Plant-earthworm partnership to increase crop productivity in the tropics.

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Malagasy soils like many other tropical soils are nutrient-depleted in their mineral form, which constrains crop growth. Therefore any process leading to the mineralization of N and P trapped into the soil organic matter need to be studied in order to develop sustainable agricultural tools in a near future. Priming effect (PE) is defined as a stimulation of the chemically stabilized organic matter (CSOM) mineralizationby the amendment of fresh organic matter (FOM). This microbially-mediated process is not completely understood(Fontaine et al, 2003, Kuzyakov et al., 2000). In theory, organic amendments could stimulate nutrient recycling from the CSOM stock, provided that they will be managed under the right conditions. Endogeic earthworms are ecosystem engineers known to influence the dynamics of soil organic matter (SOM) as they ingest high amount of soil and assimilate one part of the organic carbon in relation with microbial activity stimulation(Lavelle and Martin, 1992). During the gut transit, microorganisms are awaked and their activities are stimulated by several mechanisms (Fig A):

1- the small energy-rich molecules contained in the mucus,
2- the physical optimal conditions brought by the mucus like neutral pH, water…,
3- the meeting with fresh residues induced by the gut mixing.

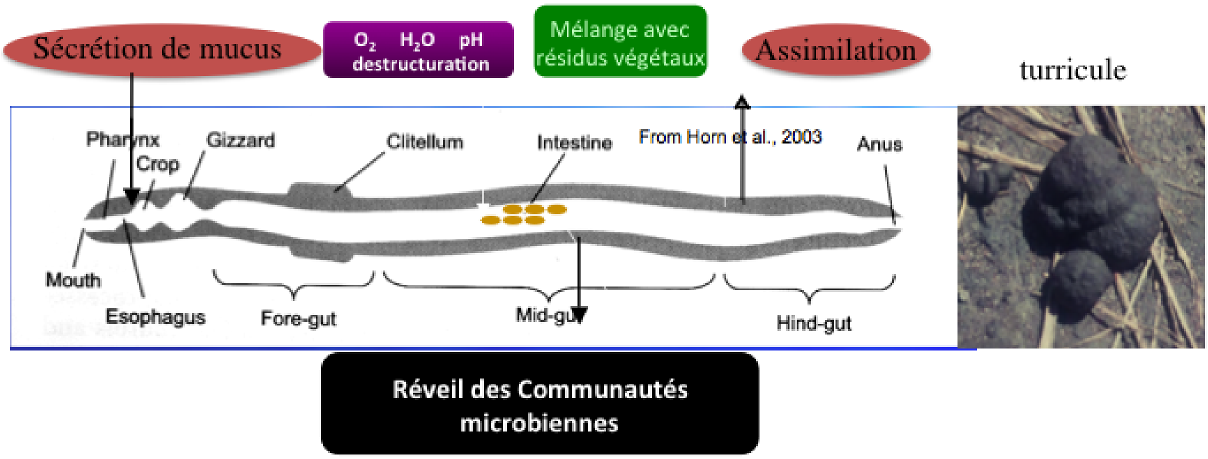


Fig. A

Those conditions have shown to be favorable to PE generation (Bernard et al. 2012), which could be the reason why fresh casts are more concentrated in nutrients (N and P) than the bulk soil around (Chapuis-Lardy et al. 2010). Therefore, earthworm could be seen as a potential agricultural tool to furnish nutrients to crops along the culture *via* PE processes.

During a first laboratory study we wanted to determine which earthworm was able to generate the most intense PE among three different species collected in the Highlands of Madagascar (Andranomanelatra): *Pontoscolexcorethrurus*, *Kynotusparvus*and *Dichogastersaliens*. We wanted also to identify microbial groups stimulated by worms, which could be ivolved in such PE.

