The effect of peatland drainage and rewetting (ditch blocking) on extracellular enzyme activities and water chemistry

Journal Item

The effect of peatland drainage and rewetting (ditch blocking) on extracellular enzyme activities and water chemistry

Mike Peacock[ac], Timothy G. Jones[a], Belinda Airey[a], Anna Johncock[a], Chris D. Evans[b], Inma Lebron[b], Nathalie Fenner[a], Chris Freeman[a].

[a]Wolfson Carbon Capture Laboratory, School of Biological Sciences, Bangor University, Deiniol Road, LL57 2UW, UK.

[b]Centre for Ecology and Hydrology, Environment Centre Wales, Deiniol Road, LL57 2UW, UK.

[c]Corresponding author. Centre for Earth, Planetary, Space and Astronomical Research (CEPSAR), Department of Environment, Earth and Ecosystems, The Open University, Walton Hall, Milton Keynes, MK7 6AA. mikepeacocknin@yahoo.co.uk

Abstract

Extensive areas of European peatlands have been drained by digging ditches in an attempt to improve the land, resulting in increased carbon dioxide fluxes to the atmosphere and enhanced fluvial dissolved organic carbon (DOC) concentrations. Numerous peatland restoration projects have been initiated which aim to raise water tables by ditch blocking, thus reversing drainage-induced carbon losses. It has been suggested that extracellular hydrolase and phenol oxidase enzymes are partly responsible for controlling peatland carbon dynamics, and that these enzymes are affected by environmental change. The aim of this study was to investigate how drainage and ditch blocking affect enzyme activities and water chemistry in a Welsh blanket bog, and to study the relationship between enzyme activity and water chemistry. A comparison of a drained and undrained site showed that the drained site had higher phenol oxidase and hydrolase activities, and lower concentrations of phenolic

compounds which inhibit hydrolase enzymes. Ditch blocking had little impact upon enzyme activities; although hydrolase activities were lowered 4-9 months after restoration, the only significant difference was for arylsulphatasearylarylsulphatase. Finally, we noted a negative correlation between β-glucosidase activity and DOC concentrations, and a positive correlation between arylsulphatase activity and sulphate concentration. Phenol oxidase activity was negatively correlated with DOC concentrations in pore water, but for ditch water phenol oxidase correlated negatively with the ratio of phenolics to DOC. Our results imply that drainage could exacerbate gaseous and fluvial carbon losses, and that peatland restoration may not reverse the effects, at least in the short term.

Key words: ditch blocking, peatland restoration, phenol oxidase, β-glucosidase, dissolved organic carbon, phenolics,

1. Introduction

Northern peatlands are important carbon stores, but many have been drained for forestry, agriculture, and peat harvesting. In the UK drainage ditches were predominantly dug during the 19th and 20th centuries. The size and spacing of ditches varies but in UK blanket bogs they are typically around 0.5 m deep, with 7-20 m spacing (Stewart & Lance, 1991). It has been suggested that blanket bogs are somewhat resistant to drainage, with water table drawdown occurring only in the immediate vicinity of ditches (Stewart & Lance, 1991), and the magnitude of drawdown will depend on ditch spacing and the hydraulic conductivity of the peat (Armstrong, 2000). Nevertheless, long-term drainage can lead to the establishment of deeper water tables (Holden *et al.*, 2011), and even slight changes in water tables can have ecological effects (Price *et al*., 2003).

Blanket bogs are largely ombrotrophic, and often found at the headwaters of river catchments, making them sources of potable water as well as sources of dissolved organic carbon (DOC) (Hope *et al*., 1999). The quality of water draining these systems thus has relevance for aquatic ecosystems (Karlsson *et al.*, 2009), water treatment (McDonald *et al*., 1991), and human health issues (Chow *et al*., 2003). DOC is a natural export from peatlands, but there is evidence that DOC concentrations are higher in drained bogs (Glatzel *et al*., 2003, Wallage *et al*., 2006). The drainage of ombrotrophic bogs generally leads to an increase in carbon dioxide (CO_2) emissions and a decrease in methane (CH_4) emissions (Bussell *et* al., 2010).

In an attempt to reverse these drainage-induced biogeochemical changes, numerous peatland restoration projects have been initiated. Sites that have been ditched are restored by blocking the ditches with dams. The aim is to return the water table to pre-drainage levels. Some success has been observed on blanket bog; 6-7 years after rewetting, Holden *et al.* (2011) observed that a ditch-blocked site had hydrological functioning intermediate between an undrained site and drained site. Similarly, Wilson *et al.* (2011a) and Worrall *et al.* (2007) both noted increases in the water table after blocking

One aspect of drainage that has received little attention is the activity of soil extracellular enzymes. Extracellular enzymes are involved in peatland carbon cycling (Freeman *et al.*, 1997) but their activities are constrained by the conditions that exist in peat soils. Recalcitrant phenolic compounds are released by plants (Wetzel, 1992) and degraded by phenol oxidase, which has limited activity in northern peatlands due to the acidic pH, low temperatures and low oxygen content (Pind *et al.*, 1994, Freeman *et al.*, 2001a, Tahvanainen & Haraguchi, 2013). The build-up of phenolics in turn inhibits the activity of hydrolase enzymes (Freeman *et al*., 1990, Wetzel, 1992); resulting in low rates of decomposition. Conversely, increased peat aeration stimulates phenol oxidase activity, lowers phenolic

concentrations, and removes the inhibitory effect on hydrolase enzymes (Freeman *et al*., 2001a). It can therefore be hypothesised that long-term drainage would lead to increased phenol oxidase activity, reduced phenolic concentrations and increased hydrolase activity, thereby resulting in greater overall soil decomposition rates and contributing to carbon loss (*hypothesis 1*). Theoretically, ditch blocking would reverse this by raising the water table, and leading to suppressed phenol oxidase activity, increased phenolic concentrations and reduced hydrolase enzyme activity (*hypothesis 2*). The aim of this study was to test these hypotheses using two sites located within a large peatland. A further aim was to examine enzyme activities and to determine if they were related to DOC or phenolic concentrations, as past studies have shown contradictory results.

2. Materials and Methods

2.1. Study sites

The study was carried out on the Migneint blanket bog, North Wales (UK). According to the JNCC National Vegetation Classification (NVC), it includes areas of mire habitat of classes M18, M19 and M20. Mean annual rainfall is 2.2 m and mean annual temperature 5.6 °C (Billett *et al*., 2010).

The primary field site was the Afon Ddu catchment (latitude 52.99 N, longitude 3.82 W, 490 m above sea level) which was drained during the 1970s and 1980s. The ditches run downslope and were blocked in February 2011. A replicated experiment was established in August 2010 which comprises four ditches that have been left open as controls, and eight that have been blocked using two different methods. Four have been blocked using peat dams, for which the peat is extracted from 'borrow pits' adjacent to each ditch. The other four have been blocked using a reprofiling technique, which involves the ditch vegetation being removed, and the peat bottom being compressed to destroy any natural pipes that may be

present. The ditch is then infilled with peat from borrow pits and the vegetation is replaced. As in the previous treatment peat dams are also constructed along the ditch.

