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Title: Evaluating the use of dominant microbial consumers (testate amoebae) as indicators of blanket peatland restoration

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Keywords: Drainage; Drain-blocking; Peatlands; Human disturbance; Restoration; Bioindicators; Testate amoebae.

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Abstract: Peatlands represent globally-important ecosystems and carbon stores. However, large areas of peatland have been drained for agriculture, or peat has been harvested for use as fuel or in horticulture. Increasingly, these landscapes are being restored through ditch blocking and rewetting primarily to improve biodiversity and promote peat accumulation. To date we have little knowledge of how these interventions influence the microbial communities in peatlands. We compared the responses of dominant microbial consumers (testate amoebae) to drainage ditch restoration relative to unblocked ditches in a UK upland blanket peatland (Migneint, North Wales). Two techniques were used for restoration: (i) dammed ditches with re-profiling; and (ii) dammed ditches with pools of open water behind each dam. Testate communities in the inter-ditch areas changed markedly over time and between treatments illustrating the potential of this group of organisms as indicators of blanket peatland restoration status. However, the responses of testate amoebae to peat rewetting associated with restoration were partially obscured by inter-annual variability in weather conditions through the course of the experiment. Although there was considerable variability in the response of testate amoebae communities to peatland drain blocking, there were clearly more pronounced changes in samples from the dammed and reprofiled treatments including an increase in diversity, and the appearance of unambiguous wet-indicator species in relatively high abundances (including *Amphitrema stenostoma*, *Archerella flavum*, *Arcella discoides* type, *Diffflugia bacillifera* and *Diffflugia bacillarum*). This reflects a shift towards overall wetter conditions across the site and the creation of new habitats. However, water-table was not a significant control on testate amoebae in this case, suggesting a poor relationship between water table and surface moisture in this sloping blanket peatland. Our findings highlight the potential of testate amoebae as bioindicators of peatland restoration success; however, there is a need for caution as mechanisms driving change in the microbial communities may be more complex than first assumed. Several factors need to be taken into account when implementing biomonitoring studies in peatlands

including: (i) the natural variability of the peatland ecosystem under changing weather conditions; (ii) any disturbance connected with the restoration procedures; and (iii) the timescales over which the ecosystem responds to the management intervention. Our results also suggest an indicator species approach based on population dynamics may be more appropriate for biomonitoring peatland restoration than examining changes at the community level.

Response to Reviewers: Please see separate file.

1 **Evaluating the use of dominant microbial consumers** 2 **(testate amoebae) as indicators of blanket peatland** 3 **restoration**

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27 **Key-words:** Drainage; Drain-blocking; Peatlands; Human disturbance; Restoration; Bioindicators; Testate
28 amoebae.

29 Manuscript R1 for *Ecological Indicators*

30 **Highlights**

- 31 • Testate amoebae communities changed after ditch blocking in a blanket peatland.
- 32 • Significant drivers of change include type of ditch-blocking treatment and time.
- 33 • Pronounced changes in diversity across site relate to creation of new habitats.
- 34 • First appearance of key wet indicator taxa is after ditch blocking on the site.
- 35 • Water table is not controlling testate amoeba communities in this blanket peatland.

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47 **Abstract**

48 Peatlands represent globally-important ecosystems and carbon stores. However, large areas of peatland have
49 been drained for agriculture, or peat has been harvested for use as fuel or in horticulture. Increasingly, these
50 landscapes are being restored through ditch blocking and rewetting primarily to improve biodiversity and
51 promote peat accumulation. To date we have little knowledge of how these interventions influence the
52 microbial communities in peatlands. We compared the responses of dominant microbial consumers (testate
53 amoebae) to drainage ditch restoration relative to unblocked ditches in a UK upland blanket peatland
54 (Migneint, North Wales). Two techniques were used for restoration: (i) dammed ditches with re-profiling;
55 and (ii) dammed ditches with pools of open water behind each dam. Testate communities in the inter-ditch
56 areas changed markedly over time and between treatments illustrating the potential of this group of
57 organisms as indicators of blanket peatland restoration status. However, the responses of testate amoebae to
58 peat rewetting associated with restoration were partially obscured by inter-annual variability in weather
59 conditions through the course of the experiment. Although there was considerable variability in the response
60 of testate amoebae communities to peatland drain blocking, there were clearly more pronounced changes in
61 samples from the dammed and reprofiled treatments including an increase in diversity, and the appearance
62 of unambiguous wet-indicator species in relatively high abundances (including *Amphitrema stenostoma*,
63 *Archerella flavum*, *Arcella discoides* type, *Diffflugia bacillifera* and *Diffflugia bacillarum*). This reflects a
64 shift towards overall wetter conditions across the site and the creation of new habitats. However, water-table
65 was not a significant control on testate amoebae in this case, suggesting a poor relationship between water
66 table and surface moisture in this sloping blanket peatland. Our findings highlight the potential of testate
67 amoebae as bioindicators of peatland restoration success; however, there is a need for caution as
68 mechanisms driving change in the microbial communities may be more complex than first assumed. Several
69 factors need to be taken into account when implementing biomonitoring studies in peatlands including: (i)
70 the natural variability of the peatland ecosystem under changing weather conditions; (ii) any disturbance
71 connected with the restoration procedures; and (iii) the timescales over which the ecosystem responds to the
72 management intervention. Our results also suggest an indicator species approach based on population

73 dynamics may be more appropriate for biomonitoring peatland restoration than examining changes at the
74 community level.

75 **1. Introduction**

76 Peatlands represent globally important habitats and carbon stores which are under threat from human
77 activity and climate change (Holden et al., 2004; Charman et al., 2013; Swindles et al., 2015a). They store
78 approximately one third of global soil carbon, whilst covering only approximately 3% of the land and
79 freshwater surface (Holden, 2005). However, human activity has degraded peatlands through drainage and
80 harvesting of peat in many parts of the world including NW Europe, North America, Russia and SE Asia
81 (e.g. Baldock et al., 1984; Holden et al., 2004; Hooijer et al., 2010, 2012). This has led to recent efforts to
82 re-wet peatlands in order to restore active peat-forming plant communities and promote carbon sequestration
83 (e.g. Ramchunder et al., 2009; Parry et al., 2014).

84 Blanket peatlands are found in hyperoceanic regions such as those of northern Europe, Alaska,
85 Newfoundland, Tasmania, New Zealand, South America and Eastern Russia (Gallego-Sala and Prentice,
86 2012; Parry et al., 2014). There has been much research interest in blanket peatlands as it has been suggested
87 they are at risk of progressive erosion and vegetation change as a result of climate change (Gallego-Sala et
88 al., 2010; Li et al., 2015). In the UK, large areas of blanket peatland have become degraded from the effects
89 of atmospheric pollution (Smart et al., 2010), peat extraction (Cruickshank et al., 1995), artificial drainage
90 (Holden et al., 2006), grazing (Ellis and Tallis, 2001), prescribed burning and wildfire (Davies et al., 2008),
91 afforestation (Wellock et al., 2011), and the construction of buildings and access tracks (Holden, 2005).
92 Since the 1940s, many upland blanket peatlands in the UK have been drained through the excavation of
93 ditches which aimed to lower water-table levels and increase land productivity (Holden et al., 2006). The
94 excavation of ditches in blanket peatlands has driven a series of ecosystem-level changes to biodiversity,
95 hydrology, and carbon sequestration, and in some locations has increased the amount of dissolved organic
96 carbon (DOC) flux to water courses at some sites (Holden et al., 2006; Mitchell and McDonald, 1995;
97 Ramchunder et al., 2012; Parry et al., 2014). To reduce the impacts of such management practices, ditch

98 blocking with dams is now a commonplace restoration technique. The blocking of ditches is thought to lead
99 to shallower water tables in peatlands, which can have positive effects on ecological diversity and carbon
100 sequestration (e.g. Beadle et al., 2015). However, the timescales involved for any effects to become apparent
101 after re-wetting are poorly understood, and the effects may be subtle (e.g., within the boundaries of natural
102 variability). As large-scale field experiments are unlikely to exceed two-five years duration due to the
103 availability of financial resources, bioindicators can be used to detect small changes that may not be
104 apparent in hydrological or biogeochemical data (i.e., instrument-based monitoring).

105 There have been several studies examining the effects of peatland restoration on different groups of
106 organisms including beetles, rotifers, microcrustaceans and macroinvertebrates (Van Duinen et al., 2003,
107 2006; Watts et al., 2008; Więcek et al., 2013; Beadle et al., 2015). Testate amoebae are a polyphyletic group
108 of amoeboid protists characterised by the presence of a shell (test), and represent an important component of
109 the soil microbial community. Testate amoebae are dominant microbial consumers in peatlands, representing
110 5–30% of the total microbial biomass, and can have a major influence on the ecological functioning of
111 peatland ecosystems through nutrient cycling (Gilbert et al., 1998; Mitchell et al., 2003; Jassey et al., 2014).
112 They have also been shown to be sensitive hydrological indicators in peatlands (Charman and Warner, 1992;
113 Tolonen et al., 1994; Swindles et al., 2009, 2015b; Turner et al., 2012). The response of testate amoebae to
114 peatland restoration has been investigated previously based on analysis of cores from peat accumulated post-
115 restoration (Buttler et al., 1996; Jauhiainen, 2002; Davis and Wilkinson 2004; Valentine et al., 2013). There
116 have also been some experimental studies examining the response of testate amoebae to hydrological change
117 (e.g. Marcisz et al., 2014a,b). However, to date, there have not been any studies on blanket peatlands, and,
118 critically, no time-series investigations of changes in surface testate amoebae before and after management
119 intervention have been carried out relative to control systems. Here we investigate the responses of surface
120 testate amoeba communities to restoration treatments in a UK upland blanket peatland (Migneint, North
121 Wales). We examine changes in community composition, ecology, diversity and use these data to examine
122 their potential as bioindicators of peatland restoration.

124 **1.1 Hypotheses**

125 We tested the following three hypotheses:

126 [H1] Ditch blocking drives a change in testate amoebae at the community-level owing to the restoration
127 activity.

128 [H2] Key wet-indicator taxa (e.g. wet indicators from the genera *Arcella* and *Archerella*) increase in
129 response to restoration;

130 [H3] An increase in the diversity of testate amoebae is observed following restoration reflecting the greater
131 variety of habitats.

