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Enzymes and biogeochemical cycling in wetlands during a simulated drought

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Abstract. Possible interactions between soil enzymes and the biogeochemistry of wetlands were investigated during a field-based drought simulation. Under control (waterlogged) conditions, correlations were found between the activity of the enzyme $\mathcal{B}$-glucosidase and two properties associated with carbon cycling, namely i) CH_4 release ($r = 0.79$, $p < 0.01$) and ii) dissolved organic carbon concentration ($r = -0.81$, $p < 0.01$). In contrast, the transition to drought conditions resulted in correlations between $\mathcal{B}$-glucosidase activity and certain mineralisation processes, namely the release of mg and Ca ($r = 0.72$, $p < 0.05$). Sulphatase activity correlated with changes in sulphate concentration during the drought simulation ($r = 0.73$, $p < 0.05$). Further support for the suggested enzymic involvement in biogeochemical processes was found in laboratory studies. These experiments indicated that increasing the abundance of $\mathcal{B}$-glucosidase could stimulate trace gas emissions ($p < 0.001$) and increase the concentration of magnesium and calcium ($p < 0.05$). Increased sulphatase abundance caused a suppression of methane emissions ($p = 0.053$).

Introduction

Wetlands cover just 6% of the Earth's land area (Mitsch & Gosselink 1993). However, these wet or waterlogged soils may produce c. 20% of terrestrial methane release to the atmosphere (Cicerone & Oremland 1988) and 20% of dissolved organic carbon (DOC) exports to the oceans (Lugo et al. 1989). Recent estimates claim the world's peat-accumulating wetlands contain an amount of carbon (450Gt) equal to that of the entire atmosphere (Immirzi et al. 1992). Furthermore, many studies have indicated that these wetlands possess the valuable ability to ameliorate water quality (Braekke 1981; Kadlec & Tilton 1979). Clearly, from a biogeochemical perspective, wetlands are of far greater global significance than their small geographical area suggests.

It is widely accepted that waterlogging is the dominant factor regulating wetland biogeochemistry. When waterlogging is reduced by lowering the water table, wetland soils begin to exhibit characteristics which resemble those of other ecosystems: Methane and DOC release are suppressed, while mobilisation of the store of carbon as CO_2 and the release of sequestered inorganic nutrients, both increase (Moore & Knowles 1989; Heathwaite 1990; Freeman et al. 1993c; Freeman et al. 1994a). Recent concern that climate change could increase the frequency of droughts (Manabe & Weatherald 1986; Mitchell & Warrilow 1987) and thus reduce waterlogging, has served to heighten scientific interest in the mechanisms associated with the changes in wetland biogeochemical exports under drought conditions.

As with all ecosystems, the decomposition of dead biomass and the subsequent export of gaseous and dissolved materials from wetlands, is affected by microbial activity. Microbial enzymes play a pivotal role in the process of decomposition (Tabatabai 1982) and have been studied in soils since the turn of the century (Skujins 1978). However, enzymes have rarely been studied in wetlands (Pind et al. 1994; Freeman et al. 1995; Martikainen et al. 1994), a site in which enzyme activity appears unusually low (Küster, 1993). The paucity of enzyme activity has the potential to affect all major wetland functions:

1) Partially decomposed plant materials would be allowed to accumulate as peat (carbon storage).
2) Inorganic nutrients, incorporated into the biomass during plant growth, would remain sequestered within the poorly degraded peat matrix. This impairment of nutrient cycling would cause inorganic nutrients to accumulate and to be retained within the wetland (water quality amelioration, where eutrophication is a problem).
3) The rate of enzymic cleavage of labile organic substrates (energy sources) from the predominantly recalcitrant peat matrix could regulate microbial production of trace gases such as CH_4, CO_2 and N_2O.

Recent experiments suggest that each of the three biogeochemical properties could be modified by environmental conditions associated with potential future changes in climate (Freeman et al. 1993b; Freeman et al. 1993c). Our study aimed to determine whether the putative biogeochemical modifications were associated with changes in enzyme activity. Associations were sought between $\mathcal{B}$-glucosidase (an example of an enzyme involved in carbon cycling) and i) the production of trace gases (e.g. CH_4, CO_2), ii) the release of DOC, and iii) the release of inorganic nutrients (e.g. Ca, Mg). Secondly, relationships between the enzymes phosphatase and sulphatase, were sought relative to the release of phosphate and sulphate respectively.

