Article

Determining the Impact of Riparian Wetlands on Nutrient Cycling, Storage and Export in Permeable Agricultural Catchments

Penny J. Johnes[1], Daren C. Gooddy[2], Timothy H. E. Heaton[3], Andrew Binley[4], Michael P. Kennedy[5], Paul Shand[6], Hannah Prior[7]

[1] School of Geographical Sciences, University of Bristol, Bristol, BS8 1SS, UK; pj13302@bristol.ac.uk

[2] British Geological Survey, Maclean Building, Wallingford, Oxfordshire OX10 8BB, UK; dcg@bgs.ac.uk

[3] NERC Isotope Geosciences Laboratory, British Geological Survey, Keyworth, Nottingham NG12 5GG, UK; theh@nigl.nerc.ac.uk

[4] Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster LA1 4YQ, UK; a.binley@lancaster.ac.uk

[5] School of Energy, Construction and Environment, Coventry University, Priory Street, Coventry, CV1 5FB; michael.kennedy@coventry.ac.uk

[6] College of Science and Engineering, School of the Environment, Flinders University, PO Box 2100, Adelaide SA 5001; paul.shand@flinders.edu.au.

[7] Smith School of Enterprise and the Environment, University of Oxford, Oxford, OX1 3QY, UK; hannah.prior@smithschool.ox.ac.uk

* Correspondence: pj13302@bristol.ac.uk;

Abstract: The impact of riparian wetlands on the cycling, retention and export of nutrients from land to water varies according to local environmental conditions and is poorly resolved in catchment management approaches. To determine the role a specific wetland might play in a catchment mitigation strategy, an alternative approach is needed to the high frequency and spatially detailed monitoring programme that would otherwise be needed. Here, we present a new approach using a combination of novel and well-established geochemical, geophysical and isotope ratio approaches. This was developed and tested against a 2-year high-resolution sampling programme in a lowland permeable wetland in the Lambourn catchment, UK. The monitoring programme identified multiple pathways and water sources feeding into the wetland, generating large spatial and temporal variations in nutrient cycling, retention and export behaviours within the wetland. This complexity of contributing source areas and biogeochemical functions within the wetland were effectively identified using the new toolkit approach. We propose that this technique could be used to determine the likely net source/sink function of riparian wetlands prior to their incorporation into any catchment management plan, with relatively low resource implications when compared to a full high frequency nutrient speciation and isotope geochemistry-based monitoring approach.

Keywords: nitrogen, phosphorus, nutrient cycling, biogeochemistry, geochemistry, geophysics, wetlands, catchment management

1. Introduction

As the international scientific literature produced over the past three decades attests, biogeochemical cycling in wetland systems shows a significant degree of inter- and intra-site variability, leading to highly variable rates, patterns and speciation of C, N and P within and from these wetlands to adjacent surface waters [1-8]. Such variability may be related to the geomorphology and specific soil physical, soil organic, chemical and vegetation character of the

46 wetland, the nature and extent of seasonal, short-term and inter-annual wetting-drying cycles, the
47 specific nutrient speciation of inflowing waters, or to anthropic modifications [9-14]. In permeable
48 catchments, there is added complexity in terms of vertical and lateral nutrient exchange with
49 adjacent surface waters and groundwater via the hyporheic zone [4,5,15,16]. Whilst riparian
50 wetlands have been shown to act as biogeochemical cycling hotspots, with the potential to
51 transform and attenuate the nutrient and geochemistry signal from the catchment as it moves from
52 land to water [1,3,17-19], the efficacy of this function in real terms is controlled by the specific
53 distribution of functional zones or biogeochemical cycling hotspots within the wetland. These in
54 turn are controlled by the chemical character and distribution of contributing source areas of flow
55 to the wetland between and within a water year, flow routing through the wetland, the residence
56 time of water within the wetland and contact or exposure time between the microbial community
57 and the nutrient parcel transitioning through the wetland, while soil organic carbon content and
58 dissolved organic matter in porewaters acts as an energy resource to support denitrification, with
59 redox status varying laterally, vertically and over time [7,11,20,21].

