Fate, biodegradation and ecotoxicological impact of the bioherbicide leptospermone on soil bacterial community

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Biopesticide an alternative to pesticides?

Increasing interest for **biopesticides** [(i)microorganisms, (ii) biochemicals or (iii) semiochemicals] seen as an alternative to pesticides because of:

▷ Emergence of resistance to pesticides
▷ Low rate of delivery on the market of ‘safer pesticides’,

▷ Reduction of the use of pesticides and the promotion of the biocontrol within the framework of integrated pest management

ÉCOPHYTO
RÉDUIRE ET AMÉLIORER L'UTILISATION DES PHYTOS

Biopesticides are viewed as ‘**environmental-friendly compounds**’ but until now much is known on their environmental fate and ecotoxicological impact

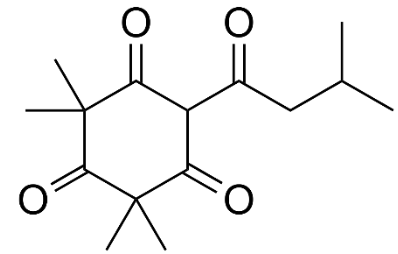
Santé Environnement

▷ Monitoring of the environmental fate and ecotoxicological impact

Leptospermone, a natural β-triketone herbicide purified from Manuka oil

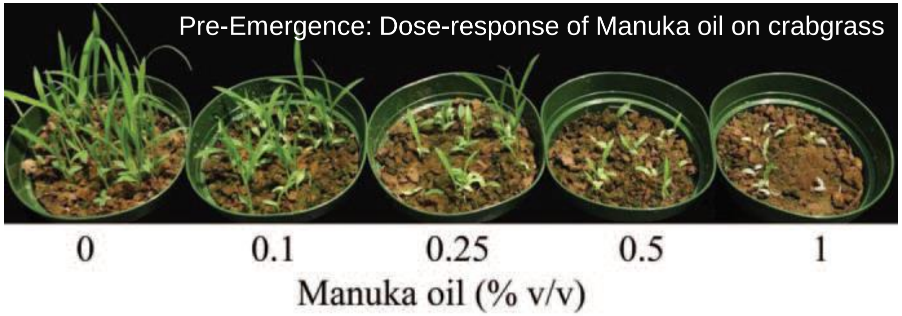
Leptospermum scoparium

Manuka oil



Leptospemone
1-hydroxy-2-isovaloryl-4,4,6,6,-tetramethyl cyclohexen-3,5-dione

Dayan et al. (2011) Weed Science 59:464-469



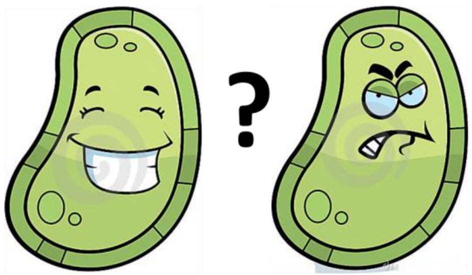
- **Mode of action**: Inhibition of the **p-hydroxyphenylpyruvate dioxygenase** (HPPD)
- **Effects** : Bleaching of the plants and perturbation of the photosynthesis

Fate, biodegradation and ecotoxicology of leptospermone to soil microorganisms

Biopesticide application to agricultural soil

- tolerance
- degradation

- inhibition
- toxicity



soil microbial communities

Experimental set-up

Soil microcosms (20g dwt): Perpignan (**P**), Saint-Jean de Fos (**S**)

Treatments: Leptospermone applied at 1x (5 µg.g^{-1}, **D1**) or 10 x (50 µg.g^{-1}, **D10**) the agronomical dose and control

Sampling times : T0-T2-T4-T8-T15-T30-T45 (n=3)