A second nearby field site, was used to provide a comparison with undrained conditions; the Bryn Du site (latitude 52.97 N, longitude 3.82 W, 460 m above sea level) includes four control plots on intact blanket bog that has not been drained.

2.2. Soil Sampling

At the Afon Ddu soil samples were taken from each of the twelve ditches in June, July, August, September and November 2011. These samples were used to test the effect of ditch blocking on enzyme activities. Additional soil samples were taken from areas of bog between ditches to examine the effects of enzyme activities on DOC and phenolic concentrations. At Bryn Du, soil samples were taken from each of the four control plots in June and September 2011. All soil samples were taken to 10 cm depth. Each soil sample comprised 2-4 sub-samples of soil (taken from an area of approximately 1 m^2) to minimise the influence of small-scale spatial variation in enzyme activity. Samples were stored in the dark at 4^{o}C. Soil water content was determined by weighing 1 g of sample, drying for 24 hours at 105^{o}C and re-weighing.

2.3. Water sampling and water tables

Water samples were taken from the ditches at the Afon Ddu and from piezometers 2-3 m adjacent to ditches (i.e. water and soil samples were taken from approximately the same locations for 'ditch' and 'bog' samples). Piezometers were constructed from PVC pipe with intakes at 10-15 cm depth. Water samples at Bryn Du were extracted using Rhizon samplers (Rhizosphere Research Products) at a depth of 10 cm. Water samples were collected in 60 ml Nalgene ® bottles and were stored in the dark at 4^{o}C.

125 Water tables were measured using dipwells constructed from PVC pipe; for each
126 ditch, a dipwell was positioned 2 m either side of the ditch. Water tables were manually
127 recorded on an approximately monthly basis from April to November 2011. Dipwell length
128 was 1000 mm. Every 100 mm, four drilled holes of 8 mm diameter were evenly spaced
129 around the pipe to allow water entry.
130
131 *2.4. Laboratory analysis*
132 Phenol oxidase activity was measured using a method modified from Pind *et al.*
133 (1994), using 1 cm^3 of soil. Analysis of hydrolase activity was measured using a method
134 modified from Freeman *et al.* (1995), using 1 cm^3 of soil. Further information concerning the
135 enzyme assays can be found in Dunn *et al.* (2013).
136 Water samples were filtered at 0.45 μm. Ion concentrations were determined using
137 either a DX-120 Ion Chromatograph (Dionex), or an 850 Professional IC (Metrohm). DOC
138 concentrations were analysed using a Thermalox Total Carbon analyser (Analytical
139 Sciences). Phenolic concentrations were determined using a method adapted from Box
140 (1983), and were derived from a standard curve using phenol standards.
141
142 *2.5. Statistical analysis*
143 Statistical analysis was carried out using SPSS v16.0.1 (IBM Corporation). The
144 Shapiro-Wilk test was used to test the normality of data, and log 10 or square root
145 transformations were attempted on any data that failed this. For the comparisons of the
146 drained and undrained site, t-tests were used, or the non-parametric Mann-Whitney test (for
147 any data that could not be transformed to normality). To compare unblocked ditches to the
148 two ditch blocking treatments, repeated-measures ANOVAs with Tukey HSD post-hoc tests
149 were carried out. If transformations failed to produce normal data, then the non-parametric

Kruskal-Wallis test was used. Linear regression was used to test for relationships between variables

3. Results

3.1. Site comparison – effect of long term drainage

A comparison of the Bryn Du data with that from the open ditches at the Afon Ddu shows that the drained site had higher hydrolase (driven by arylsulphatase and β-glucosidase) and phenol oxidase activity (Figure 1 and 2). Additionally, Bryn Du displays a significantly higher phenolic concentration; 5.6 mg L^{-1} compared with 4.8 mg L^{-1} at the Afon Ddu (one-tailed t-test, $p = 0.02$). There was no significant difference in pH; 4.27 at Bryn Du and 4.18 at the Afon Ddu. Despite the significant difference in arylsulphatase activity, there was no significant difference in pore water sulphate concentrations between the two sites: mean concentrations for the period March-November 2011 (monthly sampling, $n = 4$ per site) were 2.2 mg L^{-1} at the Afon Ddu, and 1.0 mg L^{-1} at Bryn Du (with respective standard errors of 0.8 mg L^{-1} and 0.5 mg L^{-1}). The only ion for which a significant difference was found was phosphate; concentrations at Bryn Du were often below the detection limit of the analyser (Table 1). There was no significant difference in the water content of soil samples (91.0%, SE = 0.6% at Bryn Du, 90.7%, SE = 0.8% at the Afon Ddu).

3.2. Effect of ditch blocking on enzyme activity and phenolic compounds

At the Afon Ddu experimental site 4-9 months after ditch-blocking, there was no significant difference between treatments for the activity of β-glucosidase, xylosidase or chitinase. There was a significant difference for arylsulphatase; activity was higher in the control ditches compared to the reprofiled ditches (Figure 3). Sulphate concentrations were

lowest for reprofiled ditches (1.8 mg L^{-1} compared to 2.2 mg L^{-1} for open ditches and 2.5 mg L^{-1} for dammed ditches) but this difference was not significant.

There was no significant treatment effect on phenol oxidase activity (Figure 4). There was no significant difference in ditch water pH between treatments; mean values for the length of the study were 4.21 (open), 4.34 (dam) and 4.20 (reprofiled). The depth to the water table was greatest for open ditches, with a mean of 14.8 cm (SE = 1.1 cm, min = 1.2 cm, max = 46.5 cm) for the study period. Mean depth to the water table was 10.7 cm (SE = 0.8 cm, min = 1.8 cm, max = 28.7 cm) for dammed ditches, and 9.9 cm (SE = 0.7 cm, min = -1.9 cm, max = 23.9 cm) for reprofiled ditches (n = 80 for each treatment). The difference in water tables between open and blocked ditches was significant ($p < 0.01$). Mean soil water content of samples was 90.7% (open, SE = 0.4%), 89.2% (dam, SE = 0.7%) and 88.1% (reprofiled, SE = 0.6%). Repeated-measures ANOVA showed no significant difference in mean water content. There was no significant difference between treatments for phenolic or DOC concentrations (Figures 5 and 6).

3.3. Enzymatic controls on biogeochemistry

A significant negative relationship was found between β-glucosidase activity and DOC concentration in both ditch and pore waters (Figure 7). No direct relationship was found between either phenol oxidase activity and DOC ($r^2 = 0.02$) or phenol oxidase and phenolics ($r^2 = 0.09$) for ditch water, but there was a significant negative relationship between the phenolic to DOC ratio and phenol oxidase activity (Figure 8). For pore water this was not the case; there was no correlation between phenolic to DOC ratio and phenol oxidase activity ($r^2 = 0.05$), and the strongest relationship (highest r^2 value) was between phenol oxidase activity and DOC concentration (Figure 8). There was a weak positive correlation between arylsulphatase activity and sulphate concentration in ditch water (Figure 9).