60 A wide range of papers have been published on wetland biogeochemistry, demonstrating what
61 often appear to be conflicting behaviours in systems within apparently similar hydrogeological and
62 climatic zones. However, these conflicts are largely attributable to within and between site
63 variability in the size and hydrological connectivity of the major biogeochemical functional zones
64 which were not captured within the sampling programme, differences in the range of variables
65 determined in each programme, and the differences in the frequency of sample collection in both
66 space and time. All too often, a low-resolution sampling programme is implemented, with
67 sampling of wetland soil water at relatively few sites and depths, and for a limited range of nutrient
68 species, due to resource constraints or to the need to employ less invasive sampling techniques in
69 an ecologically sensitive environment. The resulting data are often misleading, suggesting for
70 example, that functions such as denitrification can account for nitrate 'loss' across large wetland
71 tracts without ever accounting for the spatial variability in the distribution of that function in the
72 subsurface zone, or over time in response to hydrological flushing events. Meanwhile the
73 catchment management literature often contains gross assumptions regarding the potential rates of
74 nutrient 'removal', 'attenuation' or 'loss' achievable in both natural and constructed wetlands and
75 buffer strips. Nutrient cycling and transformation, temporary storage within the soil matrix, plant
76 and microbial community of the wetland, and subsequent export of organic nutrient forms to
77 adjacent waters under high flow conditions are all too often missed as alternative causes of the
78 observed patterns. Reported rates of nutrient 'removal' following the installation of riparian
79 wetlands or 'buffer strips' are then erroneously low for catchment managers [8], or highly variable
80 over the hydrological year [15]. Where a high frequency sampling programme has been undertaken
81 which adopts an holistic approach to assessing C, N and P cycling and flux [22-24], very often
82 different conclusions are drawn, and the capacity of wetlands to attenuate nutrient flux from
83 catchment sources, to transform inorganic nutrient influx to organic nutrient forms stored within
84 the wetland, and to export accumulated dissolved organic nutrient stores during flushing events
85 becomes apparent [5,22,25-27].

86 The best environmental policy is underpinned by sound and holistic science, but the resources
87 required to undertake such holistic assessment are significant and often prohibitive even for single
88 site investigations. An alternative approach is required which can identify the likely distribution of
89 functional zones across large and/or multiple wetland systems without the need to undertake
90 costly, invasive and time-consuming high resolution spatial and temporal monitoring. Here we
91 present such an approach, building on the HydroGeoMorphic UNIT (HGMU) classification of
92 wetlands [28], using a novel toolkit of geophysical, geochemical and natural abundance isotopic
93 approaches to identify the distribution of functional zones in complex permeable riparian wetlands
94 and determine their likely impact on nutrient transport, transformation and export to adjacent
95 waters [29-32]. This recognises the need to understand the subsurface structure and hydrological
96 function within wetlands, given their potentially significant role in the hydrological and

97   biogeochemical behaviour of catchments, as well as their effects on the wetland habitat itself, and to
98   derive an understanding of where water comes from, how it affects soil redox status and how long
99   it is in contact with the wetland plant and microbial communities.

100 This approach was developed as part of the project on Hydrogeochemical Functioning of Lowland
101 Permeable Catchments funded from 2002-2005 under the UK NERC LOCAR programme [33-34].
102 The research was undertaken on an instrumented riparian wetland adjacent to the River Lambourn
103 with the aim of identifying the role of these wetlands in modifying the nutrient chemistry of waters
104 draining to the Lambourn from its catchment. The wetland had been the subject of a previous
105 investigation into total nitrogen (N) and total phosphorus (P) cycling dynamics in the wetland soil
106 water and the adjacent river reach [5]. Key findings from the earlier work on the Boxford wetland
107 indicated that:

108 • Wetlands in these lowland permeable catchments act as a sink for N and P under baseflow
109   conditions, with high concentrations of dissolved organic N (DON) and P (DOP)
110   accumulated in soil porewaters following plant assimilation of nitrate (NO_3-N) and
111   orthophosphate (PO_4-P), plant dieback and microbial decomposition of dead organic
112   matter.

