Biodegradation of polyphenolics (tannins) in natural soils

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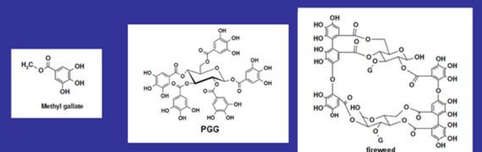
Background

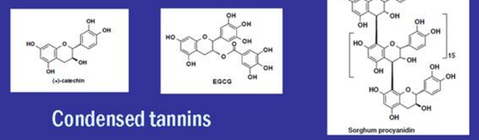
Little is known about the effects of tannins on soil microbial communities. Tannins may inhibit enzyme activity and thus exert a negative effect on microbial growth. Alternatively, tannins could serve as C sources for metabolism. We postulate that structural differences between different tannins determine the effects of tannins on soil microbes. In this experiment, we determined the effect of several tannins on soil microbial respiration.

Previous Hypotheses

Tannins are polyphenolic plant compounds which can either be classified as hydrolysable tannins (HT), which contain esterified galloyl groups, or condensed tannins (CT), which are polymeric flavonoids. It has been suggested in the literature that HT could be better substrates for growth than CT because their ester linkages could be easier to degrade. It has also been hypothesized that lower molecular weight tannins could be better substrates than larger compounds.



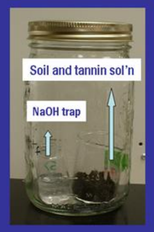
Hydrolysable tannins



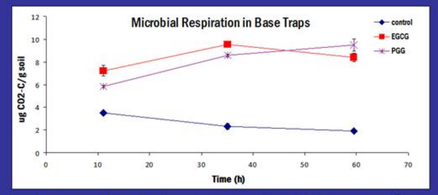
Condensed tannins

Base Trap Incubation Method

Tannin was added to 30 g soil (up to 2 mg tannin/mg soil) and sealed in a mason jar respirometer.



CO_2 released by respiration reacted with the base trap. The base traps were removed after a specific time intervals and titrated to calculate pH change as an indicator of metabolic activity. Some jars had water added as a control.

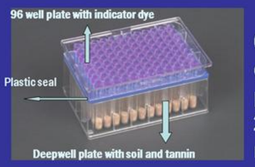


Preliminary results indicate that microbial usage of EGCG peaks between 30 h and 60 h, while PGG might peak higher and at a later time. There were many flaws with this experimental set up, particularly:

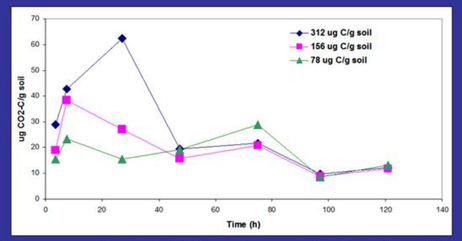
- Amount of time required per trial due to titrations
- Amount of soil and tannin required per trial

MicroResp System

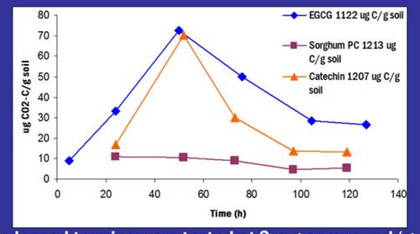
The MicroResp system is a 96 well plate set up developed by Macauley Scientific Consulting Ltd. for detection of CO_2 emitted by microorganisms. The 96 well plate system uses an automatic plate reader instead of acid-base titrations to measure the amount of CO_2 respired, and thus the metabolism taking place. Each trial requires less soil and very little tannin compared to the Mason jar respirometers.



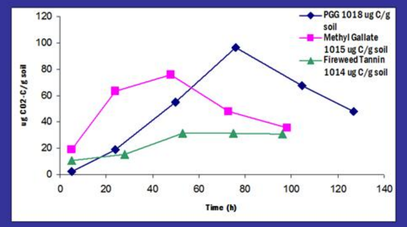
0.385 g of soil are added to each well of the deepwell plate. Wells are dosed with 25 uL of tannin solution. The respired CO_2 is funneled to a single well of the 96 well plate by a plastic seal. CO_2 changes the pH in the well, and an indicator dye changes from pink to yellow. This change in color is measured using an automatic plate reader. The color change is related to the amount of carbon dioxide emitted and the amount of metabolism taking place.



Glucose was tested at different concentrations from 0.8 mg tannin/g soil to 0.2 mg tannin/g soil. The respired CO_2 was proportional to the amount of C added. Glucose standards were included in every plate for comparability.



Condensed tannins were tested at 2 mg compound/g soil. The nontannin phenolic catechin was metabolized readily, but sorghum procyanidin, a catechin polymer was inert. EGCG, a small flavonoid-based tannin, was rapidly respired.



Hydrolysable tannins were tested at 2 mg compound/g soil. The nontannin phenolic methyl gallate was respired more rapidly than PGG, a relatively small hydrolysable tannin. The large hydrolyzable tannin from fireweed was utilized very slowly.

Conclusions

The nontannin phenolics we tested were rapidly metabolised. Some of the smaller tannins (PGG, EGCG) were good substrates but the higher molecular weight tannins (fireweed, procyanidin) were poor substrates. PGG, a hydrolysable tannin, yielded higher respiration rates than the somewhat smaller condensed tannin EGCG. Our work conrims the hypotheses that smaller phenolics are better substrated, and hydrolysable tannins are more easily metabolised than condensed tannins.

Future Work

More trials are necessary to further determine the relationship between tannin structure and effect on microbial populations. Specifically, it is necessary to run a trial with hydrolysable and condensed tannins of roughly the same size to eliminate the effect of a compound's size. Additionally, we could work with different soil samples, and thus different soil microbial communities, to determine if there is a consistent response to the same compounds across different communities.

Acknowledgements

- Miami University Undergraduate Summer Scholars
- USDA-ARS Cooperative agreement 58-1932-6-634
- Melany Fisk (Miami University)