4. Discussion

4.1. Effects of long term drainage

Results from a comparison between an undrained site and a drained site support hypothesis 1; that drainage leads to lower phenolic concentrations, and enhanced activities of phenol oxidase and hydrolases. This is in agreement with Freeman *et al.* (2001a), who showed that increased oxygen availability following drainage stimulates phenol oxidase activity, which in turn degrades phenolics and removes the inhibition on hydrolase enzymes. The enhancement of hydrolase activity was partly controlled by increased β-glucosidase activity, a response which has been observed before (Fenner *et al*., 2005). Additionally, long-term drainage leads to greater water table fluctuations (Holden *et al*., 2011) which can exacerbate the effects of seasonal drought, leading to an associated increase in oxygen availability of a magnitude to override pH controls and consequently stimulate phenol oxidase activity. As an aside, it should be noted that phenolics were measured in pore water at the undrained site and ditch water at the drained site; this will somewhat confound the results, as pore water and surface water would have some natural differences. However, this does not impinge on the enzyme data where methods were identical at both sites.

It is important to acknowledge that the observed differences in biogeochemistry may not have been due to drainage, as this was a limited comparison of two sites (i.e. with no data from before the Afon Ddu catchment was drained), with pseudoreplication (i.e. sampling over time) rather than true replication. The sites are close together and share the same climate and similar peat characteristics, and the only difference in pore-water ion concentration was observed for phosphate. Nevertheless, it could be that some other factor is responsible for the differences in enzyme activity.

224 *4.2. Effect of ditch blocking*

225 Although ditch blocking appeared to lower the activity of each of the hydrolase
226 enzymes studied, arylsulphatase was the only enzyme to show a statistically significant
227 difference. As such we are unable to find support for hypothesis 2: that ditch blocking would
228 suppress phenol oxidase activity, leading to a subsequent increase in phenolics and lowered
229 hydrolase activities. Fenner & Freeman (2011) noted that upon rewetting after drought,
230 phenol oxidase activity did not immediately decline, and remained high (for a period of
231 months to years) as a legacy from the previous aerobic conditions. It should be noted that
232 there was no significant difference in soil moisture between the blocked and open ditches,
233 despite the fact that the depth to the water table was significantly greater around open ditches.
234 It could be that a lack difference in soil moisture is due to the fact that water tables were
235 relatively high for all treatments, therefore making soil moisture insensitive to ditch blocking.
236 Additionally, Holden *et al.* (2011) suggest that ditch blocking only partially restores the
237 hydrological functioning of blanket bog, and other evidence suggests that it could be several
238 years before the rewetting suppresses enzyme activity (Fenner & Freeman, 2011). It might
239 be expected that enzyme activity would increase in the reprofiled ditches due to the
240 disturbance that this method involves; large volumes of peat are removed from the adjacent
241 borrow pits to infill the ditch, which might theoretically allow some oxygen infiltration.
242 However, the enzyme response was identical for the dammed ditches and the reprofiled
243 ditches, suggesting this was not the case. As such, it may be that the ditch blocking was on
244 wet and dense peat, and therefore very little air entered or became trapped in the peat.

245 The suppression of arylsulphatase activity in the reprofiled ditches could have
246 repercussions on CH_4 fluxes. Raising the water table will alter the redox conditions and
247 stimulate the methanogenic community, thus increasing CH_4 emissions (Komulainen *et al.*,
248 1998, Urbanová *et al.*, 2011). Coupled to this, arylsulphatase releases sulphate which is

implicated in reduced CH_4 emissions when the water table falls. The suppression of arylsulphatase following ditch blocking could result in a reduced rate of sulphate production which would then contribute to the enhanced CH_4 fluxes (Freeman *et al*., 1997). A weak but significant, positive relationship was found between arylsulphatase activity and sulphate concentrations in ditch water, but no significant difference in sulphate concentration was detected between treatments.

We observed no change in ditch water DOC concentrations immediately after ditch blocking, and this is similar to studies of blanket bogs that have noted small changes in DOC following ditch blocking (i.e. differences of approximately 1 mg L^{-1}, e.g. Gibson *et al*., 2009, Ramchunder *et al*., 2012) or even small increases (e.g. Wilson *et al*., 2011b). The lack of change in DOC concentration can be explained partly by the overall lack of response in enzyme activities. Considering that other ditch blocking studies have speculated that the action of enzymes could be involved in any restoration-induced changes in DOC dynamics (e.g. Wallage *et al*., 2006, Worrall *et al*., 2007), it is interesting to note that there has apparently been only one other study that investigated the response of enzymes to ditch blocking. Bonnett *et al.* (2008) compared hydrolase activities around a natural gully and around a ditch that had been blocked twelve years previously. They noted no difference in hydrolase activities in surface peat samples, but some differences at depth; for instance, β-glucosidase activity was lower around the blocked ditch at both 25 cm and 45 cm. Some studies have suggested that DOC concentrations are lowered following restoration; Wallage *et al.* noted substantially lower pore water DOC (60-70% compared to a drained site) concentrations at a blanket bog where ditch blocking had occurred 6 years previously. This could be indicative of supressed enzyme activities in the longer term following blocking. However, another study at the same site found similar fluxes and concentrations of DOC in ditches (Armstrong *et al*., 2010), thus adding further complexity to the issue.

It should be noted that the early post-restoration measurements of DOC concentration and water table that are reported here are part of a long-term experiment. It may well be that the short-term response of these variables is different to that of any long-term response.

4.3. Enzymatic controls on biogeochemistry

For both pore water and ditch water a weak negative relationship was observed between β-glucosidase activity and DOC concentration. Freeman *et al.* (1997) found the same relationship for a peatland in mid Wales, and concluded that DOC represented a substrate for β-glucosidase, with the metabolic products then being microbially degraded under anaerobic conditions.

There have been conflicting reports of the effect of phenol oxidase on phenolic concentrations. Freeman *et al.* (2001a) originally showed that increased phenol oxidase activity led to decreased phenolic concentrations, a result replicated by Fenner *et al.* (2005). However, Toberman *et al.* (2008) found a positive relationship between phenol oxidase activity and phenolics, and speculated that it could be possible for phenol oxidase to partially degrade complex phenolic compounds, thus releasing smaller, soluble phenolics. We found no relationship between phenol oxidase and phenolics. However, phenolics are a component of DOC, and (because DOC concentrations vary according to season and weather events) phenolic concentrations will also fluctuate. As such, by taking the phenolic to DOC ratio (as in Peacock *et al.*, 2013) then a significant negative relationship was observed with phenol oxidase, for ditch water. This observation suggests that phenol oxidase did not absolutely lower phenolic concentrations, but that it lowered phenolic concentrations relative to total DOC concentration. For pore water this relationship was not found; instead there was a significant negative relationship between phenol oxidase activity and DOC concentration. It

298 has been suggested previously that the phenolic to DOC ratio is an important factor in
299 enzymatic degradation (Freeman *et al*., 1990).

300 These results suggest that the action of enzymes on DOC/phenolics is complicated,
301 occasionally contradictory, and sometimes unrelated. Indeed, Kane *et al.* (2014) emphasise
302 the complexity of these interactions, and point out that positive feedbacks can exist between
303 the release of labile DOC and enzyme activities. Although the relationships reported here
304 between enzyme activities and DOC/phenolics are only weak, this is perhaps to be expected.
305 In a natural system there will be multiple drivers that interact in a complex way to control
306 fluvial carbon losses, with enzymes playing only a small part in the overall system.