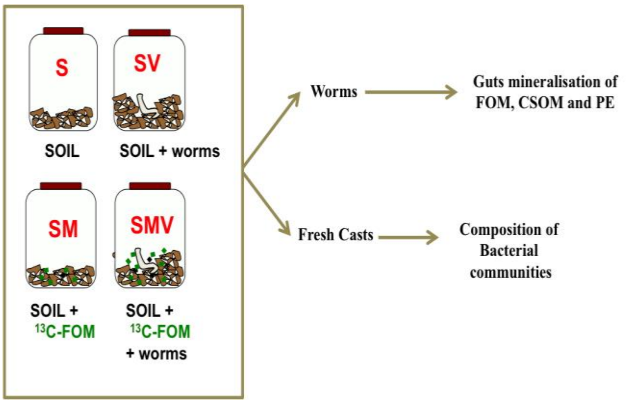
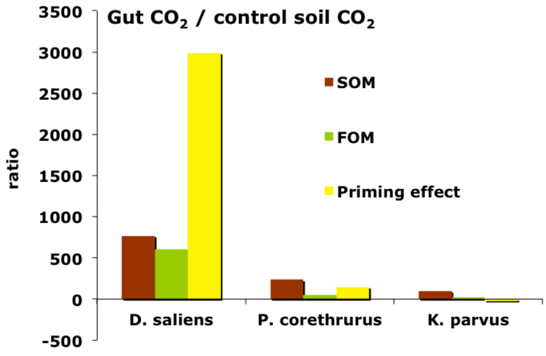


Fig. B

Ferralsol from Andranomanelatra has been incubated following the design shown on Fig B.

During six days incubation, fresh casts were collected and frozen at -20°C.Later, DNA was extracted and separated on the basis of its density using DNA-SIP approach as explained in Bernard et al. 2007. Composition of bacterial communities was analyzed in each density fraction (^{12}C and ^{13}C enriched) by high throughput pyrosequencing techniques. In parallel worms were weighted, put in small chambers and their CO_2 respiration was analyzed (total and $^{13}CO_2$), over 1h. Worms were then transferred on a humid paper over night to empty their gut before being weighted and their respiration analyzed again. The difference between both measures has been considered as "Gut CO_2". Priming effect was calculated by substracting^{13}CO2 (FOM) and control total CO_2 to FOM amended total CO_2.



We observed that the 3 earthworms tested could increase the soil and the fresh organic matter mineralization at different intensities (Fig. D). But only 2 of them (*P. corethrurus* and *D. saliens*) could increase the Priming Effect intensity resulted from the FOM amendment. *D. saliens* appeared to be the most efficient in this process.

Fig. C

Earthworms can prime SOM mineralization either by the mucus (Fig C – SOM) or by mixing with fresh organic residues (Fig C – PE). Therefore, two kinds of bacterial populations can be implicated:

1- those which are stimulated in the light DNA fractions should be involved in the mucus generating Priming Effect
2- those which are stimulated in the heavy DNA fraction should be involved in the FOM-generating Priming Effect, as they have used ^{13}C enriched FOM carbon to break CSOM and liberate nutrient.

We observed thatthe Flavobacteriaceae were stimulated by every earthworm,at different level,and more in the light than in the heavy DNA fraction. This has already been observed in a previous study with *P. corethrurus* raised in another type of soil (Bernard et al., 2012). Anyways, this family was also stimulated in the heavy DNA fraction. Therefore those organisms might have been involved in both priming mechanisms. Moreover the Flavobacteriaceaedensity strongly correlated to SOM and FOM mineralization, which definitely prove their implication in the Mucus generating Priming of FOM and SOM (Fig. D).

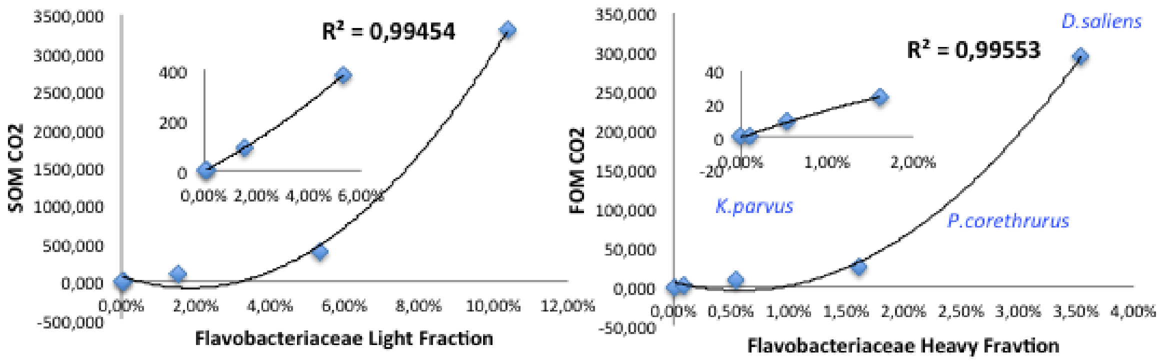


Fig. D

But their stimulation did not lead systematically to a FOM generated PE (*K. parvus* - Fig. E). We could not find any populations completely correlating to FOM generating PE intensity. Thisconfirms that PE is a facultative process for those populations but depending on environmental conditions.