113 • The wetland acts as a nutrient source under high flow conditions, with nutrient-rich
114   porewater exported to the adjacent River Lambourn, predominantly in the form of DON
115   and DOP.

116 • Wet-warm periods, with associated changes in soil redox status provide the optimum
117   conditions for gaseous N flux from the wetland via denitrification, and for the desorption
118   of P from the soil matrix and its subsequent flux in aqueous form to the adjacent River
119   Lambourn.

120 • The wetland source/sink function varies both from baseflow to high flow conditions, and
121   also from wet year to dry year, with greater rates of nutrient export to the adjacent
122   Lambourn in wetter years, and greater retention of both N and P in the soil porewaters in
123   drier years.

124 Further work on P and sediment transport and transformations undertaken on the adjacent
125 reach provides additional context for the interpretation of P cycling trends in the current study
126 [35-37]. Allied projects funded under LOCAR on the Lambourn and its catchment provide
127 further detailed understanding for this project to draw upon on the biogeochemical process
128 controls on nutrient exchange between groundwater, surface water and the hyporheic zone
129 upstream from the Boxford wetland [30,38], on streamflow generation and surface water-
130 groundwater interactions along the length of the Lambourn [39-40] and the specific role of
131 alluvial deposits in the Lambourn valley wetlands on groundwater-surface water exchange
132 processes [41], and on process controls on nutrient exchange between surface and subsurface
133 zones in the adjacent reach of the Lambourn [42-43].

134 The limitation of this earlier work, however, was that the proportion of catchment flow routed
135 through the wetland and the specific spatial origins of the water in the wetland at any point in
136 time were not known. The latter might comprise any, or a combination, of diffuse flow into the
137 wetland from adjacent hillslopes, road runoff, diffuse influx from groundwater recharge
138 through the valley gravels underlying the wetlands in this catchment, or influx of water from
139 the adjacent river. The dominant hydraulic gradient and thus the source of wetland soil
140 porewaters is likely to vary both in time and space, thus altering the rate and origins of nutrient
141 influx to the wetland, and the contact time between the wetland plant and microbial
142 communities and the nutrient load in transit which has been reported in previous studies to be

143 a key control on processes such as soil redox and ultimately denitrification in wetland soils
144 [11,20]. This in turn would influence the speciation of the nutrient load as it moves through the
145 wetland.

146 In this programme, we therefore adopted a 3-step approach to determine:

147 1. the sub-surface structure, hydrological connectivity and function of the wetland using
148 geophysical mapping and geochemical ratio techniques to identify the likely origins and
149 residence times of waters within the wetland soil matrix as these varied in space and time. In
150 combination these would allow identification of the potential location of biogeochemical
151 cycling hotspots within the wetland.

152 2. the nutrient cycling, retention and export behaviour in the wetland using a high temporal and
153 spatial resolution sampling and analysis programme for all N species and P fractions flushed
154 to and through the wetland and out to the adjacent aquifer and river, including the use of
155 isotope ratios to discover direct evidence of the rates and locations of denitrification hotspots
156 in the wetland. From this, we could then determine:

157 a. the biogeochemical cycling hotspots within the wetland;

158 b. the extent to which inorganic N and P 'loss' could be accounted for via denitrification,
159 biotic uptake, and degradation of dead organic matter leading to the accumulation and
160 subsequent flushing of dissolved organic nutrient fractions within soil porewaters; and

161 c. the whole system nutrient attenuation behaviour and capacity of natural riparian
162 wetlands in intensively farmed permeable catchments.