Fate

Organic extraction

↓

UV-HPLC

Biodegradation

Enrichment culture

Purification of strain

Degrading activity
UV-HPLC and LC-MS

Ecotoxicology

Direct soil DNA extraction
ISO 11 063

Composition
qPCR – ISO 17 601
11 microbial groups

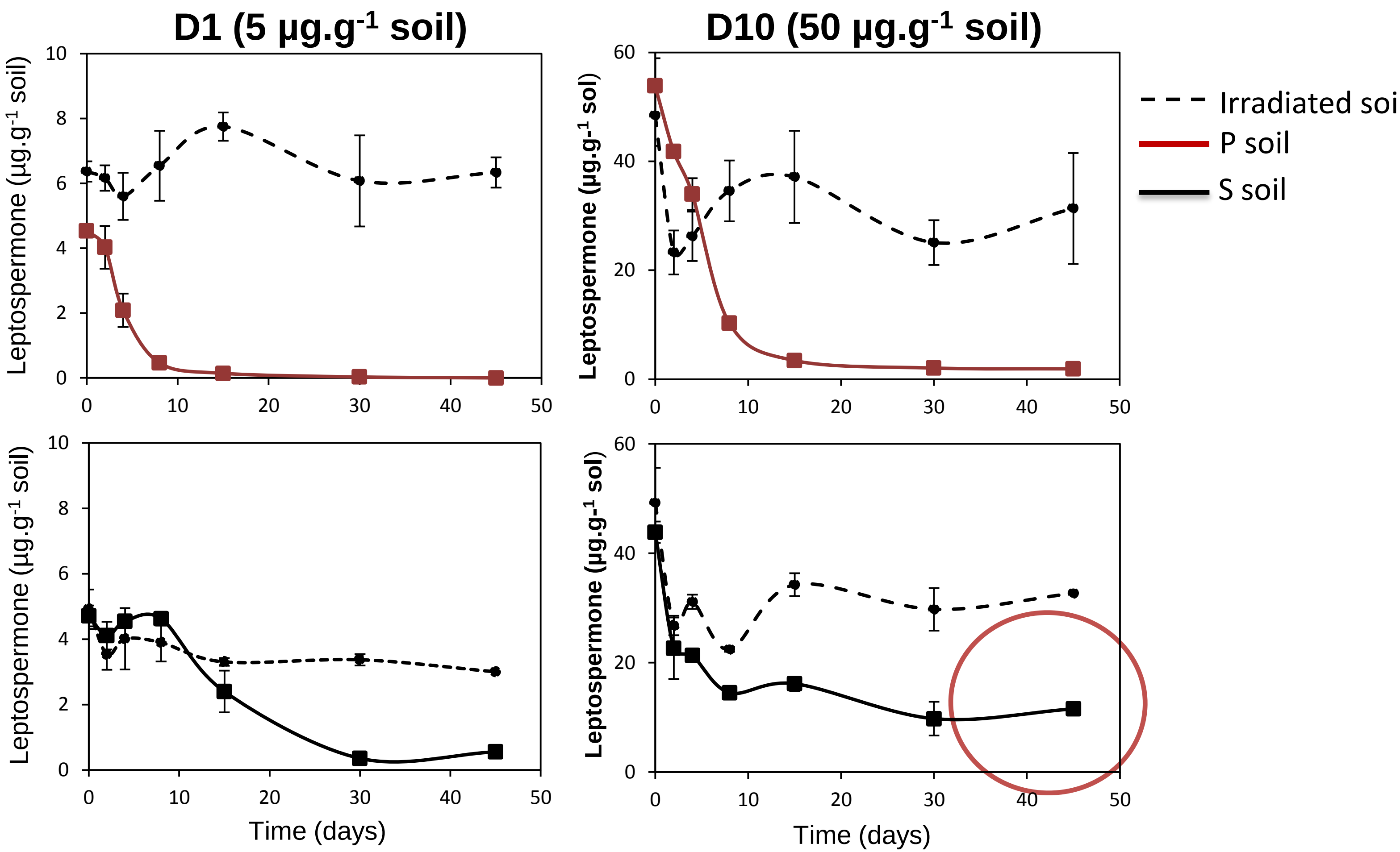
Diversity
Pyrosequencing
16S rRNA

Quantitative Insights Into Microbial Ecology

Fate of leptospermone in P and S agricultural soils