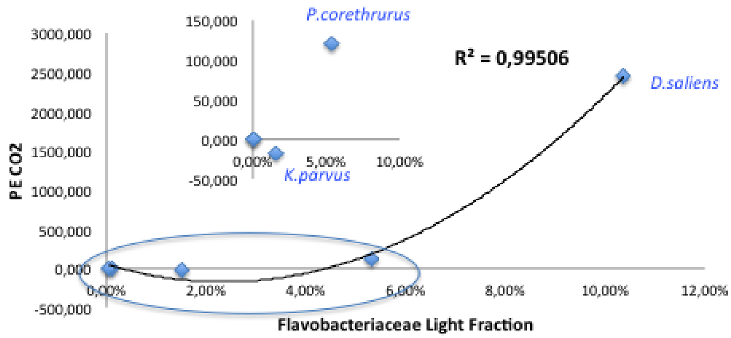


Fig. E

Other bacterial families might have been involved in PE generation but were specific to earthworm species. For example, the Paenibacillaceae were stimulated by *P. corethrurus* and by *K. parvus* but not by *D. saliens*, and the Comamonadaceae were only stimulated by *P. corethrurus*.

Dichogastersaliens demonstrated his huge capacity to mineralize FOM and SOM organic matter, and to potentially recycle nutrients for plants. Moreover, it is a small size earthworm species living at the root vicinity of *Bracharia sp.*, optimizing therefore the transfer of recycled nutrients to plant roots (Fig. F).

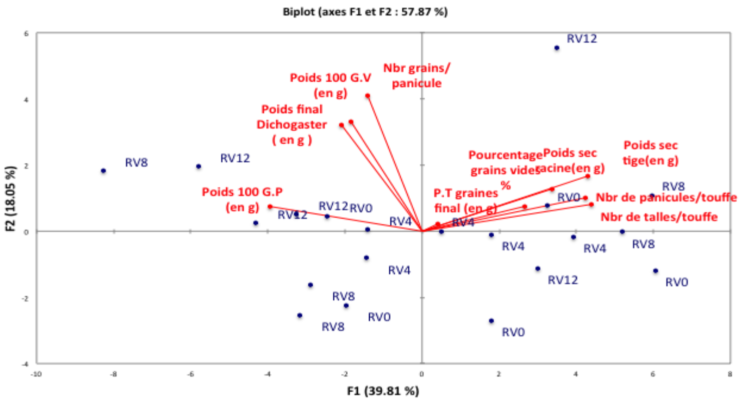
Fig. F

We decided, in a second experiment, to look at its capacity to enhance rain-fed rice production.

Rice plants cultivated in outdoor pots in Andranomanelatra agronomic trials (11 kg of 2mm sieved soil– 4 plants per pot) were inoculated with increasing number of *D. saliens* specimens (0, 4, 8 and 12 per pot). Each treatment has been repeated 5 times, and rice has been grown during 6 month (Fig. G).

Fig. G

At the rice maturity, several agronomic parameters have been measured on rice plants. Earthworms have been counted and weighted. Contamination from other unstudied earthworms, *P. corethrurus* and *Amynthasminimus*, were observed, but multivariate analyses showed that no competition occurred with *D. saliens*.



A positive relationship between the weight of *D. saliens* specimens (adults + juveniles) at the end of the experimentation and the weight of 100 grains per panicle was observed (Fig. H).

Fig H

This parameter is usually linked to climate and nutrient availability. Earthworms might have furnished some inorganic P to rice plants. In a future experiment, higher number of *D. saliens* specimens will be inoculated and P concentrations in grains will be measured. This experiment showed that as plant-fungi partnership, earthworm can also be a good partner, providing nutrients to the plant when most of nutrients are in the organic matter pool.

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