Supporting information to: *In situ* formation of environmental endocrine disruptors from phytosterol degradation: A temporal model for agricultural soils

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SI-1 Removal rate constants for beta-sitosterol from soil

It appears two different removal rates exist, one fast (0.0351 ± 0.015 day^{-1}) in lab experiments with added beta-sitosterol and in stored manure, the other one a slower under field condition (0.0022 ± 0.007 day^{-1}).

Turfitt spiked gardening soil with 2000000 µg/kg cholesterol and measured the remaining cholesterol content after 90, 180 and 360 days[1]. Based on his data, we calculated a decay rate constant of 0.0029 day^{-1}, assuming a pseudo-first-order kinetic. While cholesterol was used, the close structural similarity indicates a degradation rate constant for beta-sitosterol in the same order of magnitude.

Yager and coworkers have measured the dissipation of contaminants, including beta-sitosterol in agricultural soil, following biosolid application from secondary wastewater treatment[2]. While their triplicate measurements vary greatly, we could calculate a decay rate constant of 0.0026 ± 0.0016 day^{-1}, similar to the rate from Turfitt. The same group, this time under Kinney, measured beta-sitosterol on an agricultural field in the American Midwest, after application of manure or biosolids from a wastewater treatment facility [3]. While only two measurement points were reported, we could use them to estimate a rate constant in the same order of magnitude with 0.0021 and 0.0011 day^{-1} for biosolids and manure, respectively.

Much faster decay rates were found by us in a small laboratory experiment, spiking beta-sitosterol at 223000 µg/kg into 0.5 g of soil from a small agricultural field in Leon, Spain [Gravert, unpublished work]. After 37 days, about 74% of beta-sitosterol were removed, leading to a decay rate of 0.0361 day^{-1}.

Similar high values were observed in composting horse and cattle manure [4]. After an initial increase, beta-sitosterol degraded with an exponential rate constant of 0.053 ± 0.006 day^{-1} from day 14 to 168 of the experiment.

These high rates might be explained with higher availability of beta-sitosterol throughout the soil and manure, as well as differences in the microbial community and abundance of nutrients.

SI-2 Extended description of microbial degradation in the soil environment

The microbial community in the soil will react to an increase of beta-sitosterol with growth and require a slightly more complicated description. The count of microbes able to transform beta-sitosterol into AD will change over time with beta-sitosterol as the limiting substrate. A Monod[5] equation estimates the growth rate, as the maximum possible rate (r_{max}) multiplied by the fraction of substrate concentration over the empirical half velocity constant ($K_{[\beta St]}$) added to the substrate concentration. With a factor for the initial "background" concentration of suitable microbes ($[Microbes]_{Initial}$), the exponential growth of microbes can be described as in equation 8:

$$\frac{[Microbes]}{dt} = [Microbes]_{Initial} \cdot \left(1 + r_{max} \frac{[\beta St]}{K_{[\beta St]} + [\beta St]}\right)^t \quad \text{eq 8}$$

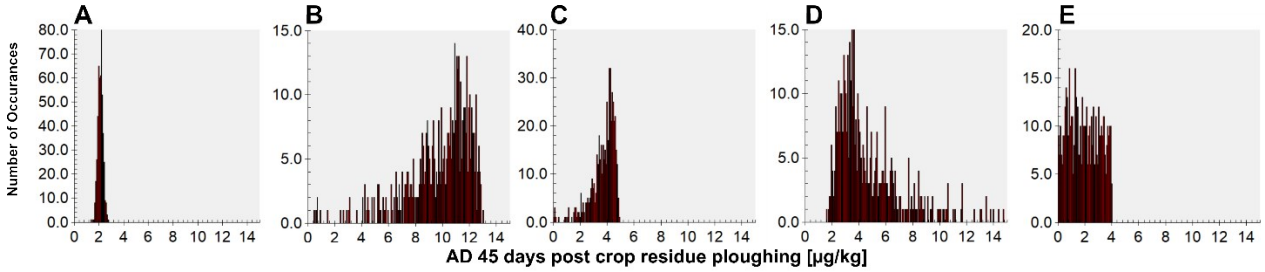
A more precise model would therefore make the decay rate constant D_T partially dependent on the count of microbes;

$$D_T = (D_{autooxidation} + D_{photodegradation}) + D_{biotransformation} \cdot [Microbes] \quad \text{eq 8}$$

Research into microbial pesticide degradation has shown that the assumption of a pseudo-first-order reaction for microbial dependent transformations is possible under general circumstances[51].

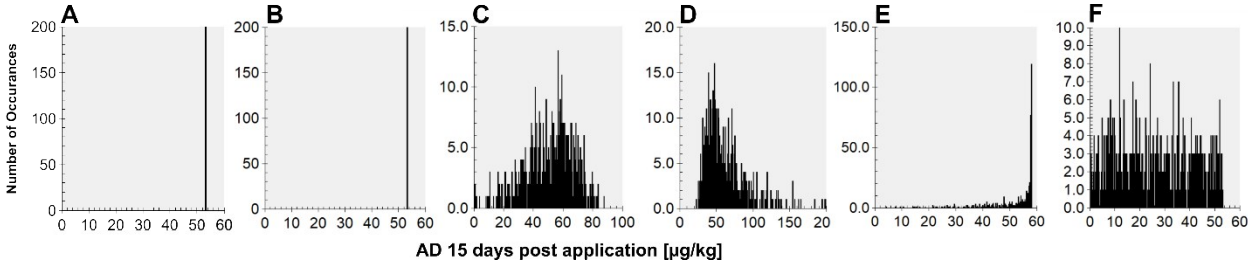
Sensitivity analysis

Sensitivity analysis for plant degradation



Supporting Information - Fig 1 Sensitivity analysis of scenario I. Histograms for the Monte Carlo analysis of (A) plant growth, (B) plant decay rate, (C) beta-sitosterol removal rate, (D) AD degradation rate, and (E) transformation ratio, with the number of occurrences over the predicted AD concentration in soil, 30 days after residual crop ploughing.

Sensitivity analysis for the transformation of beta-sitosterol added with manure



Supporting Information - Fig 2 Sensitivity analysis of scenario III. Histogram for the Monte Carlo analysis of (A) plant growth, (B) plant decay rate, (C) beta-sitosterol removal rate, (D) AD degradation rate, (E) release rate from manure, and (F) transformation ratio, with the number of occurrences over the predicted AD concentration in soil, 15 days after manure application.