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Peatland phenol oxidase:
A Microbially-Mediated Determinant of Global-Scale Atmospheric Carbon Dioxide Sequestration

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Oxygen constraints upon a single enzyme, phenol oxidase, could be considered a "latch mechanism" that holds in place a global carbon store of 455Gt - a magnitude approaching that of the entire atmosphere [1].

Of the many products of microbial metabolism in peatlands, phenolic compounds have attracted particular interest for they are potent inhibitors of microbial enzymes[2]. With the exception of phenol oxidase, few enzymes are able to degrade these highly recalcitrant materials[3]. However, the *in situ* activity of phenol oxidase is severely constrained[4]; it requires bimolecular oxygen for its activity while being located in an essentially anaerobic environment. Interestingly, the activity of the major biodegradative hydrolase enzymes are equally depressed in peatlands[5], and yet normally these enzymes remain highly active in anaerobic environments (e.g. the rumen[6] or anaerobic sludge digestor[7]). We propose that the low biodegradation rates in peatlands are due to oxygen constraints upon phenol oxidase, as these would allow phenolic materials to accumulate and inhibit those pivotal hydrolase enzymes. However, for the hypothesis to be tenable, four criteria must be fully satisfied:

The first requirement is that oxygen must stimulate phenol oxidase to a greater extent than hydrolase enzymes. The criterion was tested by comparing enzyme activities under oxygen-saturated and oxygen-free conditions. Aerated conditions decreased the activity of the hydrolase enzymes β -glucosidase (-24%, $p<0.01$) phosphatase (-32%, $p<0.01$) and sulphatase (-47%, $p<0.01$). The only enzyme to respond positively to increased oxygen availability was phenol oxidase, with a substantial 7-fold ($p<0.05$) increase in activity, lending the first measure of support for the hypothesis. Further support has been found in a Florida wetland[3], where phenol oxidase was only detectable under aerobic conditions.

Having confirmed that oxygen selectively stimulated phenol oxidase, the second requirement was that higher levels of phenol oxidase should diminish the abundance of phenolic materials. Experimental supplementation with phenol oxidase was confirmed to cause a substantial fall in phenolic material concentrations from 1.985 mg L^{-1} to 1.444 mg L^{-1} (27%; $p<0.05$) within 18 hours. Other studies have found declining water table levels (increased aeration) cause a sharp decline in phenolic concentrations [8]. When the potential for an aeration-induced rise in the activity of one of

the few enzymes able to degrade these recalcitrant materials is considered, the latter response lends additional support to the second criterion.

Once it had been established that oxygen could stimulate phenol oxidase, and that the consequently higher activity could reduce the abundance of phenolic compounds, the third requirement for tenability of the hypothesis was that the removal of phenolic compounds should stimulate hydrolase activities. Dissolved phenolic materials were selectively removed using cross-linked N-vinyl-2pyrrolidone extraction[9]. Samples of peat were exposed to either untreated water (mean 2.40 mg L^{-1} phenolic compounds) or phenolic-free waters from the wetland. Elimination of phenolic compounds stimulated enzyme activities to extents ranging between 47% ($p<0.01$) sulphatase, 26% ($p<0.05$) β-glucosidase, 22% chitinase ($p<0.05$), 18% phosphatase ($p<0.05$) and 16% xylosidase ($p<0.05$). Other studies have shown that far greater inhibition is possible (>80%) with higher phenolic concentrations[10], or following longer periods of exposure to the phenolic compounds[2].

The final postulate required that under aerobic conditions, phenol oxidase should stimulate the activity of hydrolase enzymes that would not normally be directly stimulated by the presence of oxygen. Additional phenol oxidase stimulated peat β-glucosidase (6-fold; $p<0.001$) and phosphatase (17%; $p<0.05$), a response that could be anticipated due to the enhanced removal of inhibitory phenolic compounds. Taken together, these findings all support the proposal that oxygen constraints upon a single peatland enzyme, phenol oxidase, can minimise peat decomposition and hence the re-release of CO_2 to the atmosphere. This hypothesis has profound implications in the context of climate change where it has been proposed that major peatland-dominated areas may experience an increased frequency of droughts. Under such conditions, phenol oxidase would act as the "functional switch" that transforms the ecosystem from CO_2 sink to CO_2 source, a response recently recognised in tundra peatlands[11]. Recent evidence also suggests that carbon mobilisation may also occur dissolved form [12]. Such responses are capable of substantial positive feedback to climatic warming.

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