132 **2. Method**

133 **2.1 Field site**

134 The study was undertaken in part of the Migneint in North Wales (Figure 1) close to Ffynnon Eidda (52° 58'
135 06.35" N, 3° 50' 28.67" W). Under the UK National Vegetation Classification (NVC) (Rodwell, 1991), the
136 peatland is a mix of M19 *Calluna vulgaris* – *Eriophorum vaginatum*, and M18 *Erica tetralix* – *Sphagnum*
137 *papillosum* blanket bog. The Migneint has been damaged by drainage, burning, over-grazing and, to a lesser
138 extent, afforestation. Maps compiled by Natural Resource Wales from aerial photography show that most of
139 the area was artificially drained between the 1940s and 1970s. Peat depth across the sampling area ranges
140 from 0.54 – 2.39 m, with a pH (H₂O) of 3.62 – 3.80, bulk density of 0.08 – 0.11 g cm⁻³, loss on ignition of
141 98.8 – 99.7 % and a C to N ratio of 30.0 – 36.6 (depending on depth) (Green et al., 2016). Average annual
142 rainfall is 2200–2400 mm yr⁻¹, and average January and July temperatures are 2.2°C and 12.8°C,
143 respectively.

144 **2.2 Treatments**

145 Twelve ditches, which run obliquely (at an angle of *c.* 20°) to the hillslope gradient from east (ditch 1) to
146 west (ditch 12) across the site (Figure 1), were selected for detailed study. The ditches were allocated to one

147 of three treatments, each with four replicates: (i) control (unblocked), (ii) 're-profiled' (dammed and re-
148 profiled), and (iii) dammed (dammed with pools of open water behind each dam) (Table 1). The ditches had
149 an average spacing of 16 m (range 11 to 26 m), a mean length of 99 m (range 84 to 107 m) and were on a
150 mean gradient of 4.5° (range 3.9 to 5.1°). Treatments were allocated taking into account measured pre-
151 blocking discharge rate, catchment area, surface features and position on hillslope (i.e. how the blocking
152 might affect inter-grip areas).

153 **2.3 Routine monitoring**

154 Base-line data collection at the site started on the 18th August 2010, after all the field equipment was
155 installed. All equipment was removed on the 2nd February 2011, prior to the damming/re-profiling of eight
156 of the experimental ditches. Re-installation of the equipment was completed by 23rd of February 2011, after
157 which monitoring resumed.

158 **2.4 Measurement of meteorological conditions**

159 A Davis Vantage Pro2 automatic weather station (AWS) monitored air temperature (°C) and rainfall (mm)
160 at 60-minute archive intervals (Figure 2).

161 **2.5 Measurement of water-table depths**

162 As part of a larger study of the greenhouse gas exchanges and hydrology of the site, twenty-four manual
163 dipwells were installed to monitor water-table depth in the inter-ditch zones across the field site (Figure 2).
164 Dipwells were made from 32 mm (outside diameter) × 3.5 mm (wall thickness) × 1000 mm (length)
165 polyvinyl chloride (PVC) pipe, with 8-mm diameter holes drilled at 100 mm intervals along four lines
166 running lengthwise along the pipe. These were located at 2 metres from the ditch to the west ($DW_{x,2W}$) and 2
167 metres from the ditch to the east ($DW_{x,2E}$) (Green et al., 2016).

168 **2.6 Pore-water chemical composition**

169 Twenty four piezometers for pore-water collection were installed across the site (deployed in pairs). Pore-
170 water electrical conductivity (unfiltered samples) was determined using a Jenway 4320 conductivity meter

171 (Bibby Scientific Ltd, Staffordshire, UK). Analytical grade standards were analysed at regular intervals to
172 check instrumental drift. pH (unfiltered samples) was analysed by titration using a 0.01N H₂SO₄ solution on
173 Metrohm 888 Titrando (Metrohm UK Ltd, Cheshire, UK) (two buffer standards of pH 4 and 7) (Figure 2).

174 **2.7 Vegetation survey**

175 Four vegetation surveys (October 2010, October 2011, September 2012, September 2013) were undertaken
176 to quantify the abundance (nested frequency) of the plant species in permanent 1 × 1 m quadrats across the
177 site. There were 48 permanent quadrats, 16 associated with each management type, and four associated with
178 each ditch. The quadrats were situated equal distances apart within each inter-ditch area. To determine
179 nested frequency each quadrat divided into 10 × 10 cm squares and presence-absence of each plant species
180 of interest was measured within those squares. We used data from the quadrats nearest the testate amoeba
181 sampling points.

182 **2.8 Sampling of testate amoebae**

183 Testate amoebae sampling dates corresponded to dates of routine site monitoring. The sampling dates were
184 116 (t_0 - 15/10/2010) and 6 (t_1 - 02/02/2011) days before, and 63 (t_2 - 12/04/2011) and 234 (t_3 - 30/09/2011)
185 days after ditch blocking was carried out (on 08/02/2011). These sampling dates were chosen to fit around
186 the mandatory monitoring and maintenance of the site. A further set of samples were taken 771 days after
187 ditch blocking (20/03/2013) to obtain a sample following assumed stabilisation of the blocked ditches and
188 peat surfaces.

189 Moss samples of approximately 5 cm³ were sampled from an undisturbed plot immediately beside each
190 manual dipwell ($n = 24$) and placed into Ziplock bags. All samples were returned immediately to the
191 laboratory and stored at 4°C prior to further analysis. Testate amoebae were prepared using a modified
192 version of the standard method (Booth, 2010). Sub-samples of the uppermost *Sphagnum* (containing mostly
193 live testate amoebae) were sieved through a 300-µm sieve and no fine-sieving was carried out following
194 Payne (2009). The samples were stored in deionised water. Testate amoebae containing cytoplasm (i.e. those
195 that were recently alive) were counted under transmitted light at ×200–400 and identified using morphology,

196 composition, size and colour to distinguish taxa. At least 150 specimens (mean = 173, min = 150, max =
197 225) were counted per sample to ensure a statistically significant count was achieved (Patterson and
198 Fishbein, 1989). The taxonomy uses a morphospecies approach in certain circumstances, where a
199 designation that includes other species has been classed as a 'type'. Testate amoebae were identified using
200 illustrated guides (Ogden and Hedley, 1980; Charman et al., 2000).

201 **2.9 Statistical analysis**

202 Statistical analyses were performed using R version 2.15.1 (R Core Team, 2013). Nonmetric
203 Multidimensional Scaling (NMDS) and Redundancy Analysis (RDA) were used to investigate the response
204 of testate amoebae communities using the 'vegan' package (v. 2.0-5) in R (v. 2.15.1). NMDS using the
205 Bray-Curtis dissimilarity index was used to identify the important axes of variation in the data (e.g.,
206 Legendre and Legendre, 1998). The analysis 'stress' was recorded in several runs to ensure a robust analysis
207 was achieved. Ordination hulls were used to demarcate treatment category on the NMDS plots.
208 Environmental variables were fitted to the solution post-hoc using the Envfit procedure with 999
209 permutations. Analysis of Similarity (ANOSIM) and permutational MANOVA (PERMANOVA) were
210 undertaken on the testate amoebae data to determine the significance of treatment and time factors (Bray
211 Curtis dissimilarities, 9999 permutations). Data were transformed by square root prior to ANOSIM and
212 PERMANOVA analysis. A hierarchical cluster analysis using the Bray Curtis dissimilarity index was also
213 carried out to determine the similarity-dissimilarity of the samples.

214 Gradient lengths were determined using Detrended Correspondence Analysis (DCA) and, as they were
215 found to be non-linear, species data were transformed using the Hellinger distance prior to direct ordination
216 (Legendre and Gallagher, 2001). Redundancy Analysis (RDA) was used to explore the relationships
217 between testate amoebae and environmental variables. A series of partial RDAs was used for variance
218 partitioning, and Monte-Carlo permutation tests (999 permutations) were used to test statistical significance.
219 The Shannon Diversity Index was calculated for each sample to examine the faunal diversity (e.g.,
220 Magguran, 1988) in addition to species richness and evenness. Water-table predictions from the testate
221 amoebae data were carried out using the transfer function of Turner et al. (2013). A suite of water-table

222 metrics were calculated and included in the multivariate analyses: (i) water-table depth for each well on the
223 day of sampling for testate amoebae; (ii) averages, maximum and minimum of the two, three, four, and five
224 water-table readings before sampling, and (iii) seasonal averages.

225 **3. Results**

226 In total, fifty one testate amoeba taxa were identified from 31,158 individuals (Figure 3). The most
227 commonly occurring testate amoeba taxa at the site include *Nebela tincta*, *Corythion-Trinema* type,
228 *Euglypha ciliata* type, *Assulina muscorum* and *Cryptodifflugia oviformis*. The taxa with maximum
229 occurrences include *Cryptodifflugia oviformis*, *Nebela tincta*, *Corythion-Trinema* type, *Nebela militaris* and
230 *Nebela flabellulum* (Figure 3). The Shannon diversity of the communities varies between 0.92 and 2.86 and
231 increases in all treatments after t_2 (Figure 4). Water-table predictions using the transfer function of Turner et
232 al. (2013) suggest the site has become wetter after restoration, with the most pronounced changes occurring
233 in the samples from the dammed treatment (Figures 4 and 5). The application of a testate-amoeba based
234 transfer function highlights changes in relative wetness indicated by the changing testate amoebae
235 communities (Supplementary material 1). However, transfer functions currently have little predictive skill
236 for determining the absolute magnitudes of short-term water-table changes in blanket peatlands, which is not
237 what they were designed to do (also see Swindles et al., 2015).

