# Natural Transformation of Extracellular DNA in Soil

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## What is natural transformation in soil?

- Extracellular DNA or exoDNA can enter soil after cells die or can be actively secreted during processes like biofilm formation.[1,2]
- Transformation is the integration of this exoDNA and allows living cells to acquire novel functions and facilitates bacterial evolution.

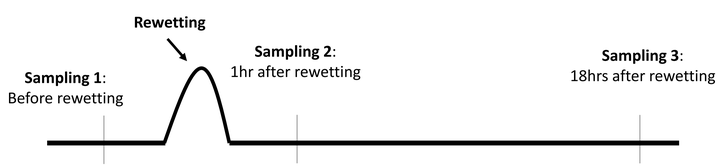
**What happens during transformation?**

1. A blue bacterium dies and releases it's DNA
2. A green bacterium could pick up the exoDNA
3. The green bacterium has now gained a new function

## Q1. Measuring exoDNA in response to rewetting

We sampled the extracellular and intracellular DNA in switchgrass soil exposed to two different drying-rewetting regimes (listed below). Soil samples were collected at three time points: before a rewetting event, 1 hour after the rewetting event, and 18 hours after the rewetting event.

*We used 2 Drying Rewetting Regimes in Switchgrass Soil*
Ambient - 2 days drying (6.67 mm rain)
Extreme - 28 days drying (80 mm rain)

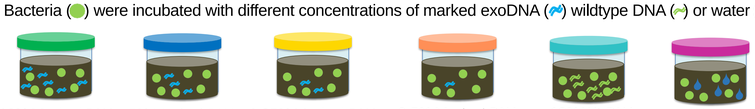


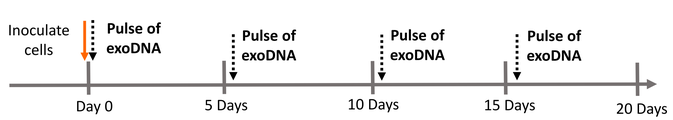

To estimate exoDNA we added the chemical PMA to soil subsamples as it binds to exoDNA and prevents amplification during qPCR. Subtracting the DNA amplified in chemically treated samples (intracellular) from the DNA amplified in untreated samples (total) provides an estimate of exoDNA.

## Q2. Measuring transformation after a pulse of exoDNA into soil

To quantify how transformation rates changed in response to the concentration of exoDNA pulsed into soil, we incubated *Pseudomonas stutzeri* cells with marked exoDNA for 20 days. We measured transformation every 5 days and then pulsed exoDNA into the soil after each measurement.

Bacteria (●) were incubated with different concentrations of marked exoDNA (〰) wildtype DNA (〰) or water (💧)




**Importance:** The integration of exoDNA may occur rapidly when it is available, making transformation an important mechanism for maintaining functional and genetic diversity in bacterial populations.

## Research Question 1

**1.** Do extreme rewetting events create a pulse of exoDNA into switchgrass soil? **NO**

### 1. ExoDNA is equal to intracellular DNA, but does not increase after rewetting

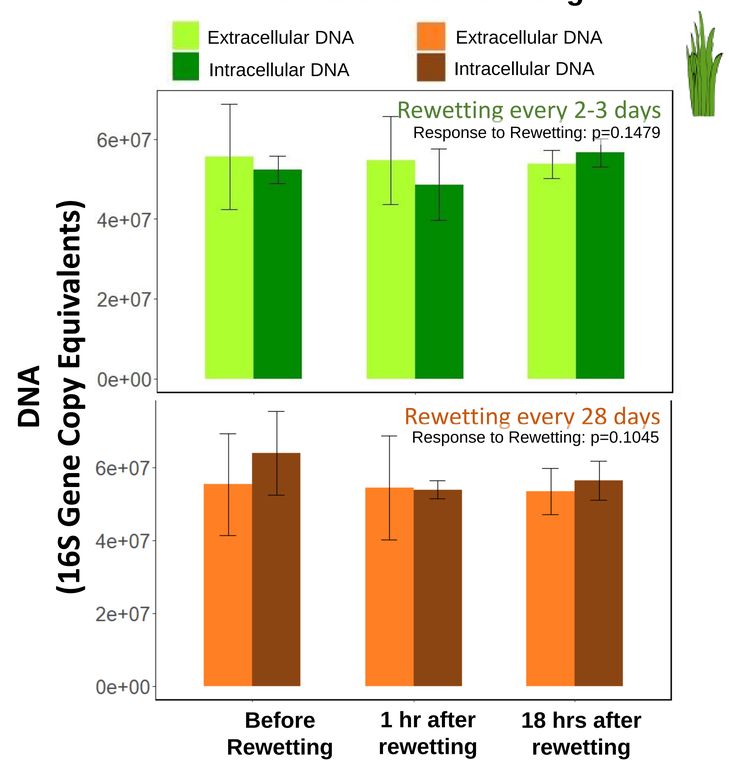



Figure 1. ExoDNA and intracellular DNA in response to ambient (2-3 days dry) and extreme (28 days dry) drying-rewetting. Bars are the mean of 4 field replicates and error bars are standard error of the mean.

Literature Cited
1. Thomas et al. 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol. 3:711–721.
2. Carini et al. 2016. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nat Microbio. 2:16242.

## Research Question 2

**2.** Can a pulse of exoDNA into switchgrass soil facilitate transformation? **YES**

### 2. Transformation in soil increases with exoDNA

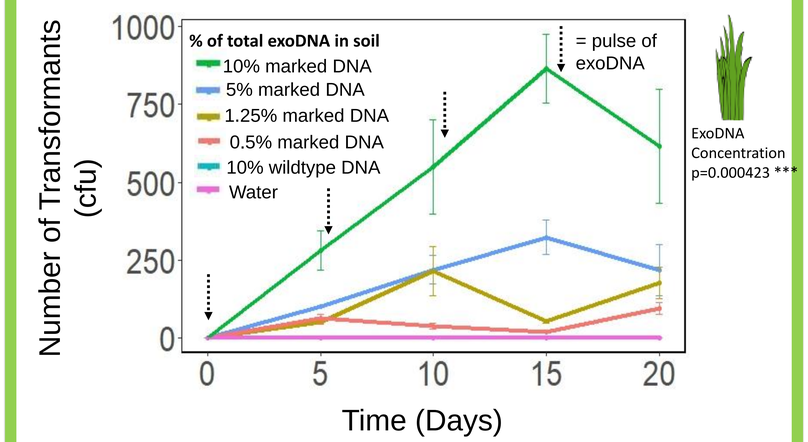



Figure 2. The number of transformants that evolved in the 5 days after a pulse of exoDNA. Points are the mean of 8 replicate soil microcosms and error bars are standard error of the mean.

## Conclusions & Discussion

- Neither ambient or extreme rewetting was associated with a pulse of exoDNA.
- However, we did find similar concentrations of exoDNA and intracellular DNA in switchgrass soils.
- We did find that transformation rates increased after a pulse of exoDNA.
- A very small amount of exoDNA facilitated transformation though, so large pulses of exoDNA into soil might not be critical for transformation.
- Ultimately, more work needs to be done to understand the release and subsequent transformation of exoDNA, as transformation could act as a form of genetic rescue by recycling genetic info back into living members of the community.