Soil P
DT_{50}= 4 and 6 days

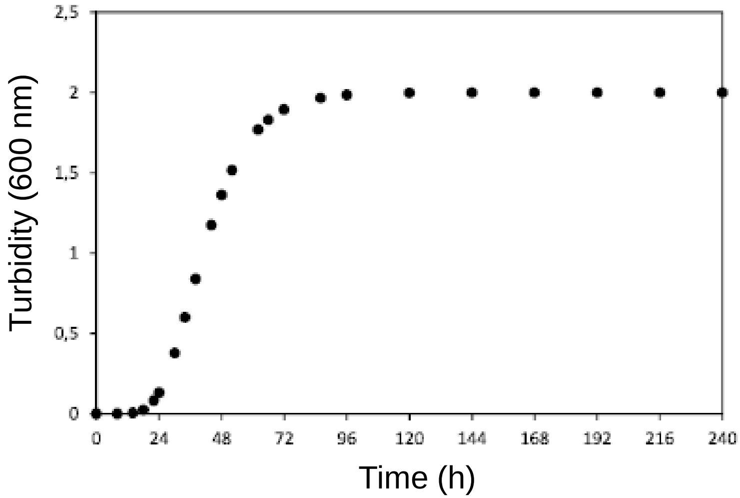
Soil S
DT_{50}= 9 days

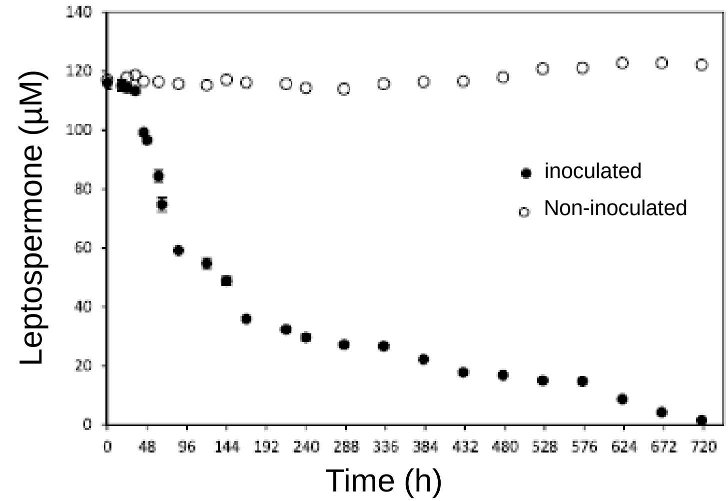


▷The dissipation of leptospermone in soil is in majority controlled by biotic processes degradation

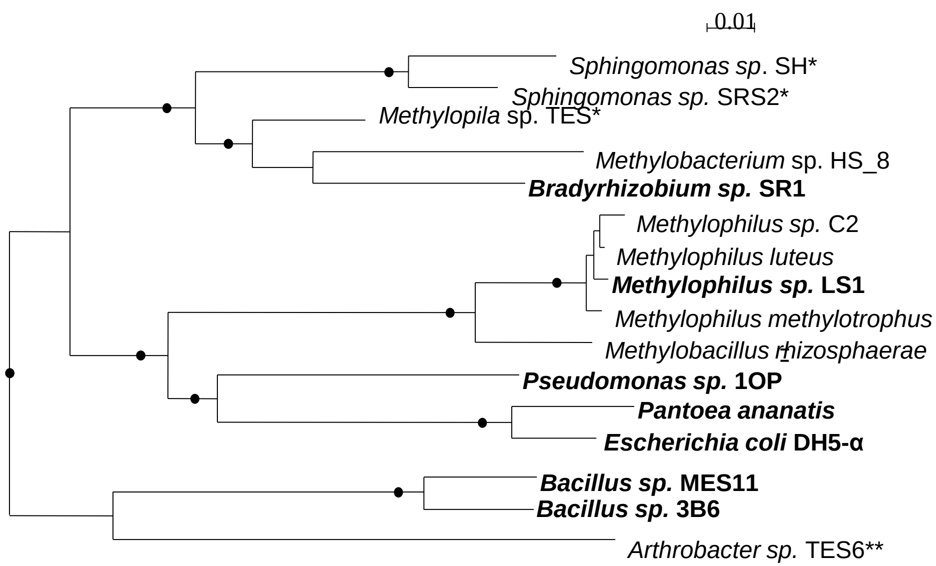
▷ Entire dissipation in **P** soil but uncomplete dissipation in **S** soil at D10 (~ 15 µg.g^{-1} remaining)