238 The following environmental variables were significantly associated with the community dataset (Figure 5):
239 time ($p < 0.0001$), rainfall ($p < 0.001$) and treatment ($p < 0.05$). ANOSIM showed that community
240 composition was significantly different with time (all treatments combined) ($R = 0.395$, $p = 0.0001$) and
241 treatment (all times combined) ($R = 0.119$, $p = 0.0001$). Treatment ($R = 0.127$) and time ($R = 0.252$) were
242 also significant when only t_{2-4} (after restoration samples) were analysed ($p = 0.0001$ in both cases). There
243 was no significant difference (95% level) between the community compositions at t_0 (t_0 : $R = 0.109$, $p =$
244 0.053). However, the difference between the communities under the different treatments changed through
245 time, becoming most significant at t_4 (t_1 : $R = 0.125$, $p = 0.036$; t_2 : $R = 0.071$, $p = 0.142$; t_3 : $R = 0.117$, $p =$
246 0.031 ; t_4 : $R = 0.162$, $p = 0.007$). PERMANOVA corroborated the results of ANOSIM: community
247 composition was significantly different with time (all treatments combined) ($F = 10.35$, $p = 0.0001$) and

248 treatment (all times combined) ($F = 4.923$, $p = 0.0001$). There was no significant difference (95% level)
249 between the community compositions at t_0 (t_0 : $F = 1.551$, $p = 0.082$). Treatment ($F = 3.55$) and time ($F =$
250 6.04) were also significant when only t_{2-4} (after restoration samples) were analysed ($p = 0.0001$ in both
251 cases). However, the difference between the communities under the different treatments changed through
252 time, becoming most significant at t_4 (t_1 : $F = 2.020$, $p = 0.021$; t_2 : $F = 1.440$, $p = 0.126$; t_3 : $F = 1.618$, $p =$
253 0.029 ; t_4 : $F = 2.027$, $p = 0.007$). Treatment*time interaction was non-significant for both ANOSIM and
254 PERMANOVA analyses. Cluster analysis also suggested a clear division before and after restoration in the
255 community characteristics (Supplementary material 2).

256 Monte Carlo permutation tests highlighted the significance of RDA axis one ($p = 0.038$) and all canonical
257 axes ($p = 0.006$) (Supplementary material 3). Axis one explained 43.2% of the species-environment
258 relationship whereas axis two explained 20.8%. A pRDA including all continuous environmental and
259 ordinal variables suggested that the following variables were most important: time (49.3%, $p < 0.001$),
260 temperature (10.5%, $p < 0.001$), treatment (6.2%, $p < 0.05$) and *Sphagnum* abundance (5.5%, $p < 0.05$). A
261 pRDA only including the continuous variables (i.e., not including treatment or time) revealed the following
262 significant environmental variables: rainfall (25.3%, $p < 0.01$), *Sphagnum* abundance (20.1%, $p < 0.01$) and
263 temperature (16.3%, $p < 0.05$). It is noteworthy that none of the water-table depth metrics were deemed
264 significant controls on the testate amoebae communities by either NMDS or RDA.

265 The response of testate amoebae communities at the site is complex. There is a very clear management
266 effect in some of the dammed and reprofiled samples including the first appearance of key wet indicator taxa
267 in high numbers by t_3 or t_4 (*Amphitrema stenostoma*, *Archerella flavum*, *Arcella discoides* type, *Diffflugia*
268 *bacillifera* and *Diffflugia bacillarum*). This suggests that changes in the testate amoebae communities are at
269 least partially driven by management intervention. However, some of the responses are more muted or
270 ambiguous, or in some cases there is little discernible effect (Figures 5 and 6). Furthermore, key wet
271 indicator taxa also appear in the control samples (albeit in smaller numbers) suggesting that there is
272 interaction between the ditches, or that management has had wider effects across the site. The interaction
273 between ditches may be supported by the observation that no wet indicators appear in ditch 7.2 - a control

274 with the least number of near-by ditches with blocking treatments. Nevertheless, a major increase in wet
275 indicator taxa in some of the reprofiled and dammed treatment plots after management intervention is
276 apparent (Figure 6).

277 Table 4 illustrates the overall changes in testate amoeba communities between t_0 and t_4 (diversity, richness,
278 evenness and abundance of wet indicator taxa). It is clear that when the data are aggregated, overall changes
279 have been greater in the re-wetted plots compared with the controls. There are also some key differences
280 between treatments; there is a greater increase in diversity, richness and evenness in the re-profiled than the
281 dammed treatments, whereas the abundance of wet indicator taxa is greater in the dammed treatments.

282 **4. Discussion**

283 Our analysis suggests that although there is high variability between sampling points, we can accept all three
284 hypotheses based on multivariate statistical analysis, the appearance of wet indicators, and changes in
285 community diversity:

286 [H1] Ditch blocking drives a change in testate amoebae at the community-level owing to the restoration
287 activity. *Accept*: there are clear changes at the community-level at least partly driven by peatland restoration
288 as illustrated by the NMDS, ANOSIM and PERMANOVA results (see Figure 5, section 3).

289 [H2] Key wet-indicator taxa (e.g. wet indicators from the genera *Arcella* and *Archerella*) increase in
290 response to restoration. *Accept*: wet-indicators appear after restoration including *Amphitrema stenostoma*,
291 *Arcella discoides* type, *Archerella flavum*, *Diffugia bacillifera* and *Diffugia bacillarum* (see Figure 6).

292 [H3] An increase in the diversity of testate amoebae is observed following restoration reflecting the greater
293 variation in habitats. *Accept*: Diversity increases in many of the sample plots after peatland restoration (see
294 Figure 4, Table 4).

295 Previous studies from other sites in Europe have shown that testate amoebae can be used for monitoring
296 habitat changes after restoration of cutover peatlands (Buttler et al., 1996; Jauhiainen, 2002; Davis and
297 Wilkinson 2004; Valentine et al., 2013). However, these studies have focussed on subfossil testate amoebae

298 in cores taken from peat formed following restoration; which may not be a practical approach for many
299 blanket peatlands owing to slow peat accumulation rates. Instead, we have focussed on generating a time
300 series of changes in testate amoebae communities through sampling of surface vegetation. To our
301 knowledge, this work represents the first study examining the responses of testate amoebae to management
302 in a blanket peatland.

303 We have shown that there have been distinct changes in testate amoebae communities in response to
304 peatland management efforts; however, the changes have been complex in that some locations show large
305 changes in community composition, whereas some do not. The complexities at the site are probably due to
306 several key factors:

- 307 1. The natural variability of the peatland ecosystem under changing weather conditions;
- 308 2. Disturbance of the sites connected with the restoration procedures, including trampling and
309 movement of machines. This may also include redistribution of testate amoebae across the site and
310 the creation of new micro-habitats;
- 311 3. The site is generally very wet with a high degree of overland flow. The management efforts may
312 have altered the surface hydrology leading to hydrological interaction of some of the ditches from
313 ponding up of ditches and subsequent overland flow. In addition, aquatic testate amoebae from the
314 pools behind the dams may have been transported into the inter-ditch areas during storm events,
315 partly complicating the signal from the inter-ditch areas.
- 316 4. A longer timescale may be needed to fully understand how the ecosystem responds to the
317 management intervention.

318 The appearance of wet-indicator taxa in the control as well as treatment samples may relate to: (i) the
319 general diversification of ecohydrological habitats across the site following management, leading to wider-
320 site colonisation by certain taxa; or (ii) hydrological modification of the site leading to the redistribution of
321 testate amoebae by overland flow. The finding that water-table depth is not a primary driver of change in the
322 testate amoebae communities contrasts with studies from raised bogs (e.g. Swindles et al., 2009; Turner et
323 al., 2013; Swindles et al., 2015c) and probably reflects a poor relationship between surface moisture and

324 water-table in this sloping blanket peatland. This poor relationship may relate to water tables being generally
325 shallow across the site even before blocking and saturated conditions leading to frequent overland flow.

326 Our study illustrates the importance of having controls before and after management intervention in
327 biomonitoring studies so that the natural variability of the site under changing weather conditions (inter and
328 sub annual) can be taken in account. Our results also suggest an indicator species approach may be more
329 appropriate for biomonitoring the early days of peatland restoration than examining change at the
330 community level. We contend that caution is needed when using biomonitors of peatland restoration
331 including testate amoebae. It could be argued that the actual sample size for any treatment here is $n = 4$ as
332 the replication within a treatment is arguable pseudo-replication. With this small sample size only the largest
333 differences will be statistically significant. In future, larger experiments (requiring considerable funding) or
334 a larger number of similar-sized experiments can be subjected to further statistical analysis and will
335 hopefully allow firmer conclusions to be drawn.

336 Our findings that peatland restoration drives significant changes in testate amoebae populations have
337 parallels from several previous studies that have documented responses amongst other aquatic biological
338 groups (Beadle et al., 2015). For example, Goodyer (2014) reported that desmid diversity recovered from a
339 situation of low richness in drained peatlands to become more similar to nearby intact peatlands, although
340 the timescales of 12 years were longer than we observed for the testate amoebae in this study. Similarly,
341 aquatic macroinvertebrates have been shown to exhibit sensitivity to peatland restoration, with Van Duinen
342 et al. (2003) highlighting how the invertebrate fauna of bogs with remnants of peat cuttings was different
343 (higher richness, and wider compositional variation) from re-wetted peats. Re-wetted peatlands hosted fauna
344 that were more characteristic of undamaged raised bogs, and there were clear successional changes over
345 time in the rewetted peatland invertebrate communities, similar to our findings for testate amoebae. These
346 different studies show that peatland restoration elicits clear responses amongst a range of biological groups,
347 yet the processes by which these groups interact to determine the nature of ecological outcomes still needs to
348 be understood before any particular group can be proposed as definitive indicators of restoration success.
349 For example, we need to understand the nature of species interactions between the testate amoebae and their

350 predators such as Chironomidae (Mieczan et al., 1995), which are often the dominant group of
351 macroinvertebrates in peatland pools (Beadle et al., 2015). This would allow us to understand the relative
352 roles of biotic (i.e., role in food webs) vs. abiotic (i.e., hydrological) drivers of testate communities when
353 trying to interpret their response to restoration.

354 Testate amoebae are highly abundant and represent a major group of predators in the microbial food web of
355 peatlands (Gilbert et al. 1998; Ogden and Hedley 1980; Jassey et al., 2012). They can exert important effects
356 on the ecological functioning of peatlands in their role as dominant microbial consumers (Jassey et al.,
357 2014). New research suggests that mixotrophic testate amoebae play an important role in modulating
358 peatland C cycle responses (fixation of C) to climate warming (Jassey et al., 2015). Future work should
359 consider the effects of changes in testate amoebae driven by peatland restoration, and how this affects the
360 functioning of the wider microbial ecosystem and carbon cycling.