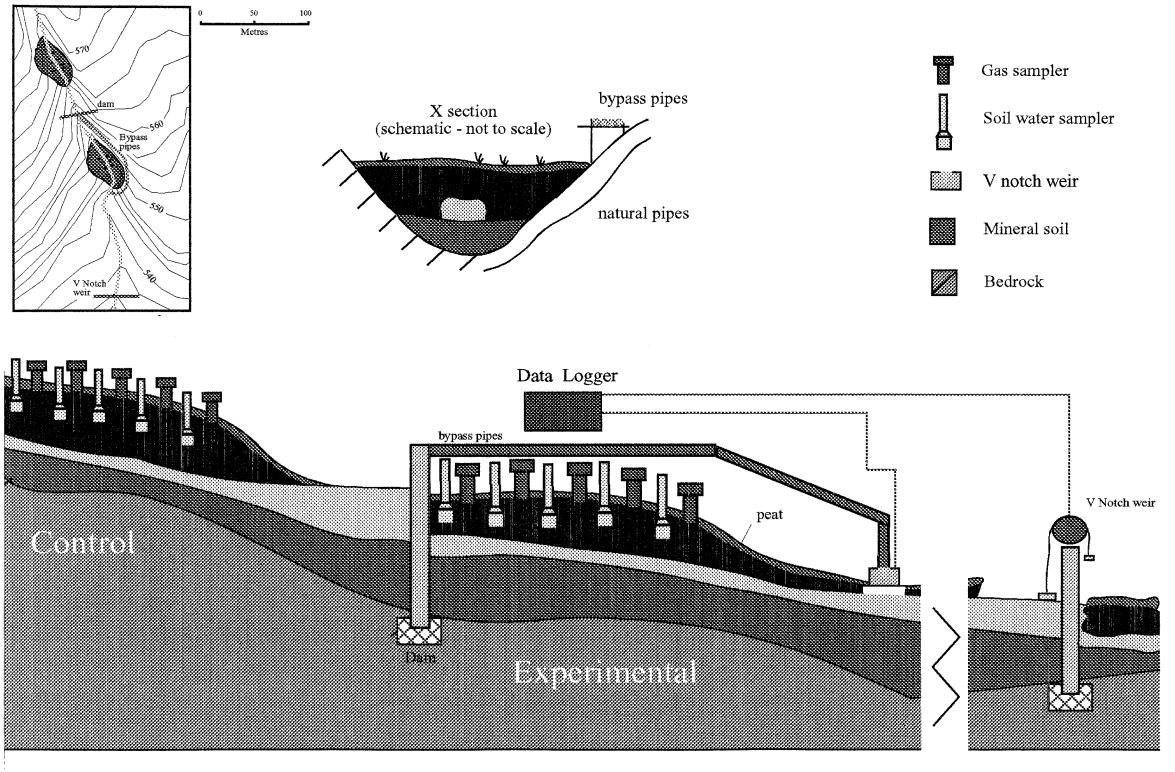
Methods

The study was carried out in two parts. First a field manipulation was undertaken, and correlations sought between enzyme activity and the release of gaseous and dissolved materials from the peat. Secondly, laboratory manipulations of enzyme activity were carried out in order to further investigate relationships found in the field study.

The field-based drought simulation was carried out at Cerrig-yr-Wyn, Plynlimon, mid-Wales (UK Nat. Grid Ref. SN 820 866) on a site that consists of a discontinuous series of *Sphagnum*- and *Juncus*- dominated wetlands (*c*. 30 m $\times$ 5 m) lying along the base of a small gully (Figure 1). Water inflow to the lower wetland was reduced by diversion through a system of pipes (Freeman et al. 1993a) for 18 weeks between May and October. Samples of released trace gases, dissolved solutes and peat soil were collected at five (2 m) intervals along transects through the centre of each bog.

