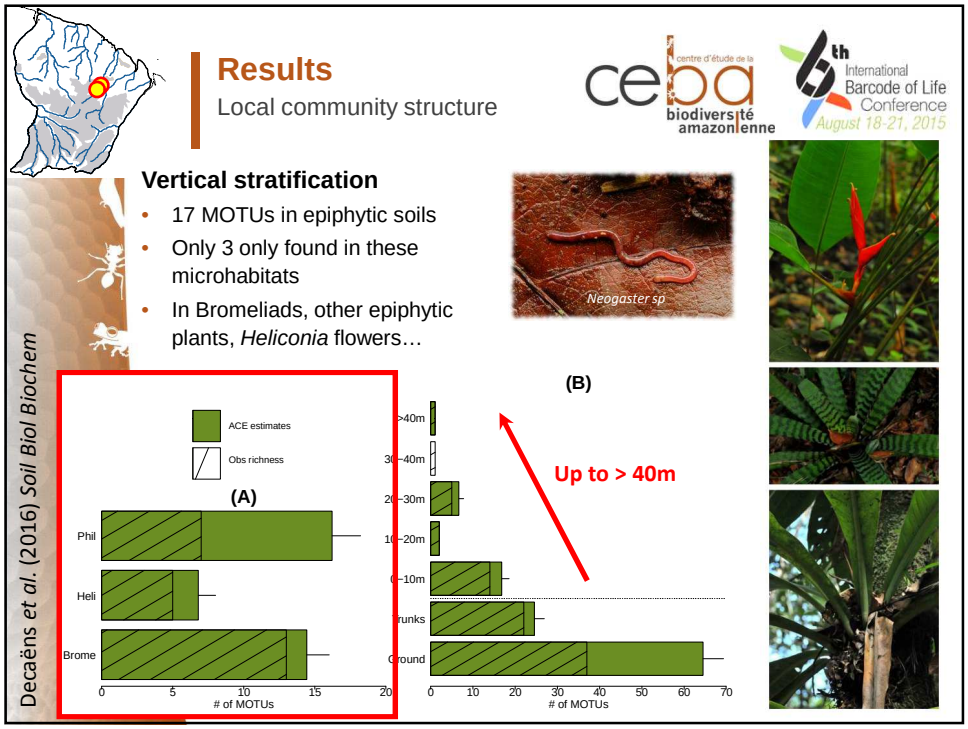
MOTU distribution among microhabitats
Dominance of specialist species for a given broad type of microhabitat
Pontoscolex corethrurus
Soil
Sedi
Surf
14
6
8
3
12
4
1
Nouraguesia sp.
Litter
Trunks
Trees
6
5
3
5
3
6
3
Surface species show little preference for litter, decaying trunks or epiphytic soils
Decaëns et al. (2016) Soil Biol Biochem