361 **Conclusions**

362 We examined the responses of dominant microbial consumers (testate amoebae) to restoration treatments in
363 a UK blanket peatland. We found that both time and treatment had a statistically-significant effect on
364 community composition; however, the testate amoebae communities across the entire site have responded to
365 changing weather conditions over the test period which partially obscures the effect of management. Despite
366 considerable variability in the response of testate amoebae communities to management intervention, there
367 were clearly more pronounced changes in several of the samples from dammed and re-profiled treatments
368 including an increase in diversity, and the appearance of unambiguous wet-indicator species in relatively
369 high abundances (including *Amphitrema stenostoma*, *Archerella flavum*, *Arcella discoides* type, *Diffugia*
370 *bacillifera* and *Diffugia bacillarum*). This reflects a shift towards wetter conditions adjacent to the
371 managed ditches as well as greater variation in habitats across the study site. Our findings illustrate the
372 potential of testate amoebae as bioindicators for the effects of peatland restoration. However, there is a need
373 for caution when using bioindicators (e.g. testate amoebae) for monitoring peatland restoration efforts as
374 ecosystem responses may be more complex than first assumed.

375

376

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379 them were part of a project funded by the UK Government's Department for Environment Food and Rural
380 Affairs (Defra). Further details of the project – SP1202: Investigation of peatland restoration (grip blocking)
381 techniques to achieve best outcomes for methane and greenhouse gas emissions / balance – may be found at:
382 <http://goo.gl/MOEV7M>.

383

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527 **Figure captions**

528 **Figure 1.** Map of study site in the Migneint, North Wales. The location of each ditch is illustrated. The
529 dipwells are located to the east (E) or west (W) of the ditch. Grey = Control; Red = Re-profiled; Blue =
530 Dammed.

531 **Figure 2.** Monitored environmental variables over the course of the experiment. Average pH and
532 conductivity are shown for each treatment type.

533 **Figure 3a.** Percentage testate amoebae data (Controls).

534 **Figure 3b.** Percentage testate amoebae data (Re-profiled).

535 **Figure 3c.** Percentage testate amoebae data (Dammed).

536 **Figure 4.** Boxplot of transfer function predicted water-table depth and Shannon Diversity Index (determined
537 from the testate amoeba communities).

538 **Figure 5.** NMDS analysis of the testate amoeba communities. The analysis is shown for each time of
539 sampling. Ordination hulls show the different treatments: Black = Control; Red = Re-profiled; Blue =
540 Dammed. Taxa and environmental vectors (fitted using 'Envfit') are illustrated in the top left panel. See
541 Table 3 for sample codes.

542 **Figure 6.** Percentage abundance of unambiguous wet indicator taxa before and after management
543 intervention. The x axis denotes the ditch number and sampling time (0-4: where 0-1 are before and 2-4 are
544 after management).

545

546 **Table 1.** Information on the *Sphagnum* moss species sampled from each ditch.

547 **Table 2.** Mean actual and predicted water table, and SDI for the three treatments (control, dammed and re-
548 profiled) (n = 120). Parentheses show standard deviation. A negative water table indicates that the water
549 table level is above the ground surface (i.e., ponding), whilst positive indicates below the ground surface.

550 **Table 3.** Ordination sample codes (for interpretation of Figure 5).

551 **Table 4.** Wet indicator taxa and changes in diversity metrics for each sample grouped by treatment.

552

553 **Supplementary material 1.** Monitored water tables compared with transfer function-predicted water tables.

554 **Supplementary material 2.** Cluster analysis of testate amoebae communities (Q-mode)

555 **Supplementary material 3.** Redundancy analysis of testate amoebae communities.

556

557

Figure 1

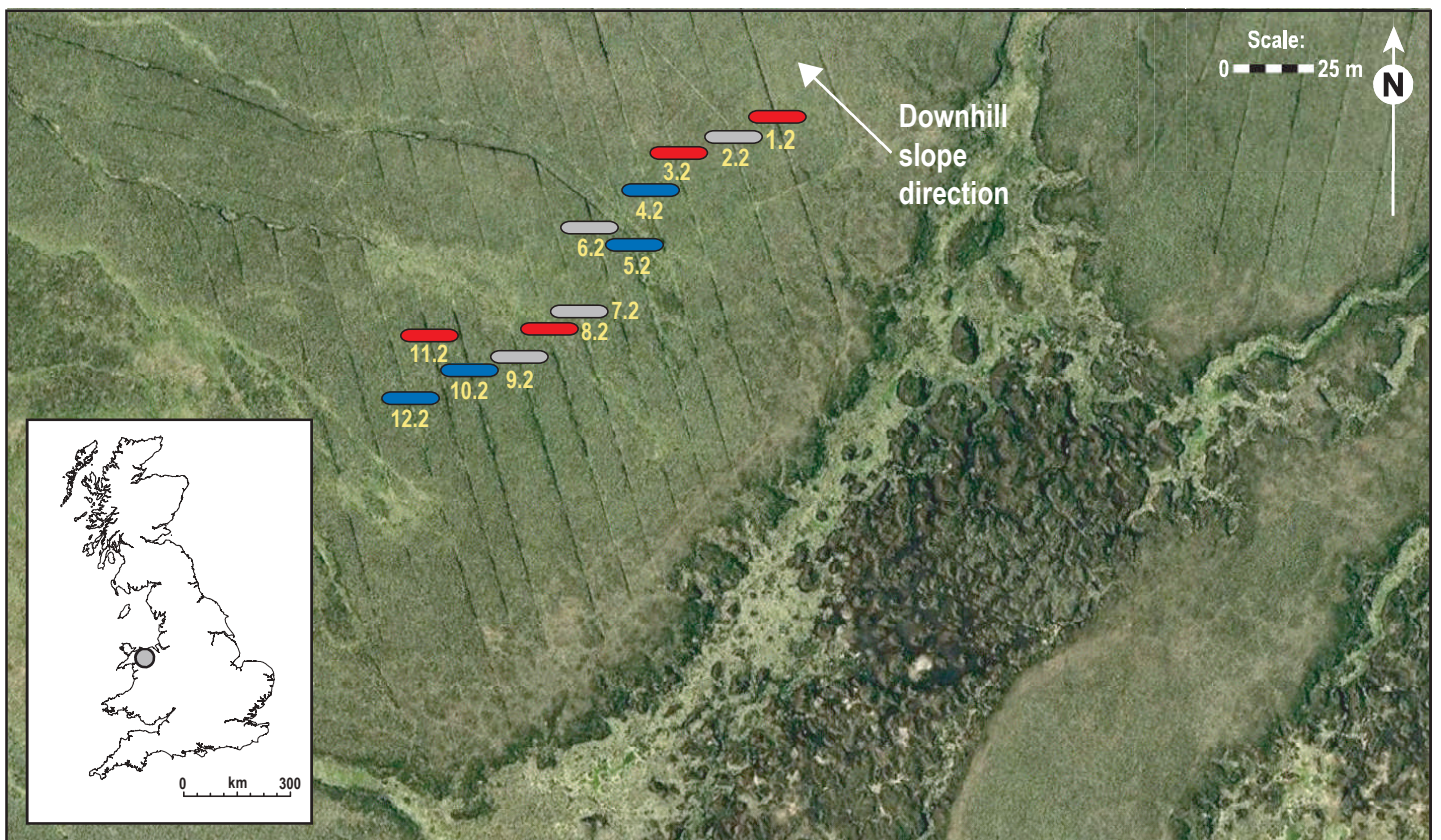
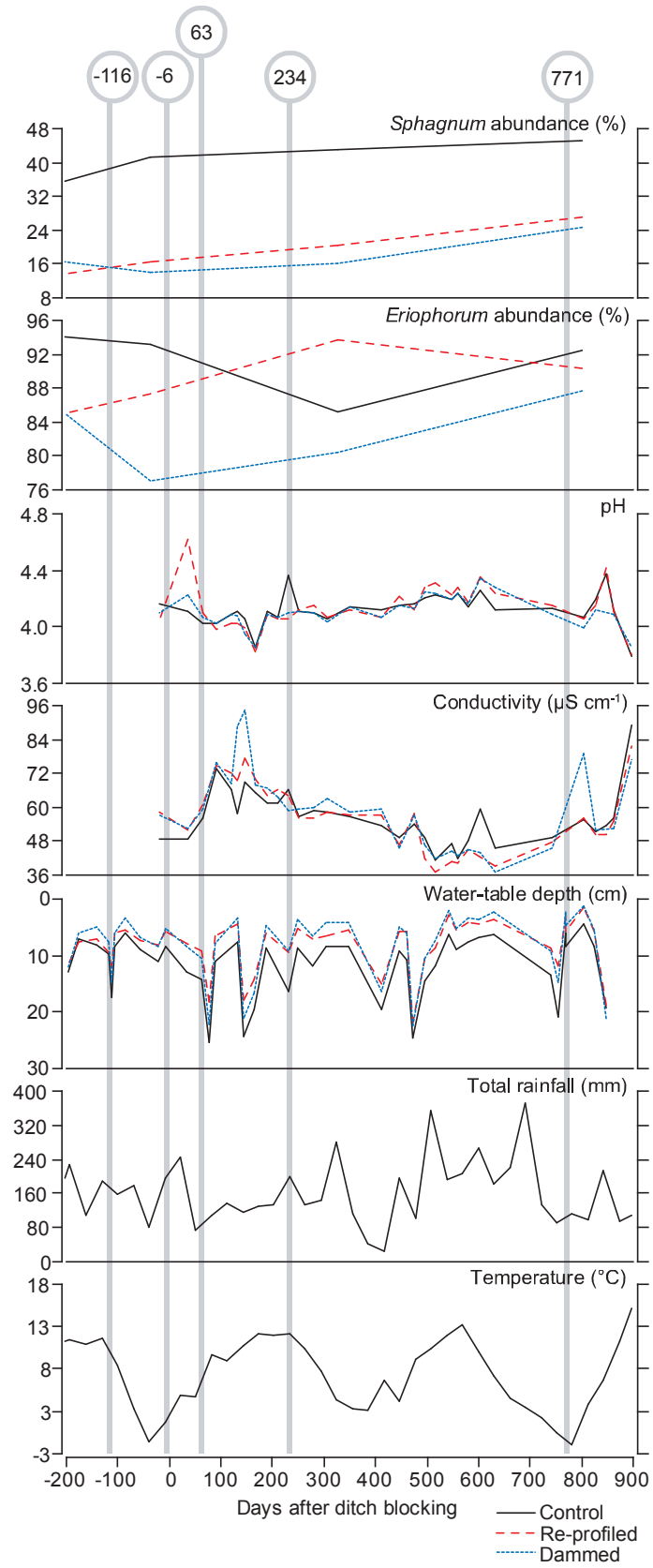
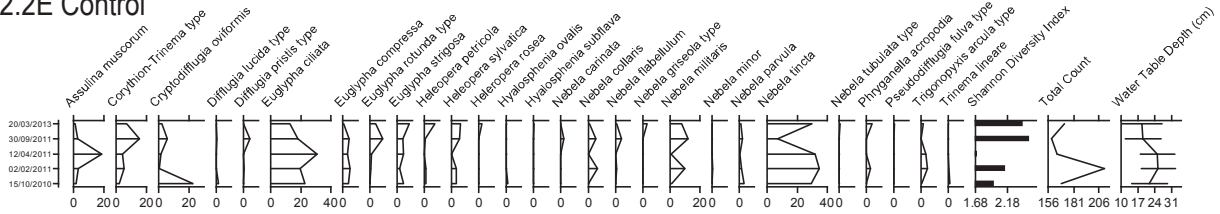