307 It is useful to consider that drainage in this context can be used an analogue for a
308 prolonged drought events. Climate change in Europe is likely to result in more frequent and
309 prolonged droughts (Alcamo *et al*., 2007). Our findings thus agree with others (e.g. Freeman
310 *et al*., 2001a, Fenner & Freeman, 2011) in suggesting that future climate change may
311 stimulate the activities of phenol oxidase, β-glucosidase and arylsulphatase. These changes
312 could result in enhanced losses of gaseous and fluvial carbon from peatlands, although the
313 increased activity of arylsulphatase in the drained site might be expected to supress CH_4
314 fluxes (Freeman *et al*., 2007). As a proxy for a recovery from severe drought, our data show
315 that the activity of carbon-cycling enzymes remain high as a legacy of the previous aerobic
316 conditions. The only significant change was a reduction in arylsulphatase activity in
317 reprofiled ditches, which might therefore contribute to the enhanced CH_4 fluxes that are
318 sometimes seen following ditch blocking (e.g. Green *et al*., 2014, Cooper *et al*., 2014).
319

320 *4.4. Conclusions*

321 Our results suggest that drainage increased enzyme activity, specifically phenol
322 oxidase, β-glucosidase and arylsulphatase. Enhanced activities of these enzymes could result

in increased losses of greenhouse gases (Freeman *et al*., 2001a) and DOC (Freeman *et al*., 2001b). Following ditch blocking there was no evidence that enzyme activities were suppressed, apart from lowered arylsulphatsase activities in reprofiled ditches. The absence of an effect on enzyme activities may have been due to a legacy of enhanced enzyme activity that was stimulated through drainage, combined with the absence of any post-blocking change in soil moisture. Furthermore, any changes may have been mediated by the weather during the monitoring period.

It is clear that long term monitoring is necessary to elucidate exactly when peatland restoration will begin to influence the activity of extracellular enzymes, as changes can create both positive and negative feedbacks to ecosystem processes (Sinsabaugh, 2010). Finally, the fact that arylsulphatase activity responded to both drainage and ditch blocking lends some evidence to suggest that it may be more sensitive to environmental change than other hydrolases.

Acknowledgements

The authors thank Trystan Edwards, Helen Buckingham and Andrew Roberts of the National Trust for their involvement in the project. DOC and water table data were collected as part of the UK's Defra (Department for Environment, Food and Rural Affairs) SP1202 project. This is a long-term project, and the analysis of early post-restoration data in this paper should not be taken to imply ditch blocking effects on DOC and water tables will be the same over two to four years as they are over the first year. Dr Richard Smart was involved with field data collection on SP1202. This work was written up through the assistance of a Knowledge Economy Skills Scholarship (KESS) PhD awarded to Mike Peacock. We thank two anonymous reviewers for their constructive comments.

References

Alcamo, J., Moreno, J.M., Nováky, B., Bindi, M., Corobov, R., Devoy, R.J.N., Giannakopoulos, C., Martin, E., Olesen, J.E., Shvidenko, A. 2007: Europe. *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden and C.E. Hanson, Eds., Cambridge University Press, Cambridge, UK, 541-580.

Armstrong, A. 2000. DITCH: a model to simulate field conditions in response to ditch levels managed for environmental aims. *Agriculture, Ecosystems and Environment*, **77**, 179-192.

Armstrong, A., Holden, J., Kay, P., Francis, B., Foulger, M., Gledhill, S., McDonald, A.T., Walker, A., 2010. The impact of peatland drain-blocking on dissolved organic carbon loss and discolouration of water; results from a national survey. *Journal of Hydrology*, **381**, 112-120.

Billett, M.F., Charman, D.J., Clark, J.M., Evans, C.D., Evans, M.G., Ostle, N.J., Worrall, F., Burden, A., Dinsmore, K.J., Jones, T., McNamara, N.P., Parry, L., Rowson, J.G., Rose, R. 2010. Carbon balance of UK peatlands: current state of knowledge and future research challenges. *Climate Research*, **45**, 13-29.

Bonnett, S.A.F., Leah, R., Maltby, E., O'Connor, M. 2008. Short-term effectiveness of drain-blocking in suppressing enzymic peat decomposition and DOC export. Report for AONB Partnership's Peatscapes project. University of Liverpool.

373 Box, J.D., 1983. Investigation of the Folin-Ciocalteau phenol reagent for the determination of
374 polyphenolic substances in natural waters. *Water Research*, **17**, 511-525.
375
376 Bussell, J., Jones, D.L., Healey, J.R., Pullin, A. 2010. How do draining and re-wetting affect
377 carbon stores and greenhouse gas fluxes in peatland soils? CEE Review 08-012 (SR49).
378 Collaboration for Environmental Evidence. www.environmentalevidence.org/SR49.html.
379
380 Chow, A.T., Kanji, K.K., and Gao, K.K.T., 2003. Production of dissolved organic carbon
381 (DOC) and trihalomethane (THM) precursor from peat soils. *Water Research*, **37**, 4475-4485.
382
383 Cooper, M.D.A., Evans, C.D., Zielinski, P., Levy, P.E., Gray, A., Peacock, M., Norris, D.,
384 Fenner, N., Freeman, C. 2014. Infilled ditches are hotspots of landscape methane flux
385 following peatland re-wetting. *Ecosystems*, DOI 10.1007/s10021-014-9791-3 .
386
387 Dunn, D., Jones, T.G., Girard, A., Freeman, C. 2013. Methodologies for extracellular enzyme
388 assays from wetland soils. *Wetlands*, **34**, 9-17.
389
390 Fenner, N., Freeman, C., Reynolds, B., 2005. Hydrological effects on the diversity of
391 phenolic degrading bacteria in a peatland: implications for carbon cycling. *Soil Biology and*
392 *Biochemistry*, **37**, 1277-1287.
393
394 Fenner, N., and Freeman, C., 2011. Drought-induced carbon loss in peatlands. *Nature*
395 *Geoscience*, **4**, 895-900.
396
397 Freeman, C., Lock, M.A., Marxsen, J., Jones, S.E. 1990. Inhibitory effects of high molecular

398 weight dissolved organic matter upon metabolic processes in biofilms from contrasting rivers
399 and streams. *Freshwater Biology*, **24**, 159-166.
400
401 Freeman, C., Lock., M.A., and Reynolds, B., 1993. Fluxes of CO2, CH4, and N2O from a
402 Welsh peatland followed simulation of water table draw-down: potential feedback to climate
403 change. *Biogeochemistry*, **19**, 51-60.
404
405 Freeman, C., Liska, G., Ostle, N.J., Jones, S.E., and Lock, M.A., 1995. The use of
406 fluorogenic substrates for measuring enzyme activity in peatlands. *Plant and Soil*, **175**, 147-
407 152.
408
409 Freeman, C., Liska, G., Ostle, N.J., Lock, M.A., Hughes, S., Reynolds, B., and Hudson, J.,
410 1997. Enzymes and biogeochemical cycling in wetlands during a simulated drought.
411 *Biogeochemistry*, **39**, 177-187.
412
413 Freeman, C., Ostle, N., and Kang, H., 2001a. An enzymic 'latch' on a global carbon store.
414 *Nature*, **409**, 149.
415
416 Freeman, C., Evans, C.D., Monteith, D.T., Reynolds, B., Fenner, N., 2001b. Export of
417 organic carbon from peat soils. *Nature*, **412**, 785-786.
418
419 Gibson, H.S., Worrall., F., Burt, T.P., Adamson, J.K. 2009. DOC budgets of drained peat
420 catchments: implications for DOC production in peat soils. *Hydrological Processes*, **23**,
421 1901-1911.
422