Biodegradation : search for leptospermone bacterial degraders in **P** soil





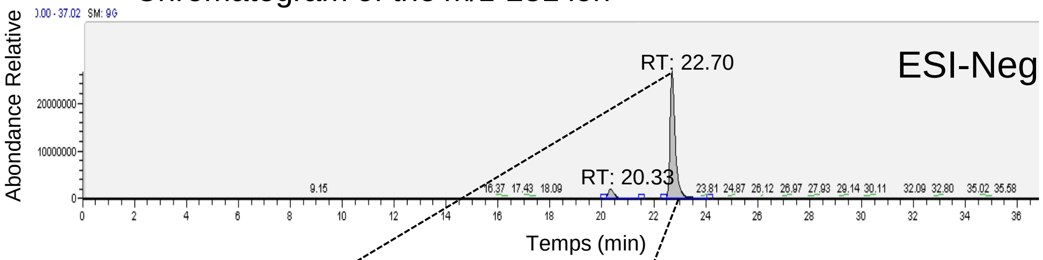
▷ After 5 enrichment cycles, isolation of a bacterial isolate able to grow (µm=0,06 h^{-1}) on MS-leptospermone (30mg.L^{-1}) and to degrade it (DT50 6 D; rate of degradation 0.005 h^{-1})



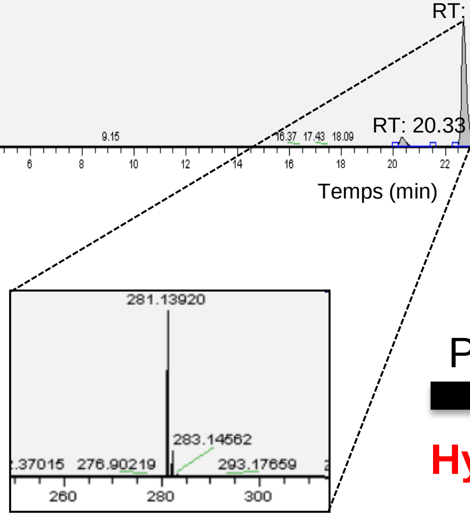
▷ 16S rRNA sequencing allowed the affiliation to the genus *Methylophilus*, and was consequently named ***Methylophilus* sp. LS1**