Five replicate peat samples were collected from each wetland for enzymic analyses every two weeks. From each, a vertical section of peat ($1\times1\times10$ homogenised for 30 seconds with 5 cm^3 deionised water. Ten cm^3 of slurry were pipetted into 20 cm^3 plastic vials and the methylumbelliferyl (MUF)-substrates added: 3 cm^3 of 500 μM MUF-$\mathcal{B}$-D-glucoside, 6 cm^3 of 1000 μM MUF-phosphate or 7 cm^3 of a 1000 μM MUF-sulphate. This suite of substrates allowed us to estimate the activity of $\mathcal{B}$-glucosidase, phosphatase and sulphatase, as examples of enzymes associated with C, P and S cycles. The samples were incubated for 60 minutes at 11 °C, and then centrifuged at 7,200 $\times$ G for 5 minutes. Five cm^3 of the supernatant were transferred to a second 20 cm^3 vial, and 0.5 cm^3 pH 11 buffer solution (0.05 M glycine/0.2 M NaOH-buffer) added to convert the MUF into the more fluorescent anionic form (Chrost & Krambeck 1986). Fluorescence was determined without delay USING a Perkin-Elmer LS50 fluorimeter at 450 nm emission and 330 nm excitation (slit-setting 1). In order to quantify product release and account for quenching effects, a set of standards was prepared according to the above procedure (methylumbelliferone replaced the MUF-substrates) for each peat sample (Freeman et al. 1995).

Methane, carbon dioxide and nitrous oxide emissions from the two bogs were estimated on a bi-weekly basis using a field-based closed chamber technique (Freeman et al. 1994b). Five replicate samples were collected from each wetland. All collections were completed within 90 minutes of midday. Gasses accumulated over 2 hours were removed through a SubaSeal septum in the 4.5 l chambers using 10 cm^3 gas-tight syringes (SGE) and transported back to the laboratory for analysis using an Ai Cambridge model 92 G.C. The system used twin Porapak QS columns at 35 °C with a N_2 carrier gas flow of 30 cm^3 min^{-1}. The GC included a flame ionisation detector incorporating a



Long profile (schematic- not to scale)

Figure 1. Plan of the field site.

$CO_2 \rightarrow CH_4$ catalytic converter and an electron capture detector. The increase in trace gas concentration (above the initial background concentration) was used to estimate gaseous fluxes from the two experimental wetlands.

Peat soil water samples were collected every two weeks from soil solution samplers placed at a depth of 10 cm (Freeman et al. 1994b). A DIONEX 2000i ion chromatograph was used to determine phosphate, sulphate, magnesium and calcium concentrations with AS4A anion and CS10 cation columns. Dissolved organic carbon (DOC) was estimated using a Skalar auto-analyser with UV digestion/colorimetric detection system (Freeman et al. 1993b). Correlations between enzyme activity and peat hydrochemistry and trace gas emissions were sought using Pearson's Correlation analyses. The enzymic data gathered in the field experiment were compared with biogeochemical parameters such as trace gas release and hydrochemistry on a cumulative activity basis (Sinsabaugh et al. 1994). Thus, each (enzymic) data point corresponded to the enzyme activity summed over all previous sampling intervals, generating units analogous to the "degree-day" units used to express the cumulative effects of temperature on ecological systems (Sinsabaugh et al. 1994).

The complementary laboratory manipulations involved placing a 1 cm^3 subsample of the peat inside 28 cm^3 glass vials sealed with air-tight caps incorporating a subaseal septum through which headspace gases could be sampled (Gammelgaard et al. 1992). Nine cm^3 of wetland water was added to each. The replicates were divided in half, with half receiving a further 1 cm^3 of water (control) while the second half received 1 cm^3 of water containing either 50 international units of $\mathcal{B}$-glucosidase (EC 3.2.1.21; Sigma) or 500 units of sulphatase (EC 3.1.6.1; Sigma). The $\mathcal{B}$-glucosidase experiment was run for a period of 2h at 8.2 °C initially and 5 cm^3 samples of trace gases were removed and analysed by gas chromatography. Rates of emission were estimated from the increase in trace gas concentration over the 2 h period. The incubations were allowed to proceed for a further 22 h, after which a 1 cm^3 sample of liquid was removed for hydrochemical analysis using the above ion chromatography and DOC analysis procedures. The impact of $\mathcal{B}$-glucosidase on the release of Mg, Ca and DOC was estimated following correction for contaminant solutes associated with the enzyme solutions. The sulphatase experiment was designed to determine whether increasing the abundance of that enzyme would suppress methane production. Samples were incubated for 2 h, and CH_4 release estimated as above. Student *t*-tests were adopted to determine whether differences between the control and enzyme-amended treatments were statistically significant in each laboratory experiment.

Results

In the control wetland, where water table levels remained above the surface, cumulative $\mathcal{B}$-glucosidase activity was found to correlate inversely with dissolved organic carbon (DOC) concentration (Figure 2a; $r = -0.81$, $p < 0.01$) but positively with methane flux (Figure 2b; $r = 0.79$, $p = 0.01$). Phosphatase activity correlated positively with phosphate concentration (Figure 2c; $r = 0.66$, $p = 0.05$). However, the relationships were no longer detectable following the imposition of a simulated drought.