423 Glatzel, S., Kalbitz, K., Dalva, M., Moore, T., 2003. Dissolved organic matter properties and
424 their relationship to carbon dioxide efflux from restored peat bogs. *Geoderma*, **113**, 397-411.
425
426 Green, S.M., Baird, A.J., Boardman, C.P., Gauci, V. 2014. A mesocosm study of the effect of
427 restoration on methane (CH_4) emissions from blanket peat. *Wetlands Ecology and*
428 *Management*, DOI 10.1007/s11273-014-9349-3.
429
430 Holden, J., Wallage, Z.E., Lane, S.N., McDonald, A.T., 2011.Water table dynamics in
431 undisturbed, drained and restored blanket peat. 2011. *Journal of Hydrology*, **402**, 103-114.
432
433 Hope, D., Billett, M.F., Cresser, M.S. 1994. A review of the export of carbon in river water:
434 fluxes and processes. *Environmental Pollution*, **84**, 301-324.
435
436 Kane, E.S., Mazzoleni, L.R., Kratz, C.J., Hribljan, J.A., Johnson, C.P., Pypker, T.G.,
437 Chimner, R. 2014. Peat porewater dissolved organic carbon concentration and lability
438 increase with warming: a field temperature manipulation experiment in a poor fen.
439 *Biogeochemistry*, DOI 10.1007/s10533-014-9955-4.
440
441 Karlsson, J., Byström, P., Ask, J., Persson, L., Jansson, M. 2009. Light limitation of nutrient-
442 poor lake ecosystems. *Nature*, **460**, 506-509.
443
444 Komulainen, V-M., Nykänen, H., Martikainen, P.J., Laine, J. 1998. Short-term effect of
445 restoration on vegetation change and methane emissions from peatlands drained for forestry
446 in southern Finland. *Canadian Journal of Forest Research*, **28**, 402-411.
447

448 Martikainen, P.J., Nykänen, H., Alm, J., and Silvola, J., 1995. Changes in fluxes of carbon
449 dioxide, methane and nitrous oxide due to forest drainage of mire sites of different trophy.
450 1995. *Plant and Soil*, **168-169**, 571-577.
451
452 McDonald, A.T., Mitchell, G.N., Naden, P.S., Martin, D.S.J., 1991. Discoloured Water
453 Investigations. Final Report to Yorkshire Water plc. 432 pp.
454
455 Peacock, M., Burden, A., Cooper, M., Dunn, C., Evans, C.D, Fenner, N., Freeman, C.,
456 Gough, R., Hughes, D., Hughes, S., Jones, T., Robinson, I., West, M., Zieliński, P., 2013.
457 Quantifying dissolved organic carbon concentrations in upland catchments using phenolic
458 proxy measurements. *Journal of Hydrology*, **477**, 251-260.
459
460 Pind, A., Freeman, C., and Lock, M.A., 1994. Enzymic degradation of phenolic materials in
461 peatlands – measurement of phenol oxidase activity. *Plant and Soil*, **159**, 227-231.
462
463 Price, J.S., Heathwaite, A.L., Baird, A.J. 2003. Hydrological processes in abandoned and
464 restored peatlands: an overview of management approaches. *Wetlands Ecology and*
465 *Management*, **11**, 65-83.
466
467 Ramchunder, S.J., Brown, L.E., Holden, J. 2012. Catchment-scale peatland restoration
468 benefits stream ecosystem biodiversity. *Journal of Applied Ecology*, **49**, 182-191.
469
470 Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidise and organic matter dynamics of soil. *Soil*
471 *Biology and Biochemistry*, **42**, 391-404.
472

473 Stewart, A.J.A., Lance, A.N., 1991. Effects of moor-draining on the hydrology and
474 vegetation of northern Pennine blanket bog. *Journal of Applied Ecology*, **28**, 1105-1117.
475
476 Tahvanainen, T., Haraguchi, A. 2013. Effect of pH on phenol oxidase activity on decaying
477 *Sphagnum* mosses. *European Journal of Soil Biology*, **54**, 41-47.
478
479 Toberman, H., Evans, C.D., Freeman, C., Fenner, N., White, M., Emmett, B.A., Artz, R.R.E.,
480 2008. Summer drought effects upon soil and litter extracellular phenol oxidase activity and
481 soluble carbon release in an upland *Calluna* heathland. *Soil Biology and Biochemistry*, **40**,
482 1519-1532.
483
484 Wallage, Z.E., Holden, J., McDonald, A.T., 2006. Drain blocking: an effective treatment for
485 reducing dissolved organic carbon loss and water discolouration in a drained peatland.
486 *Science of the Total Environment*, **367**, 811-821.
487
488 Wetzel, R.G., 1992. Gradient-dominated ecosystems: sources and regulatory functions of
489 dissolved organic matter in freshwater ecosystems. *Hydrobiologia*, **229**, 181-198.
490
491 Wilson, L., Wilson, J., Holden, J., Johnstone, I., Armstrong, A., Morris, M., 2011a. Recovery
492 of water tables in Welsh blanket bog after drain blocking: discharge rates, time scales and the
493 influence of local conditions. *Journal of Hydrology*, **391**, 377-386.
494
495 Wilson, L., Wilson, J., Holden, J., Johnstone, I., Armstrong, A., Morris, M., 2011b. Ditch
496 blocking, water chemistry and organic carbon flux: evidence that blanket bog restoration
497 reduces erosion and fluvial carbon loss. *Science of the Total Environment*, **409**, 2010-2018.

Worrall, F., Armstrong, A., and Holden, J., 2007. Short-term impact of peat drain-blocking on water colour, dissolved organic carbon concentration, and water table depth. *Journal of Hydrology*, **337**, 315-325.

Table 1. Pore water ion concentrations and standard errors (mg L^{-1}) for the undrained Bryn Du site and piezometers associated with open ditches at the Afon Du. Values are means from monthly sampling for March-July 2011 (n = 20), except for chloride, phosphate and sulphate where extra data were available; these ions were measured monthly March-November 2011 (n = 32). For each site and month n = 4. The only significant difference between sites was found for phosphate (Mann-Whitney U test, p = 0.001).