Biodegradation : identification of leptospermone transformation products

Chromatogram of the *m/z* 281 ion

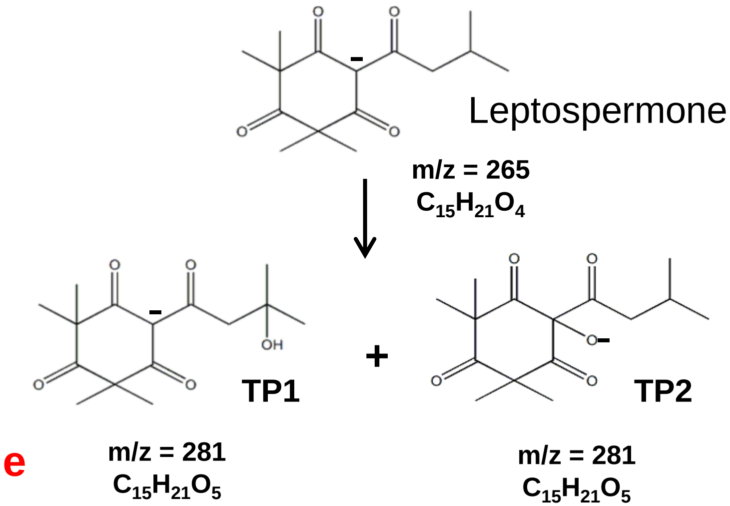


Mass spectrum (LC/MS HR) of m/z 281 ion



Proposed structures

Hydroxy-leptospermone

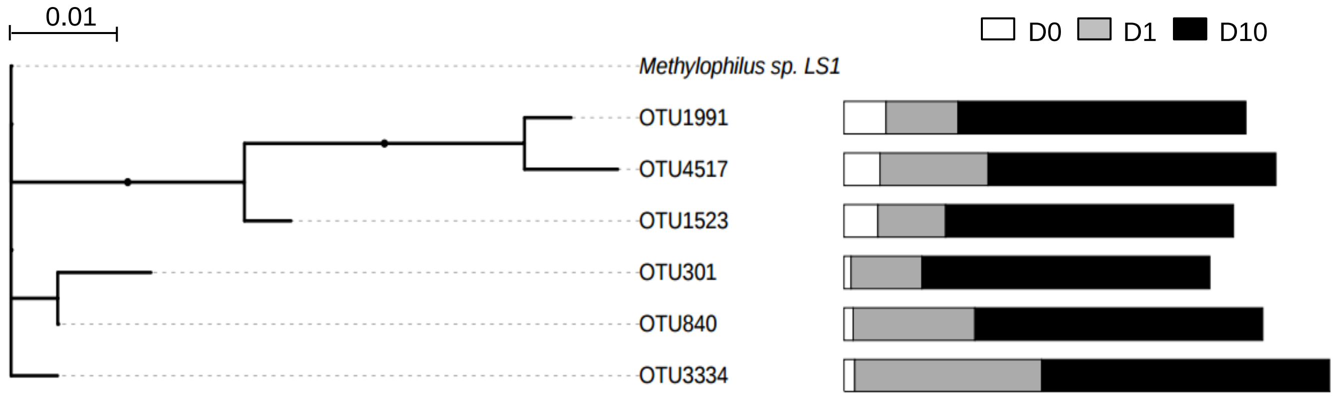


▷ Detection of several transformation products among which one dominant (RT of 22.7 min in LC, m/z = 281) with a brut formula of $C_{15}H_{21}O_5$

▷ Might be attributed to the oxidation of leptospermone leading to hydroxy-leptospermone:
- TP1: hydroxylation of the cyclohexane-1,3,5-trione
- TP2: hydroxylation of the lateral chain

▷ Formation of hydroxy-leptospermone further confirms leptospermone degrading ability of *Methylophilus* sp. LS1

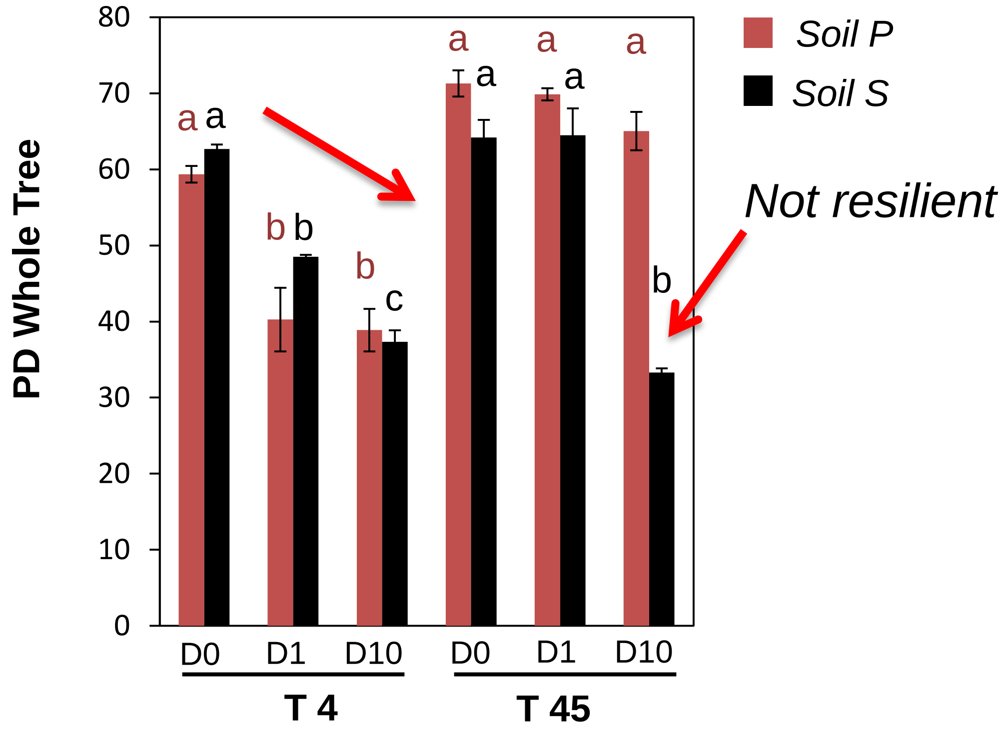
Biodegradation : abundance of OTUs related to *Methylophilus* sp. LS1 in **P** soil