Results
Local community structure
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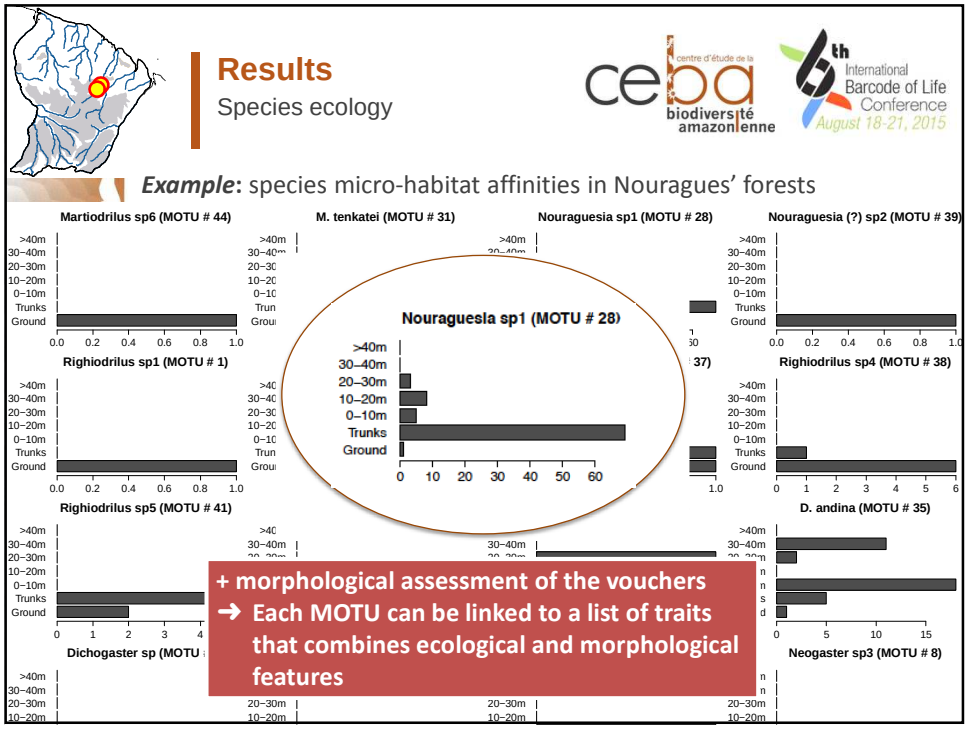
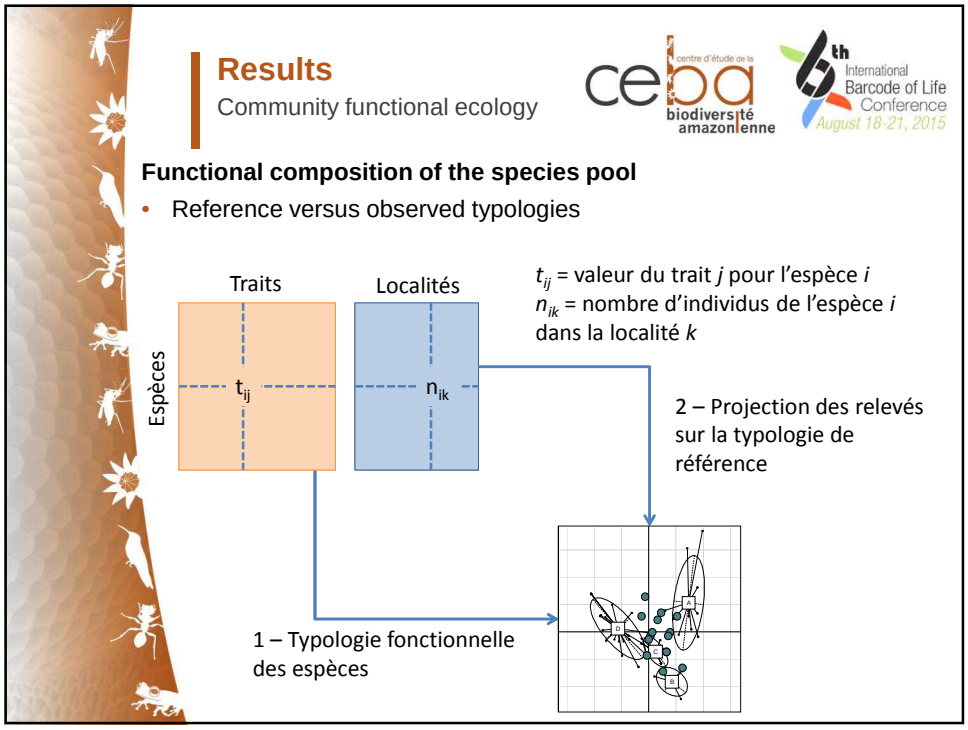
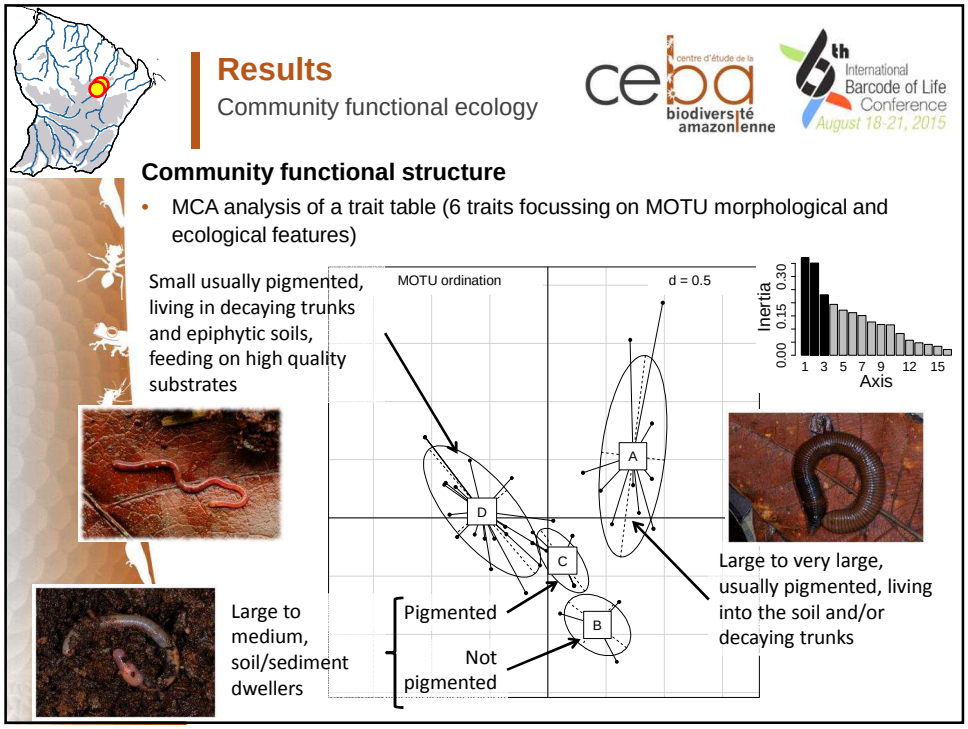
Vertical stratification
17 MOTUs in epiphytic soils
Only 3 only found in these microhabitats
In Bromeliads, other epiphytic plants, Heliconia flowers…
Neogaster sp
(A)
ACE estimates
Obs richness
Phil
Heli
Brome
0
5
10
15
20
# of MOTUs
(B)
>40m
30–40m
20–30m
10–20m
0–10m
Trunks
Ground
Up to > 40m
0
10
20
30
40
50
60
70
# of MOTUs
Decaëns et al. (2016) Soil Biol Biochem