	Bryn Du	Afon Du
Sodium	4.00 ± 0.21	4.24 ± 0.28
Ammonium	0.01 ± 0.00	0.01 ± 0.01
Potassium	0.10 ± 0.04	0.29 ± 0.20
Magnesium	0.54 ± 0.09	0.59 ± 0.07
Calcium	0.34 ± 0.08	0.60 ± 0.1
Chloride	5.26 ± 0.47	5.29 ± 0.27
Bromide	0.01 ± 0.00	0.02 ± 0.00
Nitrate	0.01 ± 0.00	0.02 ± 0.02
Phosphate	0.00 ± 0.00	0.25 ± 0.05
Sulphate	1.02 ± 0.46	2.22 ± 0.83

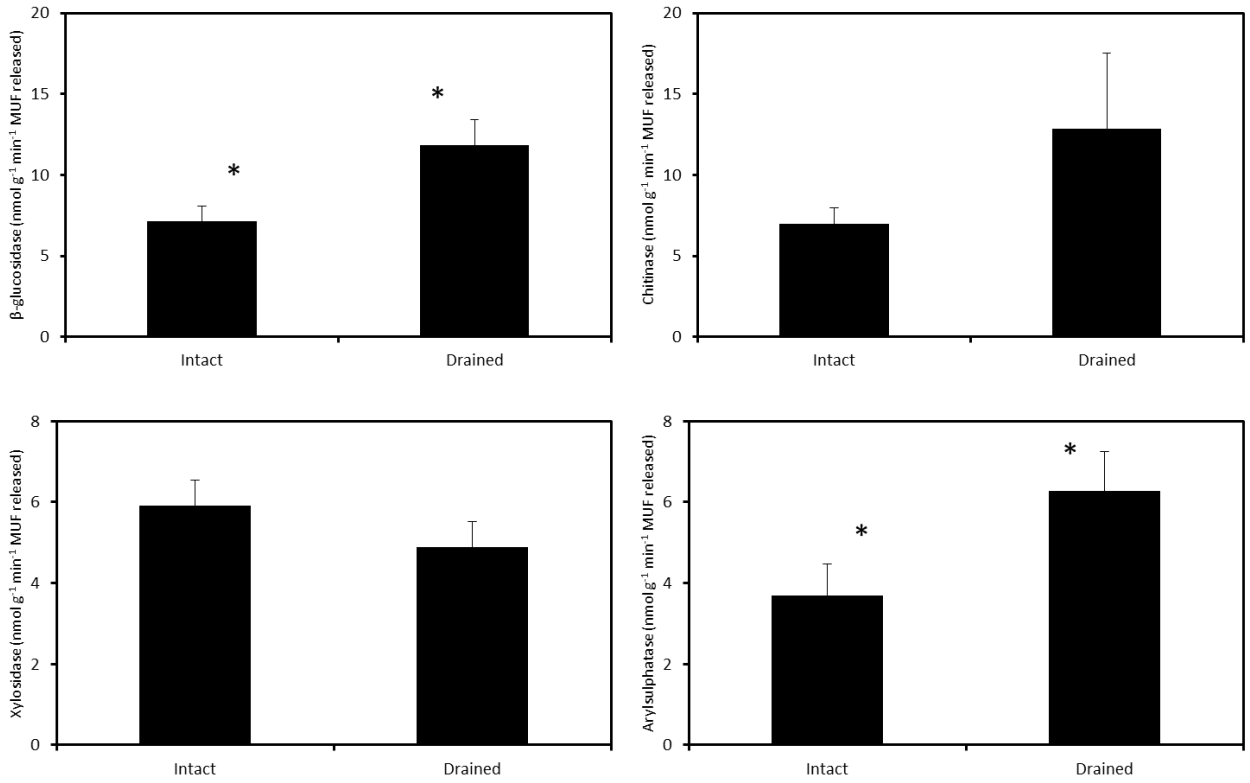


Figure 1. Mean hydrolase activities (nmol g^{-1} min^{-1} MUF released) for drained and undrained sites. Error bars show standard error of the mean. Data are mean from two sampling dates, $n = 8$ for each treatment, except chitinase which is from one sampling date ($n = 4$). There were significant differences (*) between sites for β-glucosidase (one-tailed t-test, $p = 0.01$) and arylsulphatase (one-tailed t-test, $p = 0.02$).

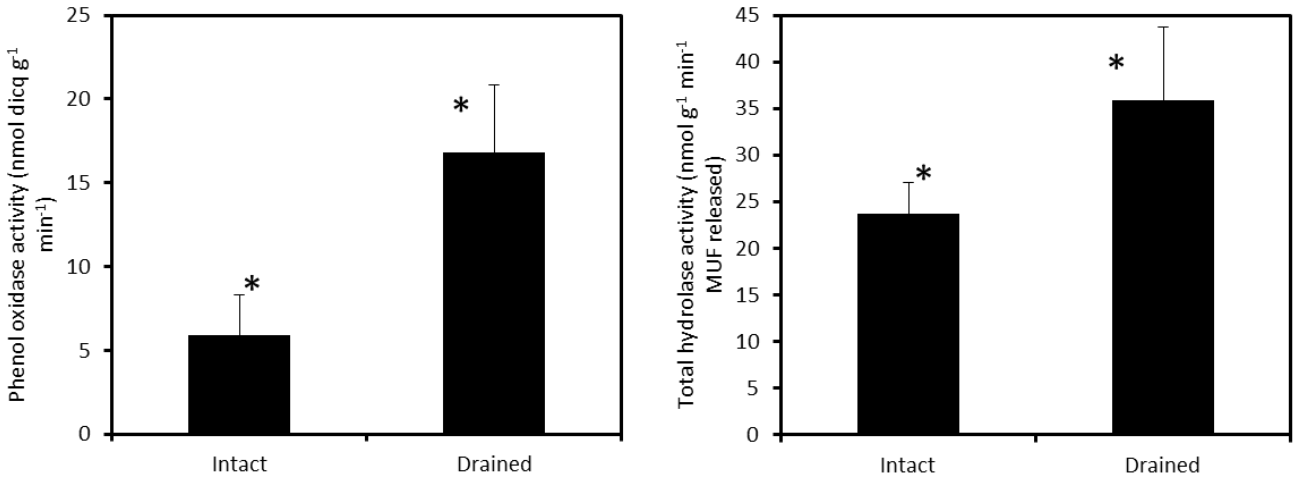


Figure 2. Mean phenol oxidase activity (nmol dicq g^{-1} min^{-1}) ($n = 8$) and total mean hydrolase activity (nmol g^{-1} min^{-1} MUF released) (i.e. sum of mean β-glucosidase, arylsulphatase, xylosidase and chitinase activity, $n = 28$) for drained and undrained sites. Error bars show standard error of the mean. The difference is significant (*) for phenol oxidase (one-tailed t-test, $p = 0.01$) and hydrolases (one-tailed t-test, $p = 0.01$).