163 3. Whether an alternative, lighter touch approach could effectively identify the wetland
164 biogeochemical functional zones and likely wetland nutrient 'removal' efficiency by using
165 geochemical, geophysical and natural abundance isotopic techniques, testing the outcomes
166 from this approach against the fine resolution but more time-consuming identification of these
167 zones achieved in step 2.

168 **2. Materials and Methods**

169 *3.1. Site description: The Boxford Wetland*

170 The Boxford experimental wetland lies adjacent to the River Lambourn which drains a
171 predominantly Chalk catchment with a complex mixture of alluvial silts and gravels overlying the
172 Seaford Chalk in the riparian zone [41]. The relationship of the Boxford wetland to local catchment
173 topography and geology is shown in Figure 1. The wetland lies on the east bank of the River
174 Lambourn just upstream from the village of Boxford, at the base of a major dry valley feature
175 orientated N-S veering NW-SE closer to the river channel. Topographically this feature appears to
176 indicate a downslope gradient for water to drain through the wetland to the River Lambourn. The
177 wetland soils comprise silty alluvium with a high proportion of nodular flint. These are underlain
178 by a flint gravel matrix of varying thickness which is in turn underlain by Seaford Chalk. These
179 alluvial gravels form an important control on surface water–groundwater interactions at the study
180 site and provide an important lateral flow pathway for pollutant transport along the river valley
181 corridor and between the wetland and the adjacent river [41]. However, there are areas of
182 discontinuity along the length of the river and, we propose, under the parts of the wetlands in the
183 river corridor, leading to complex flow pathways and domains within the riparian wetlands. This
184 in turn will generate variable hydrologic residence times within the wetlands, impacting on the
185 extent to which nutrient load delivered to these wetlands is in contact with the key biogeochemical
186 cycling process functions in the wetland.
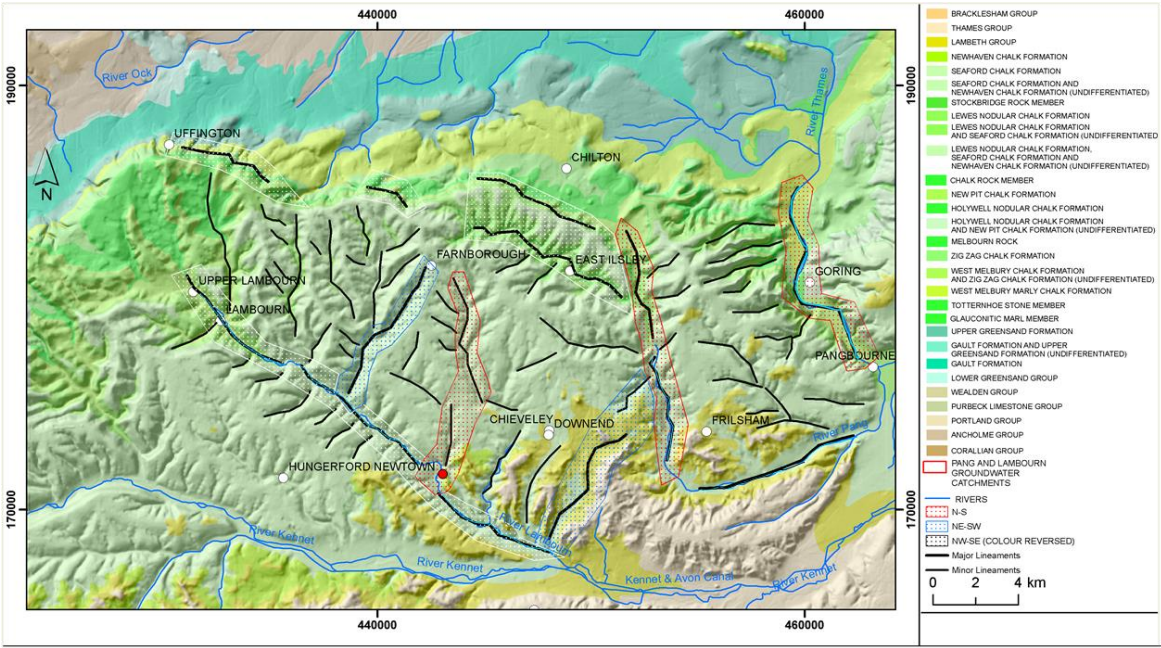
Figure 1. Dry Valley features of the Lambourn catchment. The location of the Boxford Wetland is marked with a red circle. The dry valley surface topographic catchment is shown shaded to the north of the wetland. Figure reproduced with permission of the British Geological Survey.