Results
Species ecology
Example: species micro-habitat affinities in Nouragues' forests
Martiodrilus sp6 (MOTU # 44)
M. tenkatei (MOTU # 31)
Nouraguesia sp1 (MOTU # 28)
Nouraguesia (?) sp2 (MOTU # 39)
Righiodrilus sp1 (MOTU # 1)
Righiodrilus sp4 (MOTU # 38)
Righiodrilus sp5 (MOTU # 41)
D. andina (MOTU # 35)
Dichogaster sp (MOTU
Neogaster sp3 (MOTU # 8)
>40m
30–40m
20–30m
10–20m
0–10m
Trunks
Ground
+ morphological assessment of the vouchers
➜ Each MOTU can be linked to a list of traits that combines ecological and morphological features


Results
Community functional ecology
Functional composition of the species pool
• Reference versus observed typologies
Traits
Localités
Espèces
t_{ij}
n_{ik}
t_{ij} = valeur du trait *j* pour l'espèce *i*
n_{ik} = nombre d'individus de l'espèce *i* dans la localité *k*
2 – Projection des relevés sur la typologie de référence
1 – Typologie fonctionnelle des espèces


Results
Community functional ecology
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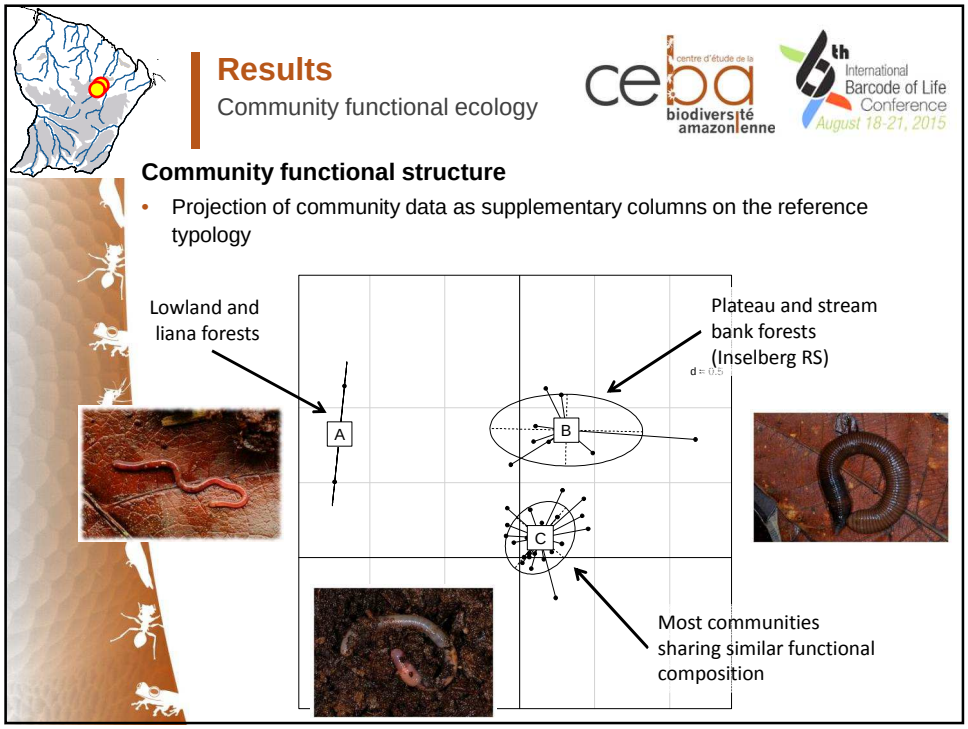
Community functional structure
• MCA analysis of a trait table (6 traits focussing on MOTU morphological and ecological features)
Small usually pigmented, living in decaying trunks and epiphytic soils, feeding on high quality substrates
MOTU ordination
d = 0.5
Inertia
0.00 0.15 0.30
1 3 5 7 9 12 15
Axis
A
B
C
D
Large to very large, usually pigmented, living into the soil and/or decaying trunks
Large to medium, soil/sediment dwellers
Pigmented
Not pigmented