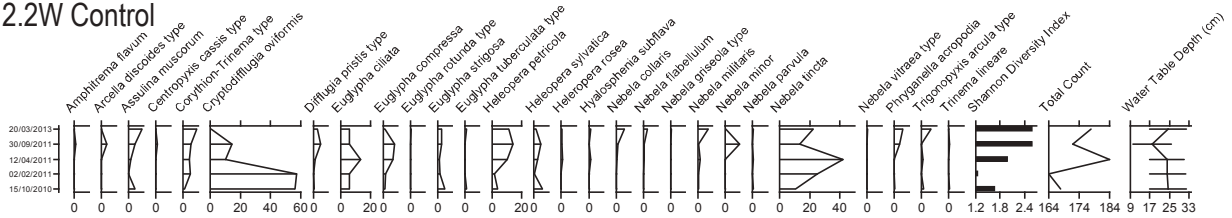
Figure 2



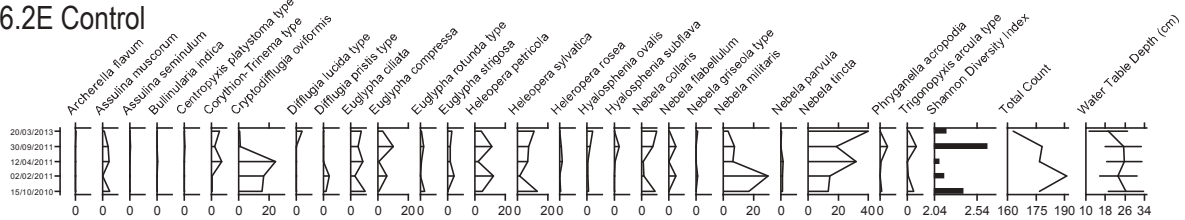
2.2E Control



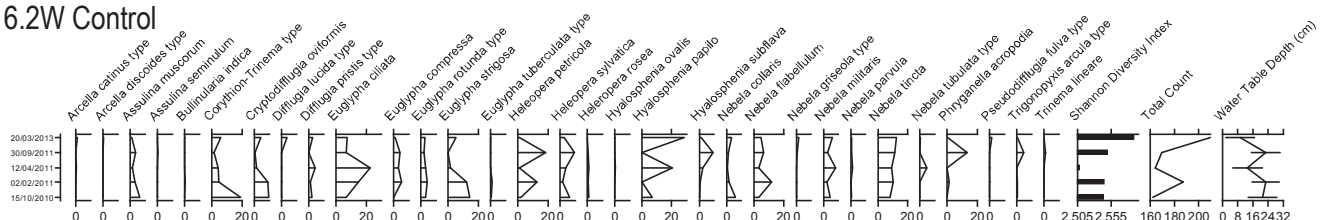
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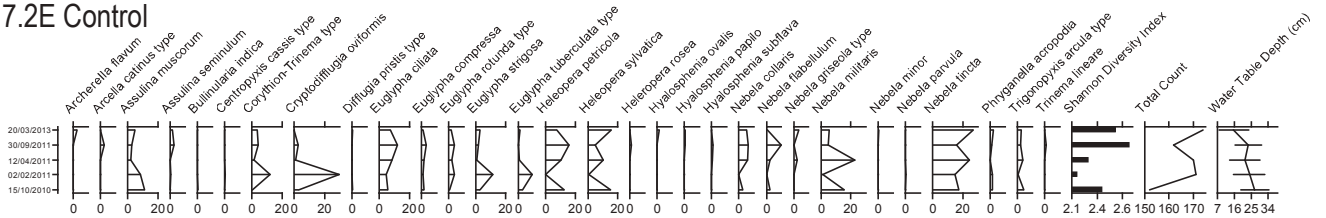
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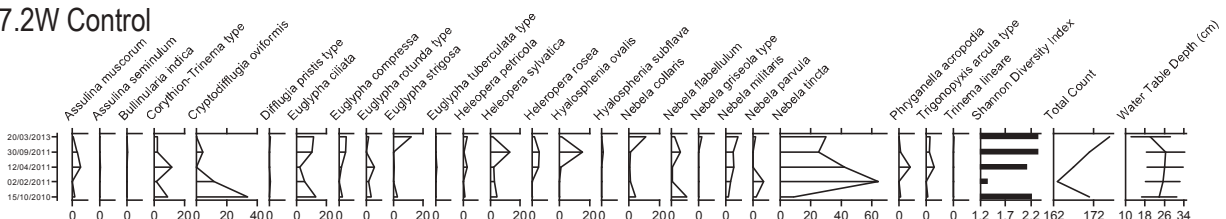
6.2W Control



7.2E Control



7.2W Control



9.2E Control



9.2W Control

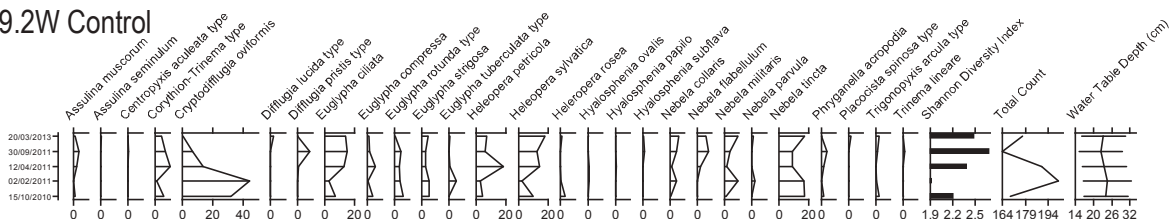


Figure 3b

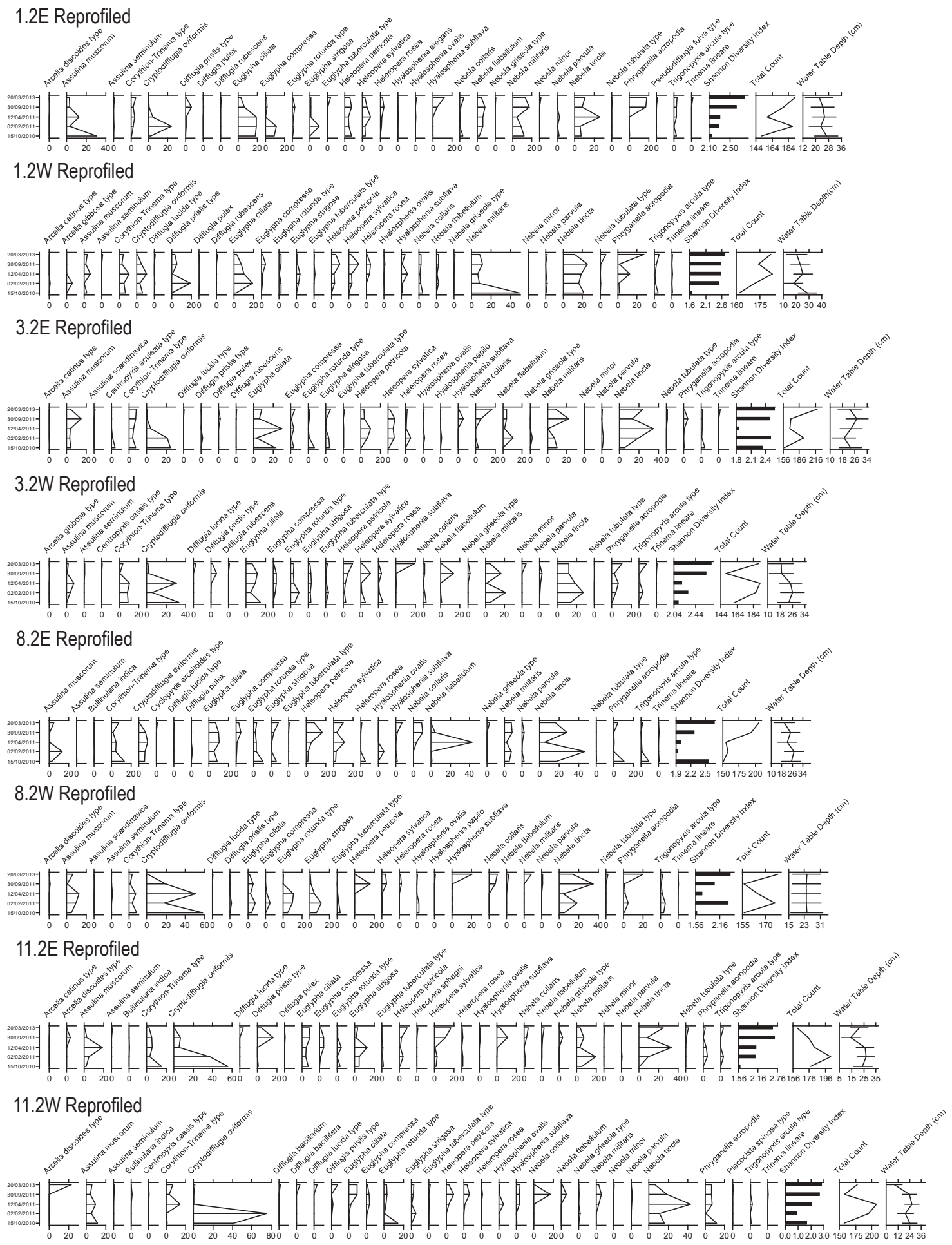
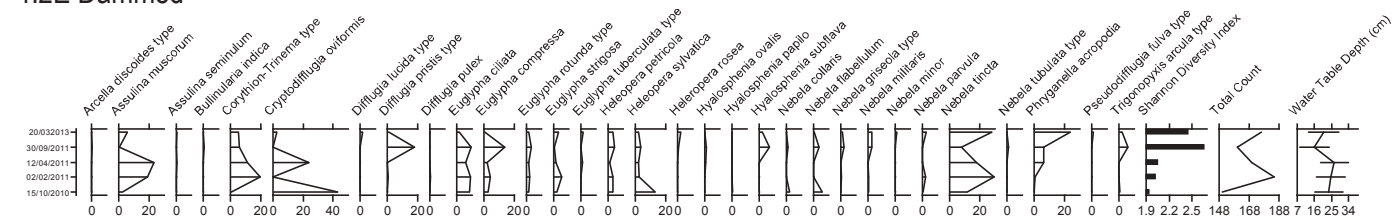
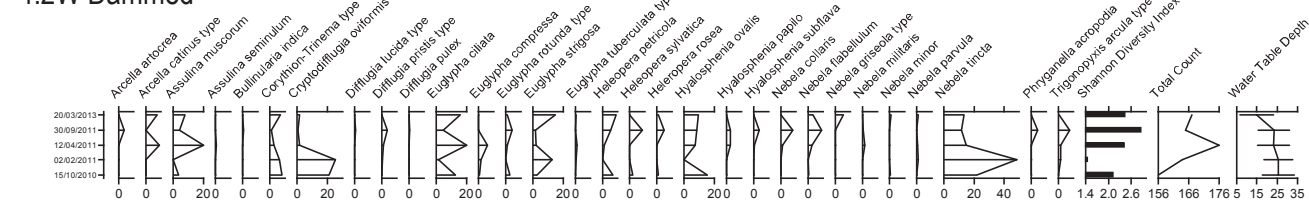