The River Lambourn flows adjacent to the site from NW to SW, with steeper hillslopes to the north and east. Site elevation varies from 1.8 m along the site margins to -0.8 m above local datum, at the base of a proto-stream channel which drains across the site from NE to SW in alignment with the dry valley feature which adjoins the site at point X (see Figure 2). The proto-stream is a dominant feature within the wetland, with intra-site topography trending downslope from the outer margins of the wetland to the proto-stream network to form a surface topographic micro-catchment. Its alignment with the dry valley feature feeding into the upslope margin of the wetland suggests a linked geomorphic origin to both features, and potentially a major flow path through the wetland from land to stream.

200
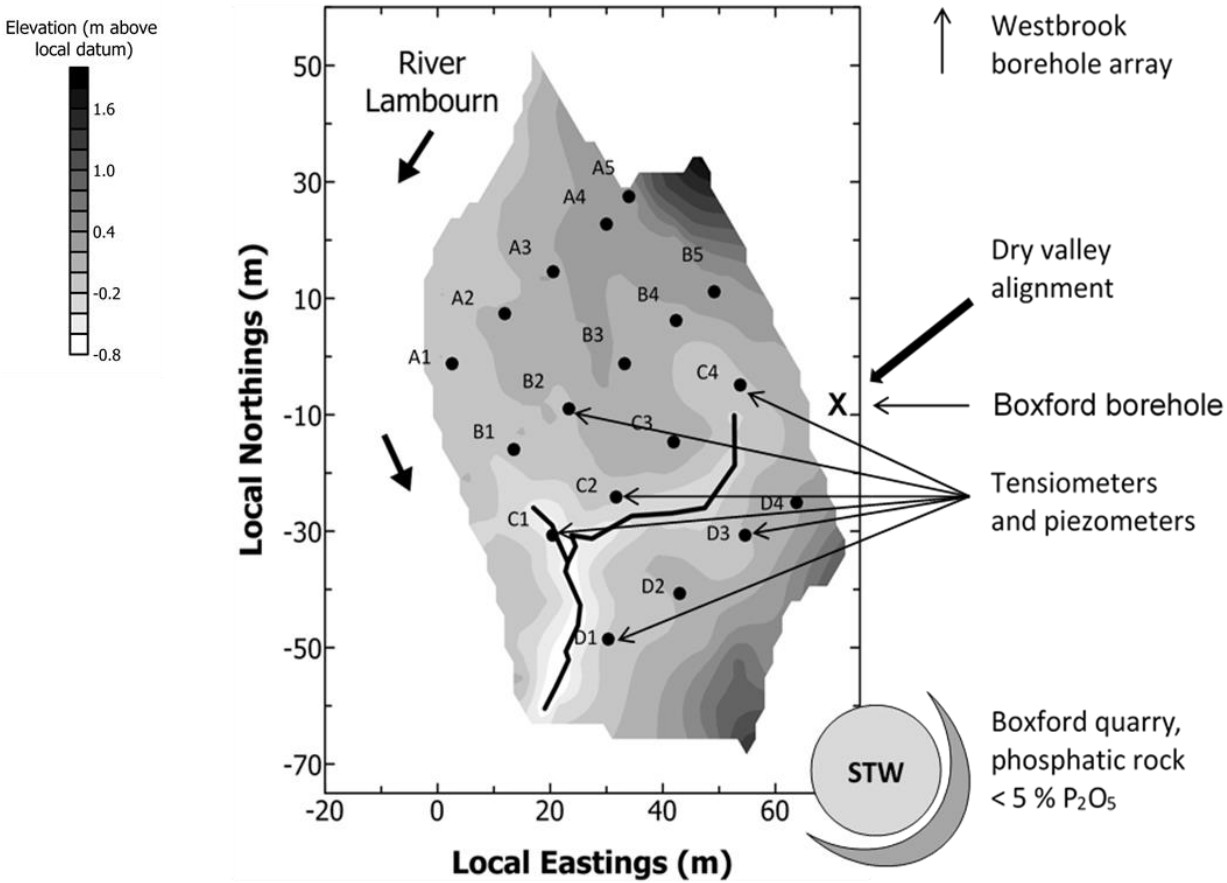
Figure 2. Site instrumentation, showing the location of the porous cup sampler nests, piezometers, and Boxford borehole.   Shading shows topographic variation across the site.