During the drought conditions, $\mathcal{B}$-glucosidase activity correlated positively with both magnesium (Figure 2d) and calcium (Figure 2e) concentrations ($r = 0.72$, $p < 0.05$). Sulphatase activity correlated positively with sulphate concentration (Figure 2f; $r = 0.73$, $p < 0.05$).

The laboratory experiments investigated the impact of elevated $\mathcal{B}$-glucosidase and sulphatase levels. Twenty four hours of $\mathcal{B}$-glucosidase treatment (Figure 3a) increased the release of magnesium (47%; $p < 0.05$) and calcium into the peat soil waters (33%; $p < 0.01$). However, the impact of the treatment on DOC concentrations was impossible to determine (see below). Addition of $\mathcal{B}$-glucosidase caused a marked stimulation of trace gas release (ANOVA, $p < 0.001$). We observed a doubling in the emissions of N_2O ($p < 0.001$) and a 47% rise in CO_2 release ($p < 0.01$). The 10-fold increase in methane emissions was only significant at the 10% level (Figure 3b).

In the field experiment, combining sulphatase and methane flux data from control and drought conditions revealed that lowest methane emissions were generally associated with highest sulphatase activities (Figure 4a) although the relationship was only significant at the 10% level. However, a further experiment conducted in the more rigourously controlled conditions of the laboratory confirmed that addition of sulphatase could cause methane emissions to fall (Figure 4b; 81%, $p = 0.053$).

Discussion

Correlations between phosphatase activity and phosphate concentration have only rarely been observed in the natural environment (Sinsabaugh 1994). In our study, such a correlation was found, although only under waterlogged (control) conditions (Figure 2c). The main difference between our study and most other studies lies in the extremely low biological activity of peatlands (Küster 1993). Lower biological activity would reduce the demand for phosphate, allowing phosphate to accumulate, thus increasing the likelihood of an observation of a positive correlation. It is interesting to note that the correlation was not detected during the simulated drought, where the higher