Figure 3c

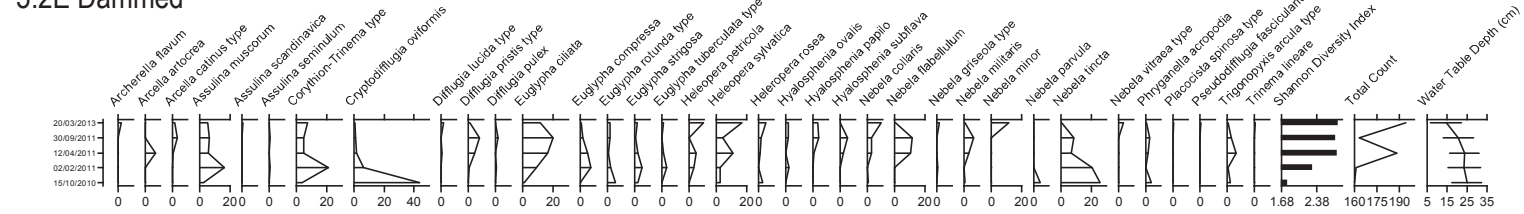
4.2E Dammed



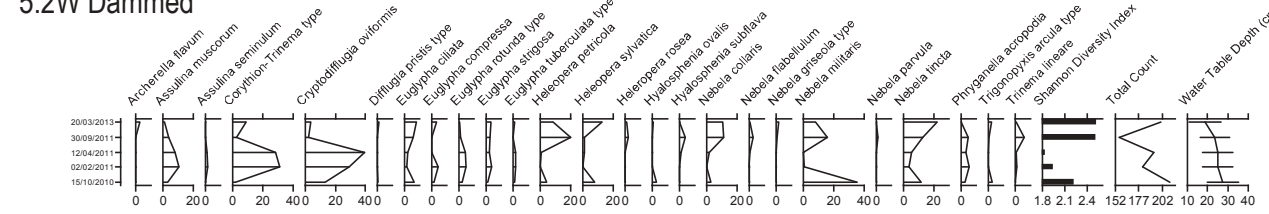
4.2W Dammed



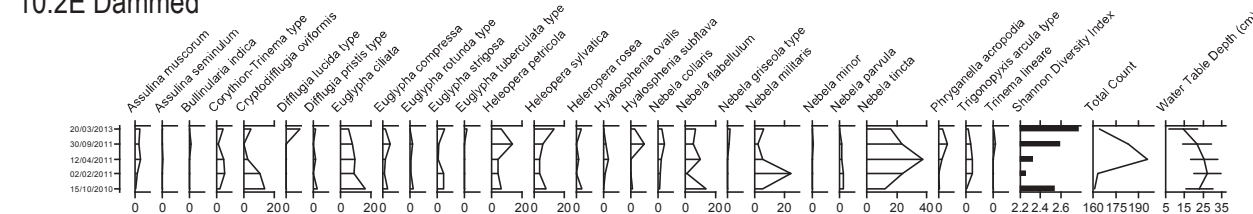
5.2E Dammed



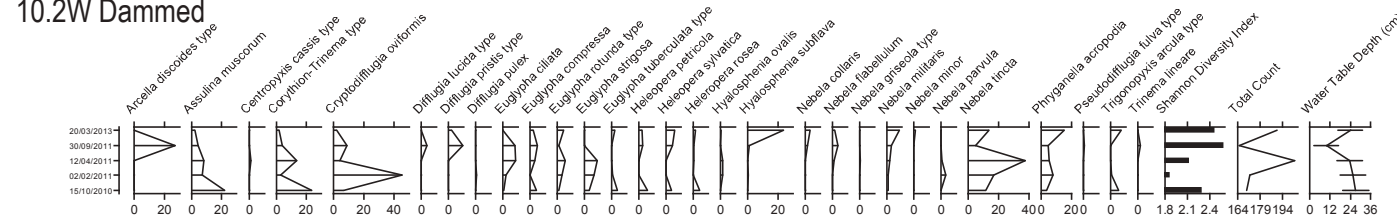
5.2W Dammed



10.2E Dammed



10.2W Dammed



12.2E Dammed



12.2W Dammed

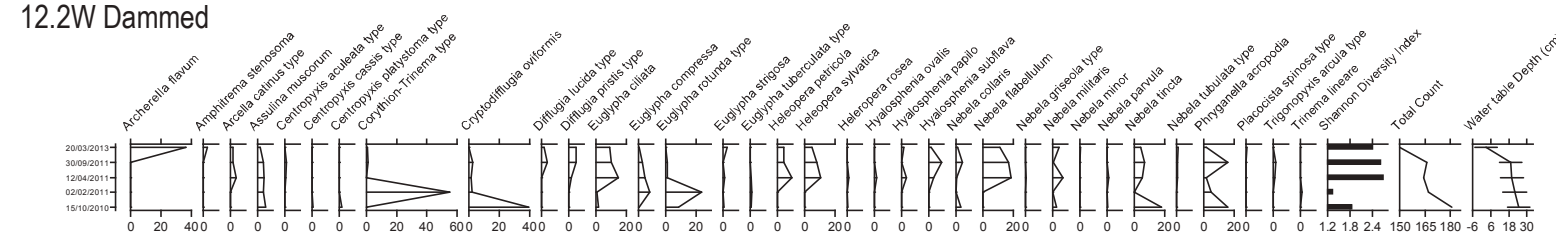


Figure 4

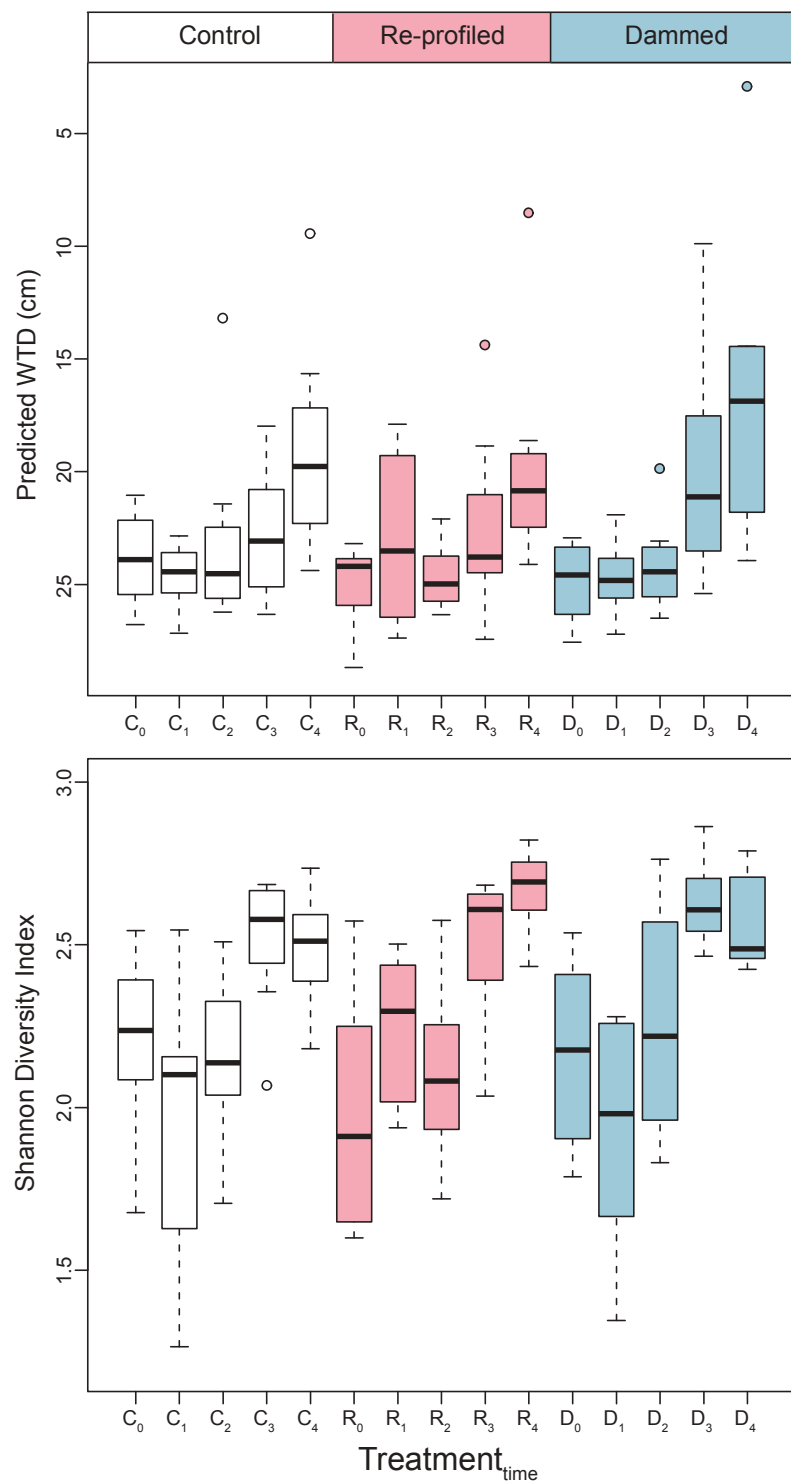


Figure 5

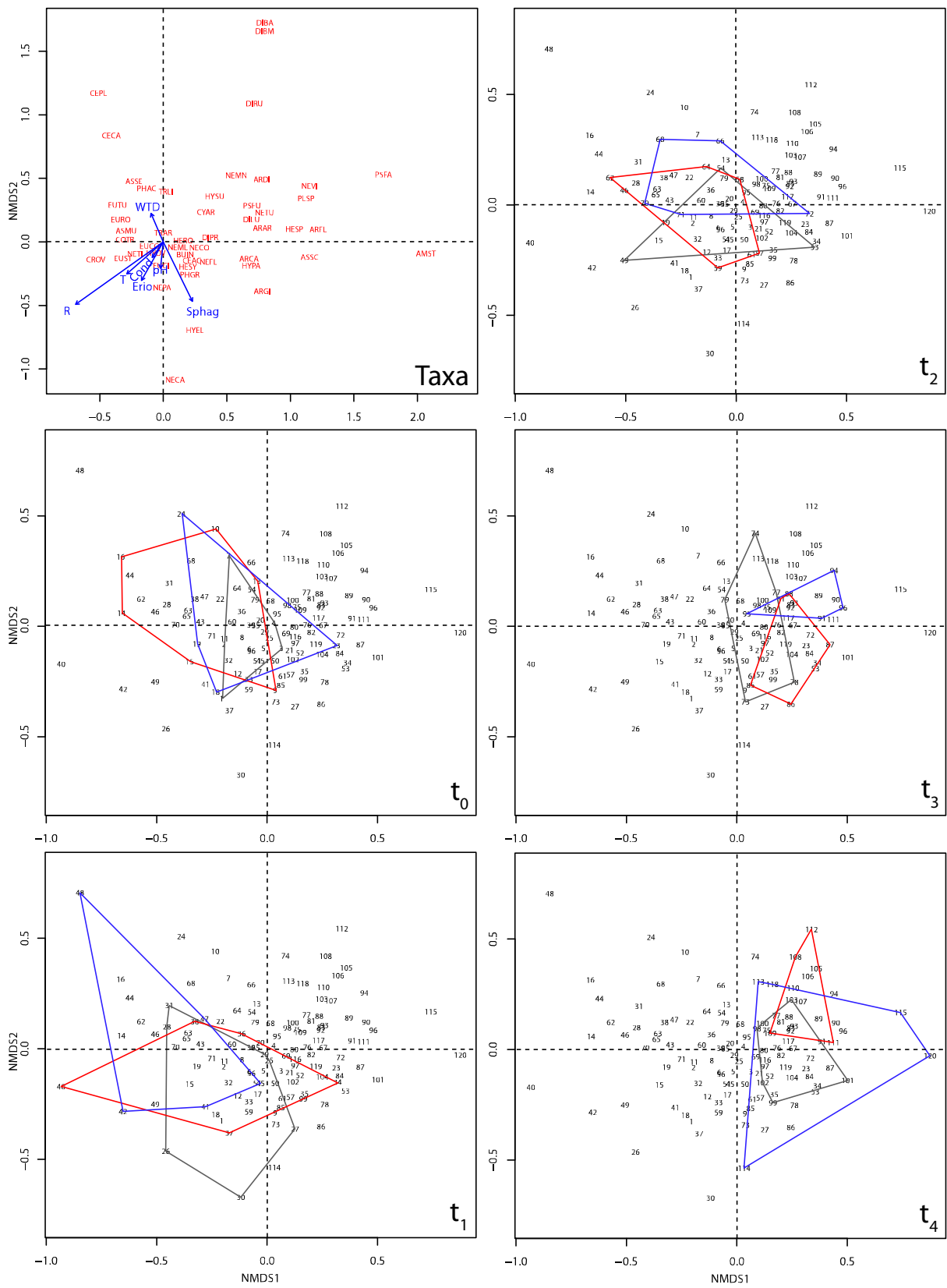


Figure 6

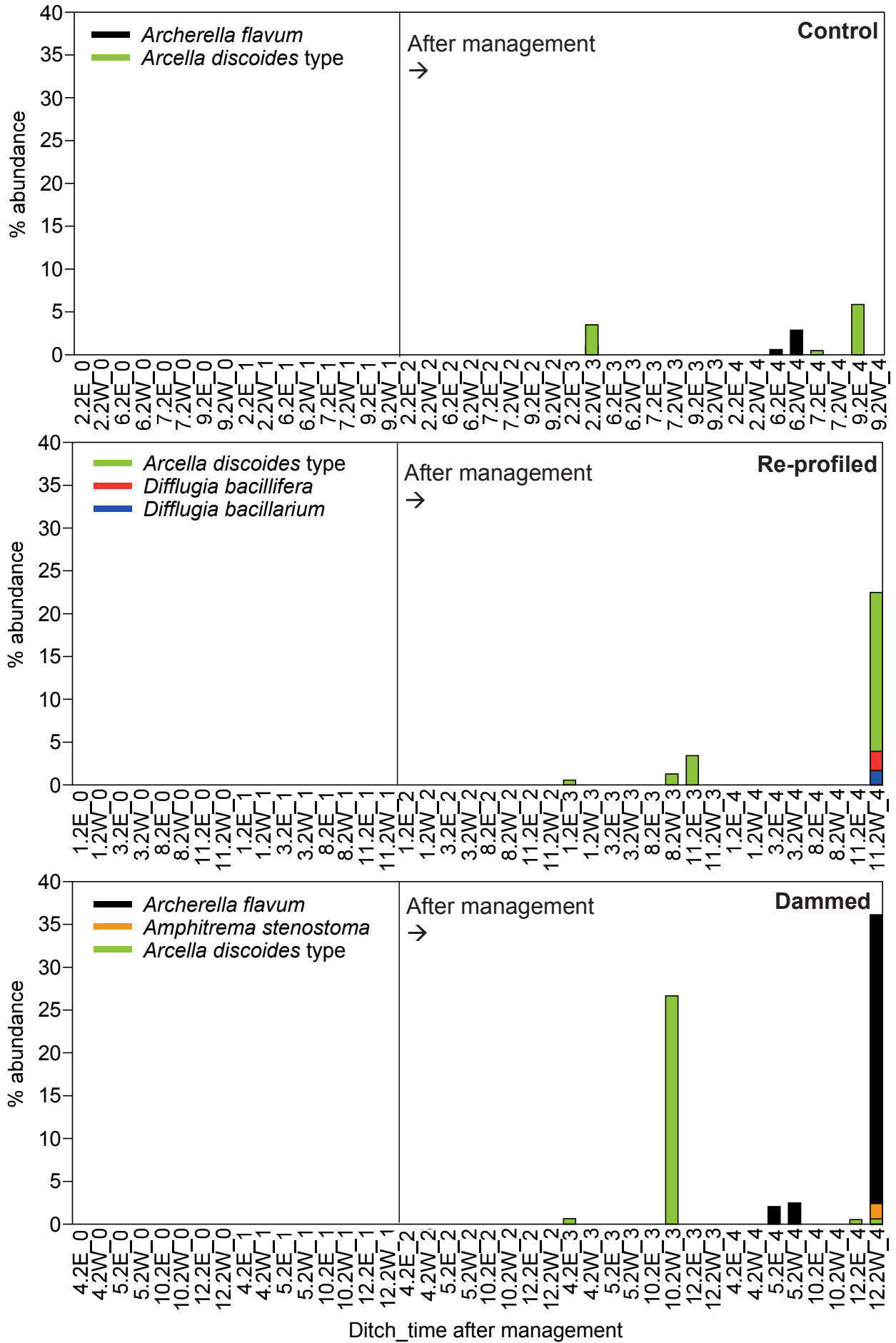


Table 1

Replicate #	Control	Re-profiled	Dammed
1	6.2 E - <i>S. capillifolium</i> W - <i>S. capillifolium</i>	3.2 E - <i>S. capillifolium</i> W - <i>S. capillifolium</i>	12.2 E - <i>S. capillifolium</i> W - <i>S. capillifolium</i>
2	2.2 E - <i>S. capillifolium</i> W - <i>S. capillifolium</i>	1.2 E - <i>S. capillifolium</i> W - <i>S. papillosum</i>	4.2 E - <i>S. fallax</i> W - <i>S. capillifolium</i>
3	7.2 E - <i>S. subnitens</i> W - <i>S. capillifolium</i>	8.2 E - <i>S. capillifolium</i> W - <i>S. capillifolium</i>	5.2 E - <i>S. capillifolium</i> W - <i>S. capillifolium</i>