3.2. Characterisation of wetland functional zones using vegetation mapping

Plant community species composition varies across wetlands as a function of variations in site hydrology, hydrochemistry, soil character and subsurface stratigraphy where this impacts on the rooting zone. It can be used as a simple indicator of the effective biogeochemical cycling functional zones within the wetland and was therefore mapped in this programme to determine the extent to which it reflected observed nutrient cycling function identified in the traditional high resolution sampling and analysis programme implemented across the site under Step 1. Similarly, hydrologic and geomorphic character have been be used to classify the major functional zones within wetlands, stemming from the HGMU classification scheme for wetlands [28]. This provides a static classification of the major functional units within a wetland from a structural perspective, and has been widely applied internationally in wetland assessment schemes, though questions remain over whether, as a stand-alone tool, it can reflect the distribution of biogeochemical function at a process scale in wetlands. Mapping of these variations, and classification using the HGMU and UK National Vegetation Classification (NVC) scheme [45] was therefore undertaken. The site is dominated by sparse stands of *Phragmites australis* along the NW-S margins of the site adjacent to the River Lambourn, with *Phalaris arundinacea*, *Glyceria maxima* and *Carex paniculata* common in the wetter and lower lying Tall Grass Washlands (NVC G18) close to the proto-stream channel and the adjacent road. To the NW of the site, Tall Herb Fen (NVC M16) is the dominant vegetation community, dominated by *Filipendula ulmaria*, *Glyceria maxima* and *Urtica dioica.*

The HGMU classification effectively separated the higher margins of the site from the agricultural land upslope, the hawthorn and blackthorn scrub which occurs along the higher upslope margins

224   of the wetland, the public footpath running NW to SE across the top of the site, the private fishing
225   bank (mowed grass) running along the western edge of the wetland, and the lower lying land of the
226   wetland proper, but it makes no distinction between the wetter and drier areas of the wetland
227   proper, nor of those areas lying along the proto-stream channel which is the dominant surface flow
228   pathway through the site, where bypass flow is likely to lead to minimal contact between the
229   nutrient load delivered from the catchment and the biological communities of the wetland. The
230   NVC provides a finer level of distinction, separating the higher and drier areas of the wetland to the
231   W of the site from the lower lying areas of the wetland to the S and E, but still does not distinguish
232   the wetter areas along the line of the proto-stream channel as a potentially distinct functional zone.

233   *3.3. Characterisation of subsurface stratigraphy using geophysical techniques*

234   Geophysics can provide spatial information on the continuity and structure of the wetland
235   subsurface zone [31, 46-48]. We therefore used electrical resistivity tomography (ERT) and ground
236   penetrating radar (GPR) to characterise the subsurface stratigraphy of the wetland [49]. The
237   geophysical surveys were undertaken at the start of the programme along a number of transects
238   across the site. Figure 2 shows the location of these transects, the results from which are reported
239   here. Electrical resistivity was measured in January 2004 using linear electrode arrays, 2 m spaced,
240   in the Wenner configuration. Data were inverted to a 2-D resistivity image an Occam's based
241   inversion [50]. GPR surveys were performed in June 2004 using 200 MHz antennae, with 0.1 m
242   spacing between 0.5 m separation common offset measurements. GPR two way travel times of
243   reflections were converted to depths using velocities derived from common mid-point (CMP)
244   surveys along the transects.