Results
Community functional ecology
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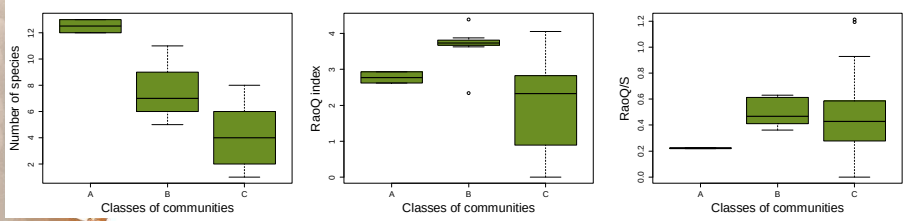
Community functional structure
• Projection of community data as supplementary columns on the reference typology
Lowland and liana forests
Plateau and stream bank forests (Inselberg RS)
A
B
C
Most communities sharing similar functional composition

Results

Community functional ecology

Community functional structure

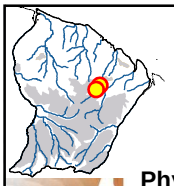
- Projection of community data as supplementary columns on the reference typology



- Lowland and liana forests (A): high sp richness and low FD
➜ Disturbances remove competition effect and filter species from a given region of the functional space

- Deep soil plateau forests (B): moderate sp richness and high FD
➜ High functional dispersion allowed by low disturbance and deep soils

- Other communities (C): low sp richness and moderate FD
➜ Competitive exclusion limiting local richness below a maximum level

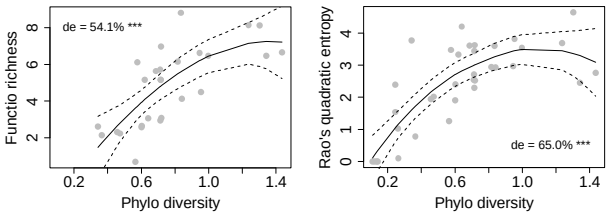


Results

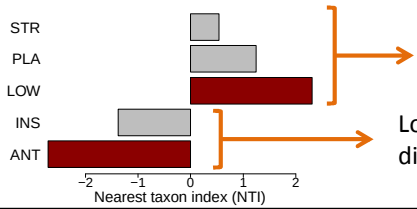
Community eco-phylogenetics

Phylogenetic diversity patterns among habitats and microhabitats

- Phylogenetic and functional diversity are significantly correlated



➜ Inferring assembly rules from community phylogenetic patterns



High PD and phylogenetic clustering
➜ Environmental filtering

Lower PD and phylogenetic over-dispersion ➜ Limiting similarity

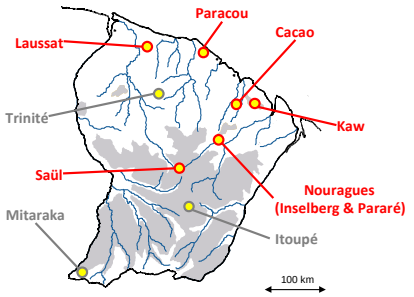
Conclusions

Highlights

- High levels of local diversity
- High levels of spatial turnover
- Consistent functional structure among communities

Perspectives

- Extend the sampling coverage
- Develop a comprehensive functional trait database
- Beta diversity patterns for TD, FD, PH
- Historical biogeography and diversification processes
- DNA barcode libraries for other studies using environmental DNA



Merci de votre attention