4	9.2 E - <i>S. capillifolium</i> W - <i>S. capillifolium</i>	11.2 E - <i>S. capillifolium</i> W - <i>S. capillifolium</i>	10.2 E - <i>S. subnitens</i> W - <i>S. capillifolium</i>

Table 2

Treatment	Time	Mean monitored water table depth (cm)	Mean predicted water table depth (cm)	Shannon Diversity Index
Control	t ₀	8.10 (± 1.8)	23.9 (± 1.3)	2.21 (± 0.11)
	t ₁	5.24 (± 1.8)	24.6 (± 1.3)	1.95 (± 0.11)
	t ₂	7.15 (± 1.8)	23.1 (± 1.3)	2.15 (± 0.11)
	t ₃	9.80 (± 1.8)	22.8 (± 1.3)	2.52 (± 0.11)
	t ₄	1.09 (± 1.8)	19.0 (± 1.3)	2.49 (± 0.11)
Reprofiled	t ₀	10.5 (± 1.8)	24.9 (± 1.3)	2.16 (± 0.11)
	t ₁	7.21 (± 1.8)	24.7 (± 1.3)	1.93 (± 0.11)
	t ₂	14.9 (± 1.8)	24.1 (± 1.3)	2.26 (± 0.11)
	t ₃	14.6 (± 1.8)	19.9 (± 1.3)	2.63 (± 0.11)
	t ₄	5.84 (± 1.8)	16.6 (± 1.3)	2.57 (± 0.11)
Dammed	t ₀	8.61 (± 1.8)	25.0 (± 1.3)	19.7 (± 0.11)
	t ₁	7.18 (± 1.8)	23.0 (± 1.3)	2.12 (± 0.11)
	t ₂	12.0 (± 1.8)	24.7 (± 1.3)	2.10 (± 0.11)
	t ₃	10.7 (± 1.8)	22.5 (± 1.3)	2.50 (± 0.11)
	t ₄	4.58 (± 1.8)	19.7 (± 1.3)	2.67 (± 0.11)