- Significant increase in the abundance of 6 OTUs (having 97% similarity with 16S rRNA sequences of *Methylophilus* sp. LS1) in **P** soil exposed to leptospermone as compared to control (D10 > D1 > D0)

- These six OTUs positively correlated with leptospermone exposure might also contribute to leptospermone biodegradation in **P** soil

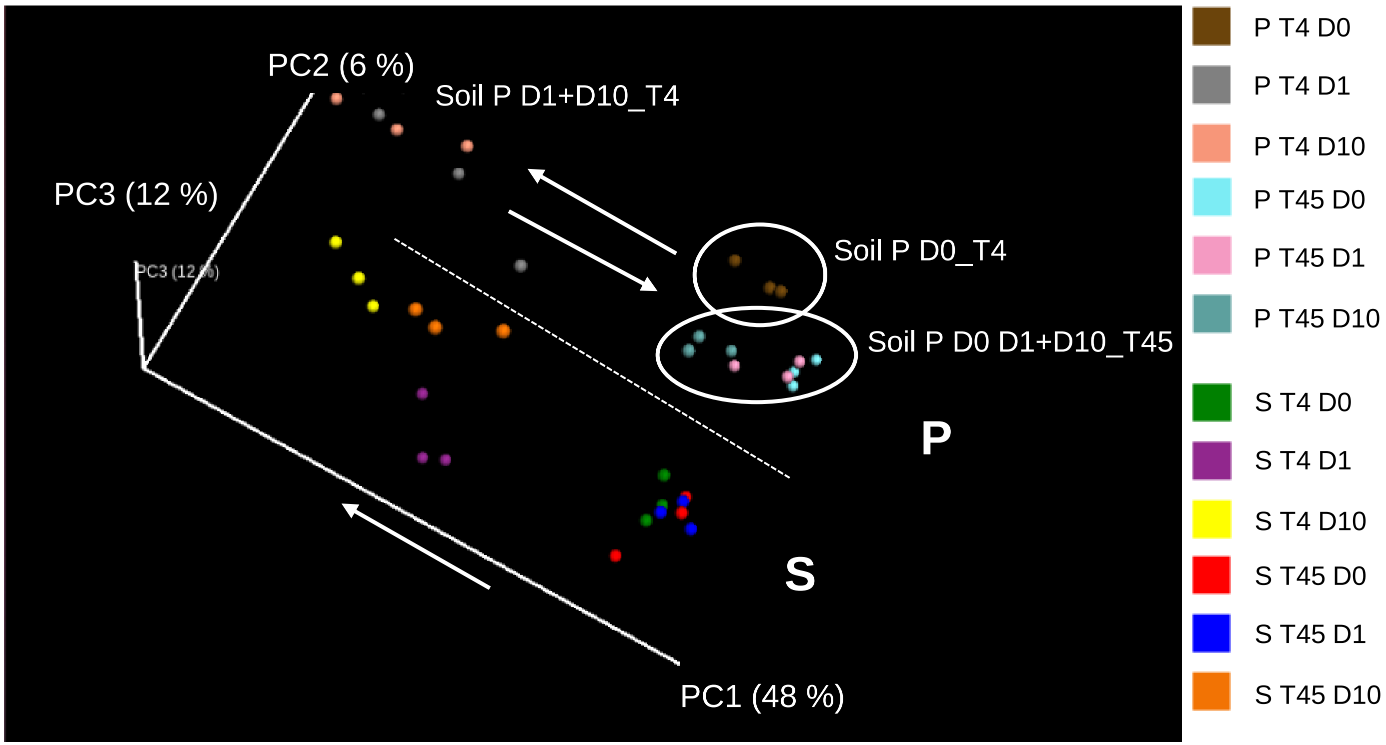
Ecotoxicology : impact of leptospermone on α-bacterial diversity