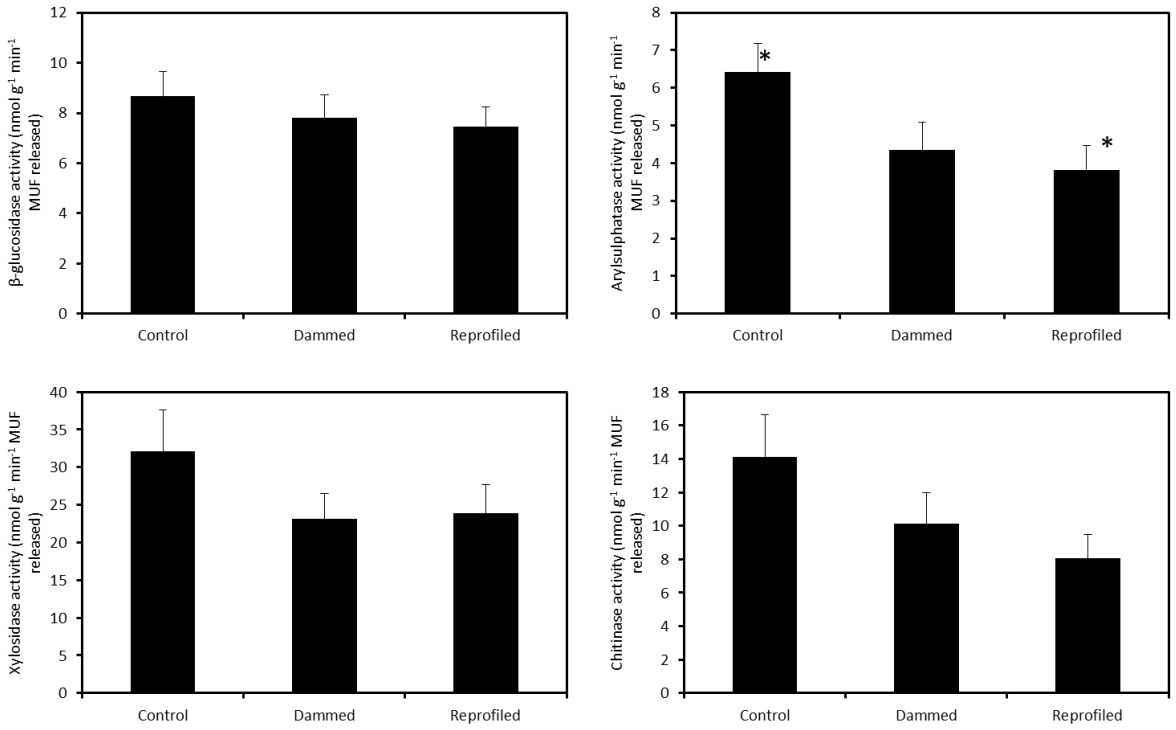


Figure 3. Mean hydrolase activities (nmol g^{-1} min^{-1} MUF released) for open control ditches, dammed ditches and reprofiled ditches. Errors bars show standard error of the mean. Data are mean of five (approximately monthly) sampling dates. n = 20 for each treatment. The only significant difference (*) was for arylsulphatase (repeated -measures ANOVA with Tukey HSD, $p < 0.05$).

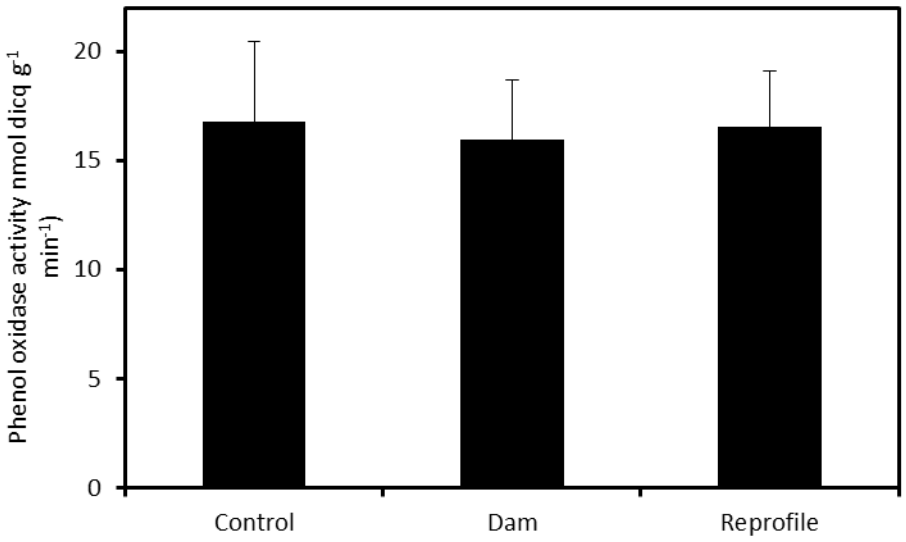


Figure 4. Mean phenol oxidase activity (nmol dicq g^{-1} min^{-1}) for open control ditches, dammed ditches and reprofiled ditches. Errors bars show standard error of the mean. Data are mean of five (approximately monthly) sampling dates. n = 20 for each treatment.

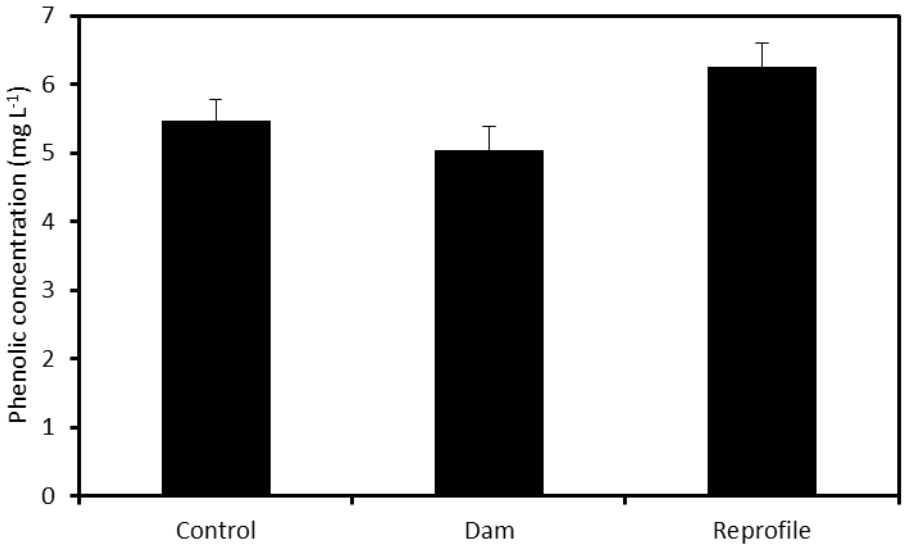


Figure 5. Mean phenolic concentrations (mg L^{-1}) for open control ditches, dammed ditches and reprofiled ditches. Errors bars show standard error of the mean. Data are mean of five (approximately monthly) sampling dates. *n* = 20 for each treatment.

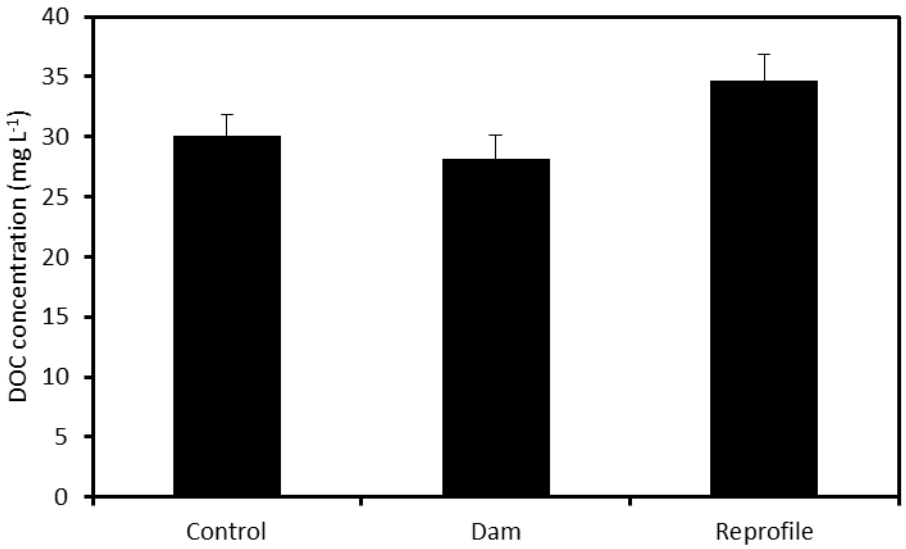


Figure 6. Mean DOC concentrations (mg L^{-1}) for open control ditches, dammed ditches and reprofiled ditches. Errors bars show standard error of the mean. Data are mean of five (approximately monthly) sampling dates. *n* = 20 for each treatment.