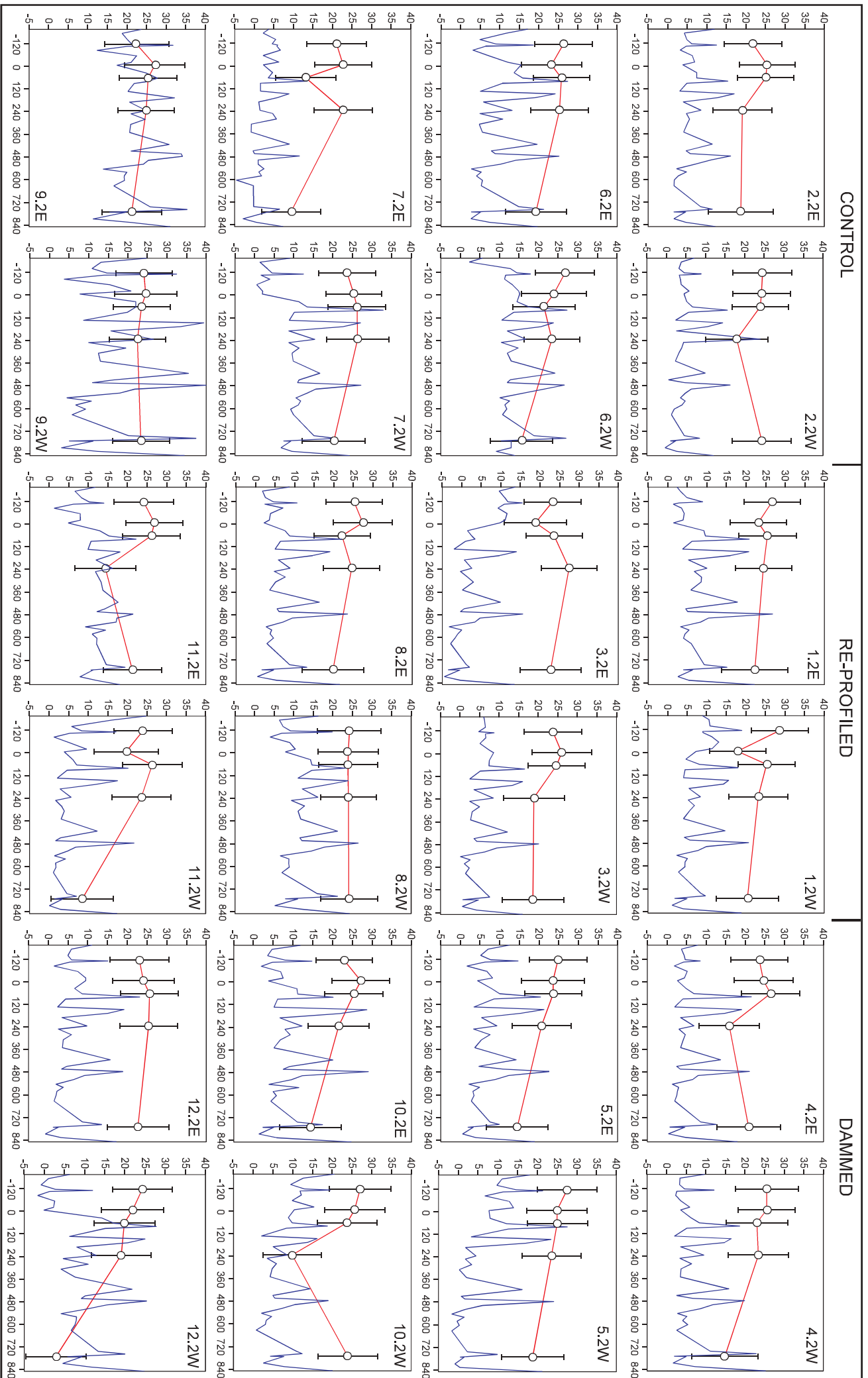
Table 3

Number	Grip_Time	Number	Grip_Time	Number	Grip_Time	Number	Grip_Time	Number	Grip_Time	Number	Grip_Time
1	2.2E_0	21	10.2E_0	41	4.2E_1	61	8.2E_2	81	1.2E_3	101	7.2E_4
2	2.2W_0	22	10.2W_0	42	4.2W_1	62	8.2W_2	82	1.2W_3	102	7.2W_4
3	6.2E_0	23	12.2E_0	43	4.2E_1	63	11.2E_2	83	3.2E_3	103	9.2E_4
4	6.2W_0	24	12.2W_0	44	4.2W_1	64	11.2W_2	84	3.2W_3	104	9.2W_4
4	7.2E_0	24	2.2E_1	44	10.2E_1	64	4.2E_2	84	8.2E_3	104	1.2E_4
6	7.2W_0	26	2.2W_1	46	10.2W_1	66	4.2W_2	86	8.2W_3	106	1.2W_4
7	9.2E_0	27	6.2E_1	47	12.2E_1	67	4.2E_2	87	11.2E_3	107	3.2E_4
8	9.2W_0	28	6.2W_1	48	12.2W_1	68	4.2W_2	88	11.2W_3	108	3.2W_4
9	1.2E_0	29	7.2E_1	49	2.2E_2	69	10.2E_2	89	4.2E_3	109	8.2E_4
10	1.2W_0	30	7.2W_1	40	2.2W_2	70	10.2W_2	90	4.2W_3	110	8.2W_4
11	3.2E_0	31	9.2E_1	41	6.2E_2	71	12.2E_2	91	4.2E_3	111	11.2E_4
12	3.2W_0	32	9.2W_1	42	6.2W_2	72	12.2W_2	92	4.2W_3	112	11.2W_4
13	8.2E_0	33	1.2E_1	43	7.2E_2	73	2.2E_3	93	10.2E_3	113	4.2E_4
14	8.2W_0	34	1.2W_1	44	7.2W_2	74	2.2W_3	94	10.2W_3	114	4.2W_4
14	11.2E_0	34	3.2E_1	44	9.2E_2	74	6.2E_3	94	12.2E_3	114	4.2E_4
16	11.2W_0	36	3.2W_1	46	9.2W_2	76	6.2W_3	96	12.2W_3	116	4.2W_4
17	4.2E_0	37	8.2E_1	47	1.2E_2	77	7.2E_3	97	2.2E_4	117	10.2E_4
18	4.2W_0	38	8.2W_1	48	1.2W_2	78	7.2W_3	98	2.2W_4	118	10.2W_4
19	4.2E_0	39	11.2E_1	49	3.2E_2	79	9.2E_3	99	6.2E_4	119	12.2E_4
20	4.2W_0	40	11.2W_1	60	3.2W_2	80	9.2W_3	100	6.2W_4	120	12.2W_4

Table 4

Samples		Wet indicators %						Diversity								
Treatment	Dipwell	ARFL	AMST	ARDI	DIBA	DIBM	Σ Wet indicators	SDI _{t0}	SDI _{t4}	Δ SDI	Richness _{t0}	Richness _{t4}	Δ Richness	Evenness _{t0}	Evenness _{t4}	Δ Evenness
Control	2.2E						0.00	1.97	2.43	0.46	15	21	6	0.48	0.54	0.06
Control	2.2W			3.49			3.49	1.68	2.60	0.92	16	23	7	0.33	0.58	0.25
Control	6.2E	0.61					0.61	2.38	2.18	-0.20	17	19	2	0.64	0.47	-0.17
Control	6.2W	2.87					2.87	2.40	2.54	0.13	18	22	4	0.61	0.57	-0.04
Control	7.2E			0.48			0.48	2.54	2.59	0.04	18	25	7	0.71	0.53	-0.18
Control	7.2W						0.00	2.23	2.35	0.12	16	19	3	0.58	0.55	-0.03
Control	9.2E			5.85			5.85	2.24	2.74	0.49	20	23	3	0.47	0.67	0.20
Control	9.2W						0.00	2.21	2.49	0.28	15	21	6	0.61	0.57	-0.03
Summary							13.29			2.25			38			0.06
Reprofilled	1.2E			0.56			0.56	2.16	2.78	0.62	15	27	12	0.58	0.60	0.02
Reprofilled	1.2W						0.00	1.70	2.70	1.00	14	27	13	0.39	0.55	0.16
Reprofilled	3.2E						0.00	2.34	2.59	0.25	18	27	9	0.58	0.49	-0.08
Reprofilled	3.2W						0.00	2.13	2.73	0.60	14	26	12	0.60	0.59	-0.01
Reprofilled	8.2E						0.00	2.57	2.69	0.12	19	21	2	0.69	0.70	0.01
Reprofilled	8.2W	1.28					1.28	1.60	2.43	0.83	11	22	11	0.45	0.52	0.07
Reprofilled	11.2E	3.43					3.43	1.62	2.62	1.00	14	25	11	0.36	0.55	0.19
Reprofilled	11.2W	22.47	3.93			1.69	28.09	1.68	2.82	1.15	11	26	15	0.49	0.65	0.16
Summary							33.36			5.57			85			0.52
Dammed	4.2E			0.63			0.63	1.95	2.46	0.51	16	24	8	0.44	0.49	0.05
Dammed	4.2W						0.00	2.14	2.46	0.32	14	17	3	0.61	0.69	0.08
Dammed	5.2E	2.06					2.06	1.79	2.79	1.00	14	30	16	0.43	0.54	0.12
Dammed	5.2W	2.49					2.49	2.22	2.52	0.30	17	21	4	0.54	0.59	0.05
Dammed	10.2E						0.00	2.54	2.77	0.24	19	21	2	0.67	0.76	0.10
Dammed	10.2W	26.67					26.67	2.29	2.46	0.17	15	19	4	0.66	0.62	-0.04
Dammed	12.2E						0.00	2.53	2.64	0.11	21	24	3	0.60	0.59	-0.01
Dammed	12.2W	36.14	2.41	0.60			39.16	1.86	2.43	0.56	15	24	9	0.43	0.47	0.04
Summary							71.00			3.22			49			0.38

Water-table depth (cm)



Days after ditch blocking