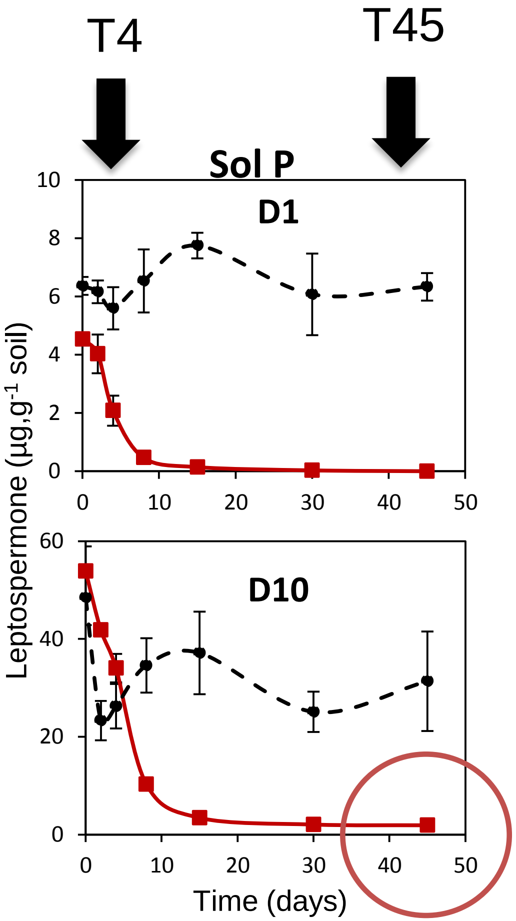
- After 4 days of exposure to leptospermone (D1 and D10) the bacterial diversity significantly decreased in both **P** and **S** soils as compared to control (D0)

- After 45 days the bacterial diversity of **P** soil was resilient for both D1 and D10 while it was not resilient in for the **S** soil exposed to D10

Ecotoxicology : impact of leptospermone on β-bacterial diversity



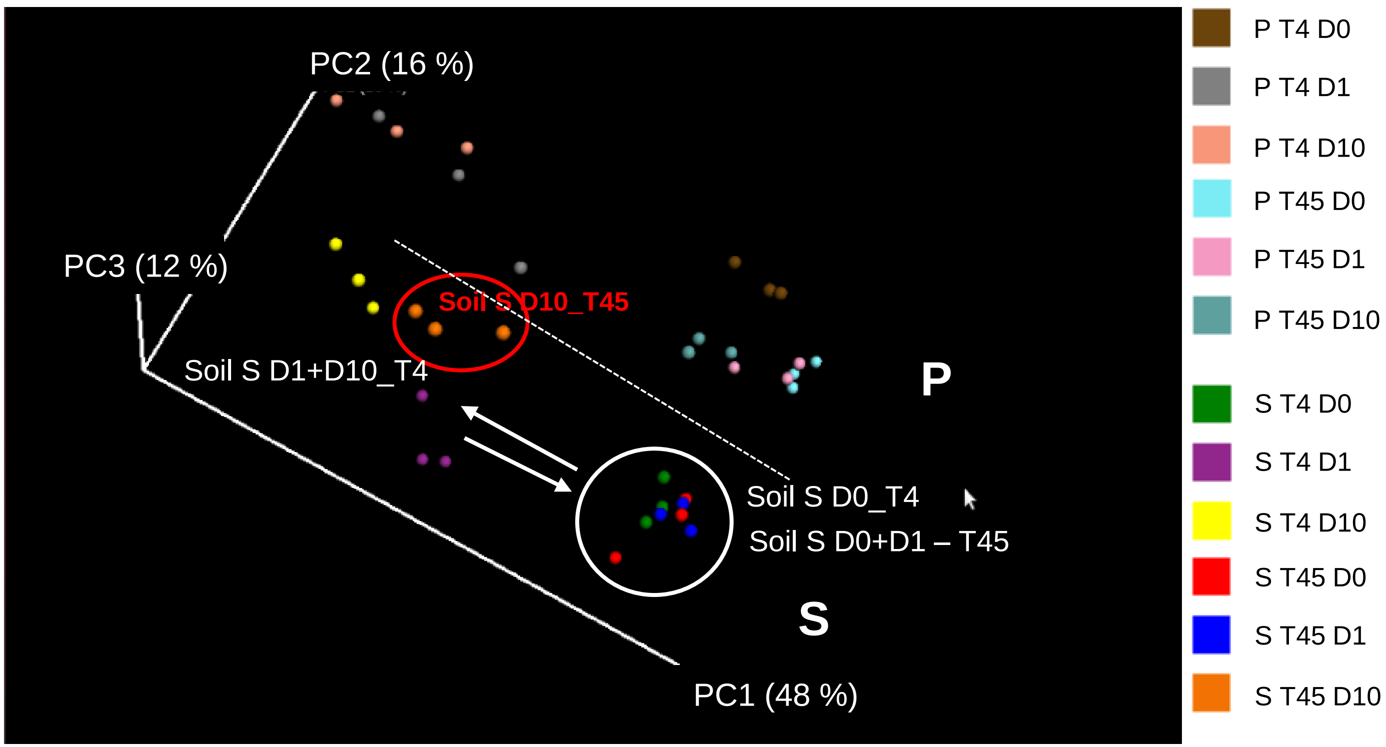
Principal component analysis (PCoA)