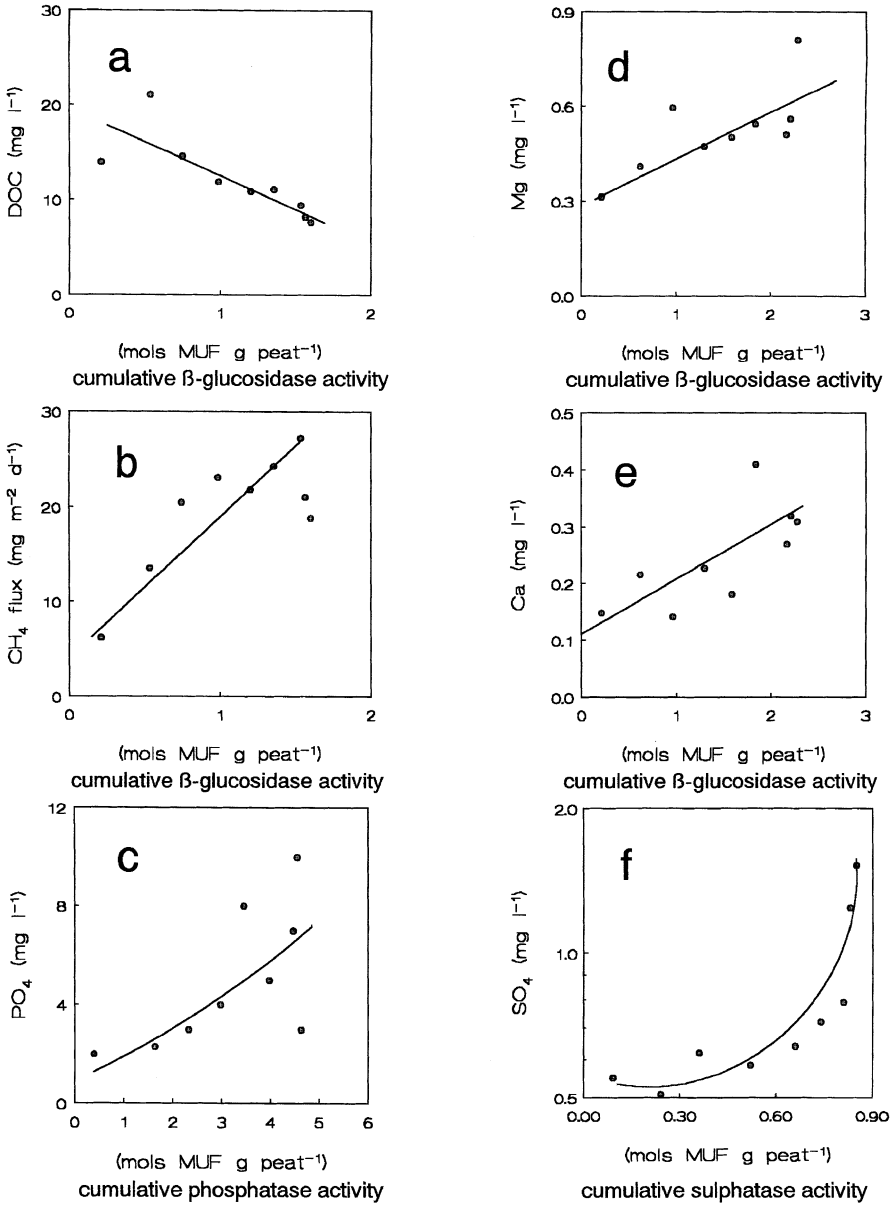


Figure 2. Correlations between cumulative enzyme activities and biogeochemical properties under waterlogged control conditions (a,b,c) and under drought conditions (d,e,f).

microbial activities (Schothorst 1977) and consequently greater phosphate uptake, would impair the detection of a relationship.

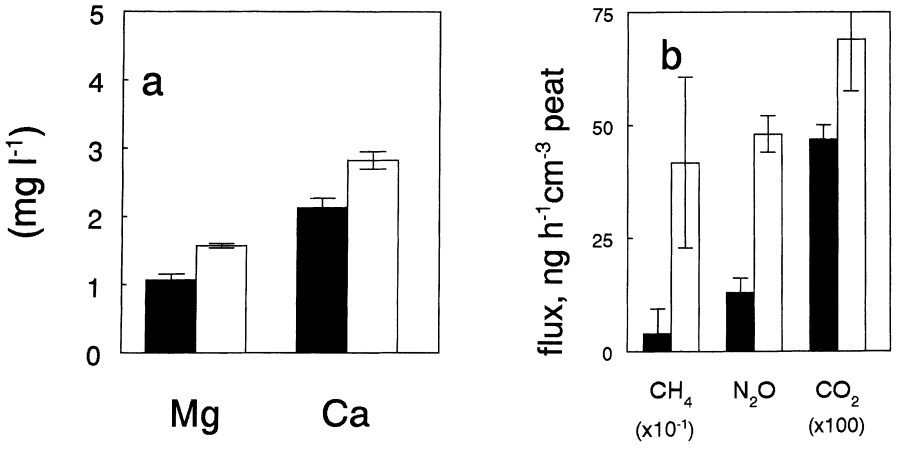


Figure 3. Magnesium and calcium concentrations (a) with trace gases fluxes (b) under control conditions (shaded bars), and following laboratory addition of $\mathcal{B}$-glucosidase (unshaded bars) (mean ± s.e.; $n = 5$).

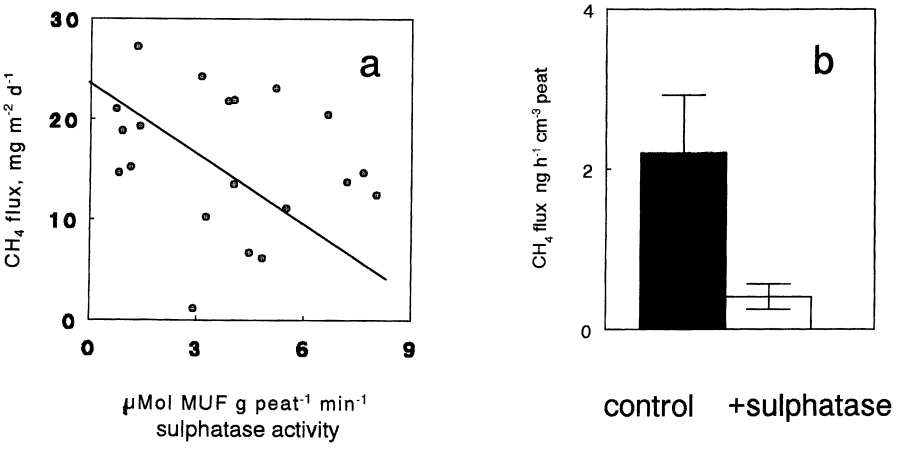


Figure 4. Methane flux vs. sulphatase activity at the field site (a) and CH_4 emissions under control conditions (shaded bars), and following the addition of sulphatase (unshaded bars) in the laboratory (b) (mean ± s.e.; $n = 5$).