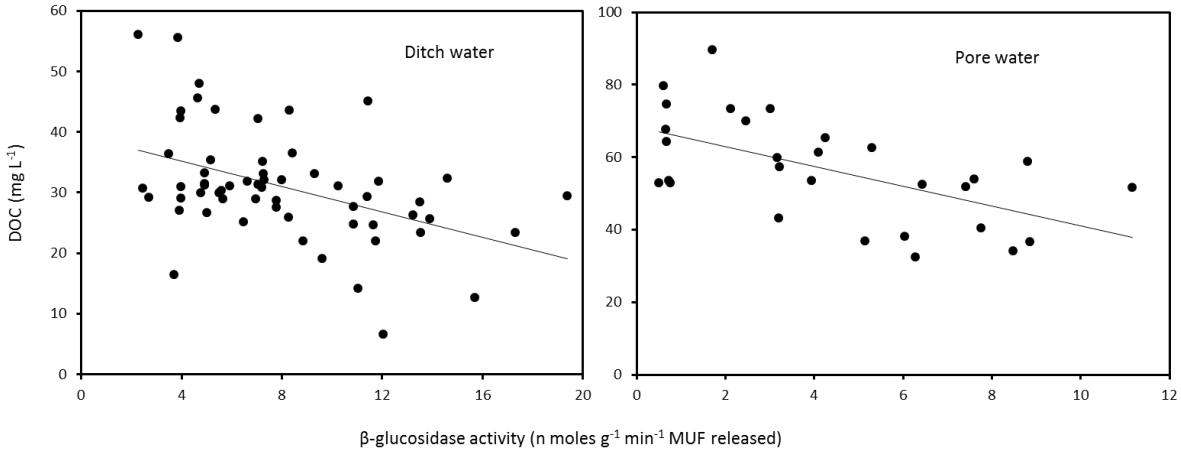


Figure 7. Relationship between β-glucosidase activity and DOC concentration in ditch and pore waters. Data are from five sampling trips between June and October 2011. For ditch water $n = 60$, $r^2 = 0.20$, $p < 0.05$, y = -1.05 x + 39.36. For pore water $n = 29$, $r^2 = 0.35$, $p < 0.01$, y = -2.74 x + 68.43.

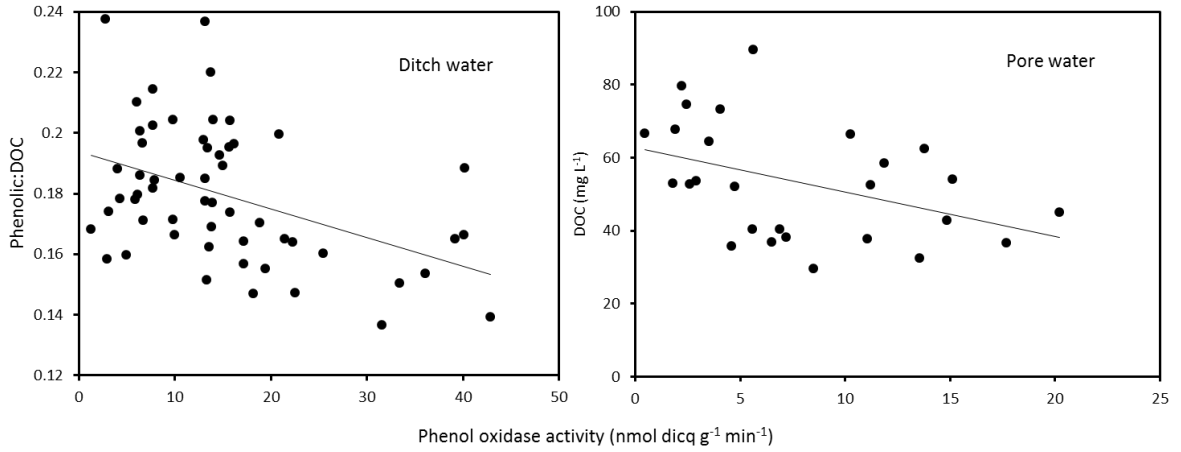
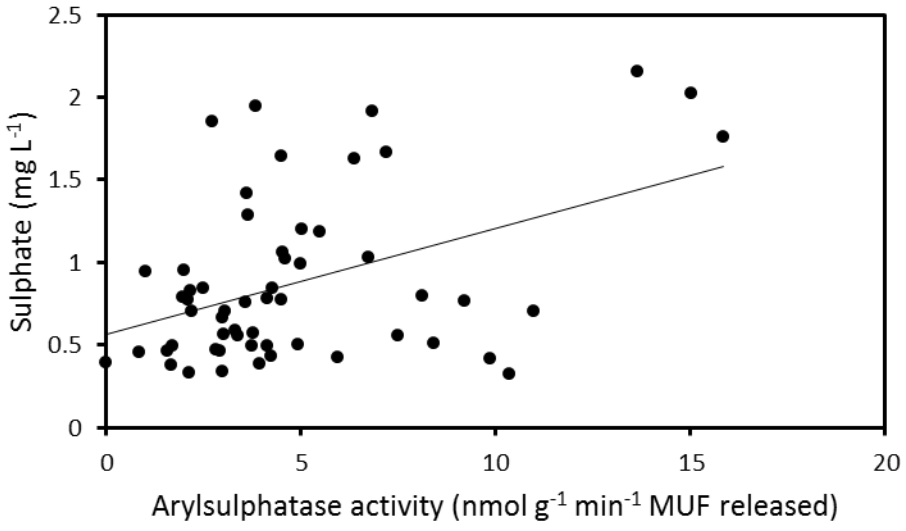


Figure 8. Relationship between phenol oxidase activity and the ratio of phenolic compounds to DOC in ditch waters, and the relationship between phenol oxidase activity and DOC concentration in pore waters. Data are from five sampling trips between June and October 2011. For ditch water $n = 56$, $r^2 = 0.19$, $p < 0.01$, y = -0.0009 x + 0.1939. For pore water $n = 27$, $r^2 = 17$, $p < 0.05$, y = -1.21 x + 62.64.



549 Figure 9. Relationship between arylsulphatase activity (nmol g^{-1} min^{-1} MUF released) and sulphate
550 concentration in ditch waters. Data are from five sampling trips between June and October 2011. n = 56, r^2 =
551 0.19, $p < 0.01$, $y = 0.064 x + 0.563$.
552