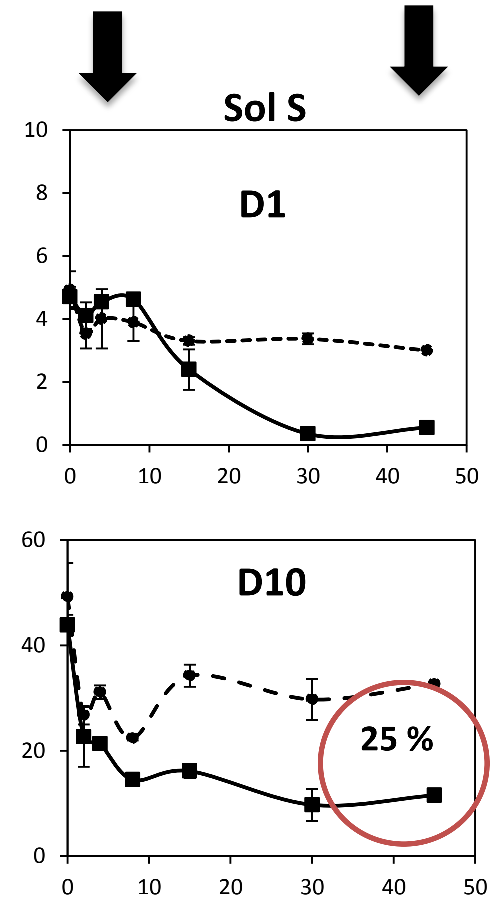
Soil P

▷ After 4 days of exposure to leptopsermone (D1 and D10) significant modification of the diversity of the bacterial community (along PC1, 48% of the variance)

▷ After 45 days the diversity of the bacterial community was resilient



Principal component analysis (PCoA)



Soil S

▷ After 4 days of exposure to leptopsermone (D1 and D10) significant modification of the diversity of the bacterial community (along PC1, 48% of the variance)

▷ After 45 days the diversity of the bacterial community of D1 was resilient but not the one of D10

Conclusions

<u>Fate:</u>

▷ Leptospermone is rapidly dissipated in **P** and **S** soils (for D1, DT50 of 4 and 9 days, respectively),

▷ At D10 dissipation is not complete in **S** soil (~ 15 µg.g^{-1} remaining)

▷ Leptospermone dissipation is mainly controlled by biotic degradation processes

<u>Biodegradation:</u>

▷ Isolation of *Methylophilus* sp. LS1 a leptospermone degrader from **P** soil

▷ Evidence for the formation of hydroxyleptospermone as one of the dominant TP

▷ Increase in the abundance of 6 OTUs related to LS1 in **P** soil exposed to leptospermone (D1 and D10)

<u>Ecotoxicology:</u>

▷ On the short term significant effect of leptospermone on α- and β-bacterial diversity in both soils at both concentrations (D1 and D10)

▷ On the long term resilience of the α- and β-bacterial diversity in **P** soil but not in the **S** soil exposed to the highest dose (D10)

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Evidence for photolytic and microbial degradation processes in the dissipation of leptospermone, a natural β-triketone herbicide

Sana Romdhane[1,2,3,4] · Marion Devers-Lamrani[3] · Fabrice Martin-Laurent[3] · Amani Ben Jrad[1,2] · Delphine Raviglione[4] · Marie-Virginie Salvia[4] · Pascale Besse-Hoggan[5] · Franck E. Dayan[6] · Cédric Bertrand[4] · Lise Barthelmebs[1,2]

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Thank you for your attention !

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