The activity of another enzyme, $\mathcal{B}$-glucosidase, correlated inversely with DOC concentration (Figure 2a) but positively with methane flux (Figure 2b) under waterlogged conditions. We had initially hypothesized that $\mathcal{B}$-glucosidase would correlate positively with DOC concentrations, on the grounds that as a carbon cycling enzyme, it was likely to attack the peat matrix and cause the release of DOC as a product. However, the observation of an inverse

relationship suggests that DOC is more likely to represent a substrate than a product. The products of $\mathcal{B}$-glucosidase activity are believed to represent an important substrate for microbial metabolism (Tabatabai 1982). The anaerobic conditions of the waterlogged wetland would favour the utilisation of these organic solutes by methanogens and other anaerobic microbes (c.f. Thomas et al. 1996). We propose that the products of $\mathcal{B}$-glucosidase activity were metabolised anaerobically, and that this led to our observation of an inverse correlation with DOC concentration, and yet positive correlation with methane flux. Some support for that hypothesis can be found in the stimulation of trace gas emissions (CH_4, N_2O and CO_2)that was observed following the laboratory addition of $\mathcal{B}$-glucosidase. However, laboratory experiments designed to confirm the depletion of the DOC pool by $\mathcal{B}$-glucosidase were less successful. $\mathcal{B}$-glucosidase is a form of DOC, thus necessitating the subtraction of the enzymic-DOC concentration from the total DOC content of the treated peat slurry. The procedure yielded a meaningless negative DOC concentration, suggesting that a large proportion of the enzymic-DOC had become adsorbed on to the peat matrix (Wetzel 1991). Adsorbed enzymes may retain their activity (Nannipieri et al. 1983) and this may explain why the enzyme addition was nevertheless able to affect trace gas release (Figure 3b).

The introduction of drought conditions in the experimental wetland, was associated with a shift from carbon cycling processes (CH_4, DOC release; Figure 2a,b) to mineralisation (Mg/Ca release; Figure 2d,e) as the dominant biogeochemical processes with which $\mathcal{B}$-glucosidase activity was correlated. Many studies have noted that drought conditions promote the release of inorganic nutrients such as Mg and Ca from wetlands (Heathwaite 1990; Freeman et al. 1993b), and our findings indicate that $\mathcal{B}$-glucosidase could contribute to that release. The hypothesis is supported by the laboratory addition of supplementary $\mathcal{B}$-glucosidase which caused an increased release of the two cations into the peat soil waters (Figure 3a).

A further correlation that was only detectable under drought conditions was that between sulphatase and sulphate concentrations (Figure 2f). Many studies have noted that sulphate concentrations increase following water table drawdown (Braekke 1981; Freeman et al. 1993b; Ogden 1982). The response has generally been attributed exclusively to the re-oxidation of sulphides. However, peatlands generally contain large quantities of organically bound sulphate ($>$20% of the total S pool (Wieder et al. 1987)). This would provide an ample supply of substrates from which sulphatase could release sulphate. It follows that sulphatase could contribute to the increased sulphate abundances that are observed during droughts. The finding is of particular interest because i) the presence of sulphate causes a lowering of methane emissions (Valiela 1995) and ii) methane emissions are known to fall during droughts (Oremland

1988; Moore & Knowles 1989). Such observations suggest that sulphatase is likely to affect methane emissions, and it is interesting to note that: i) The laboratory addition of sulphatase induced a substantial 80% fall (Figure 4b) in CH_4 emissions ($p = 0.053$) and ii) in the field, the lowest methane emissions were generally found at highest sulphatase activities (Figure 4a).

In summary, our findings lend support to the hypothesis that enzymes influence wetland biogeochemistry. The study has allowed us to propose a number of new hypotheses concerning the mechanisms by which enzymes affect the production of trace gases and hydrochemical exports. Further investigation of those hypotheses should aid our understanding of the unique functional properties of wetland ecosystems.

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