245   Example results from the ERT and GPR are presented in Figure 3 and 4, along with results from
246   shallow soil coring. The soil coring revealed a typical sequence of 0.5 to 1 m of alluvium overlying a
247   1 to 2m thick gravel layer, which lies above weathered Chalk (noted to the 4.5m maximum depth of
248   the auger profiles).

249

250
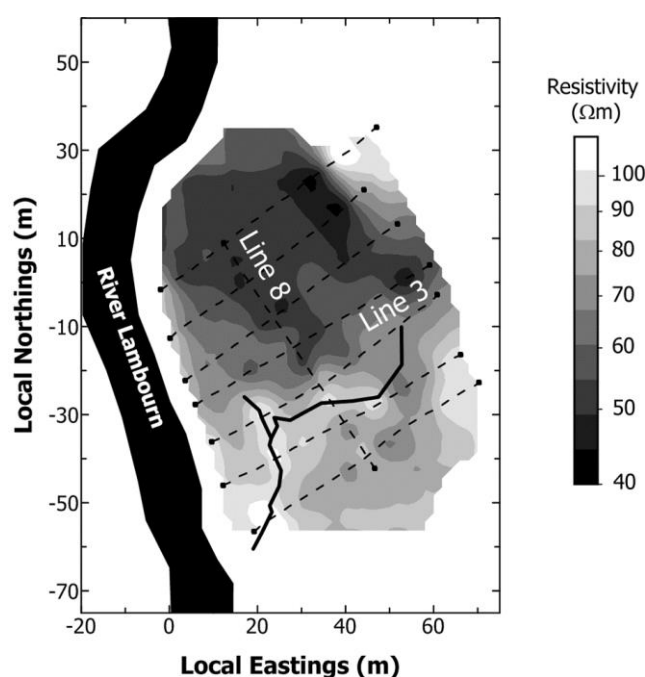
Figure 3. Resistivity variation across the site (between 0.65 m and 1.0 m below ground level)
determined from resistivity images computed from ERT surveys along the eight transects (locations
shown as broken lines).

254   The ERT images, presented in Figure 4, revealed a shallow (0.5 to 1 m thick) electrically conductive
255   (low resistivity) surface layer, which clearly coincides with the alluvial soil horizon. The ERT
256   images are, by the nature of the method, over-smoothed.   However, the GPR provides evidence of
257   a clear continuous reflector that matches the alluvium/gravel boundary. Below this boundary the
258   resistivity increases rapidly to a maximum of between 150 and 200 Ωm at 2 to 3m depth and then
259   decreases to a relatively uniform 80 Ωm. The transition from gravel to Chalk is not seen in the GPR
260   profiles because of (1) high attenuation and (2) scatter resulting from the coarse sediments in the
261   gravel layer.

262   The geophysical surveys reveal that the thickness of the alluvial soil horizon varies across the site
263   and, in particular, thins near the proto-stream channel. The ERT images show that close to the
264   stream channel the gravel layer is relatively resistive. We attribute this to a greater abundance of
265   coarser, clean gravel noted from soil cores near the channel. The gravels away from the channel
266   were noted to contain much greater fines content, with clear layers of putty chalk, which will lead
267   to a similar low resistivity electrical signature as the underlying Chalk. In fact Musgrave and
268   Binley, through time-lapse monitoring of one ERT transect over one year, revealed evidence of
269   potential groundwater connection to the proto-stream channel through observations of the
270   suppression of subsurface temperature dynamics, attributed to direct connection to stable
271   groundwater temperatures [49].

272
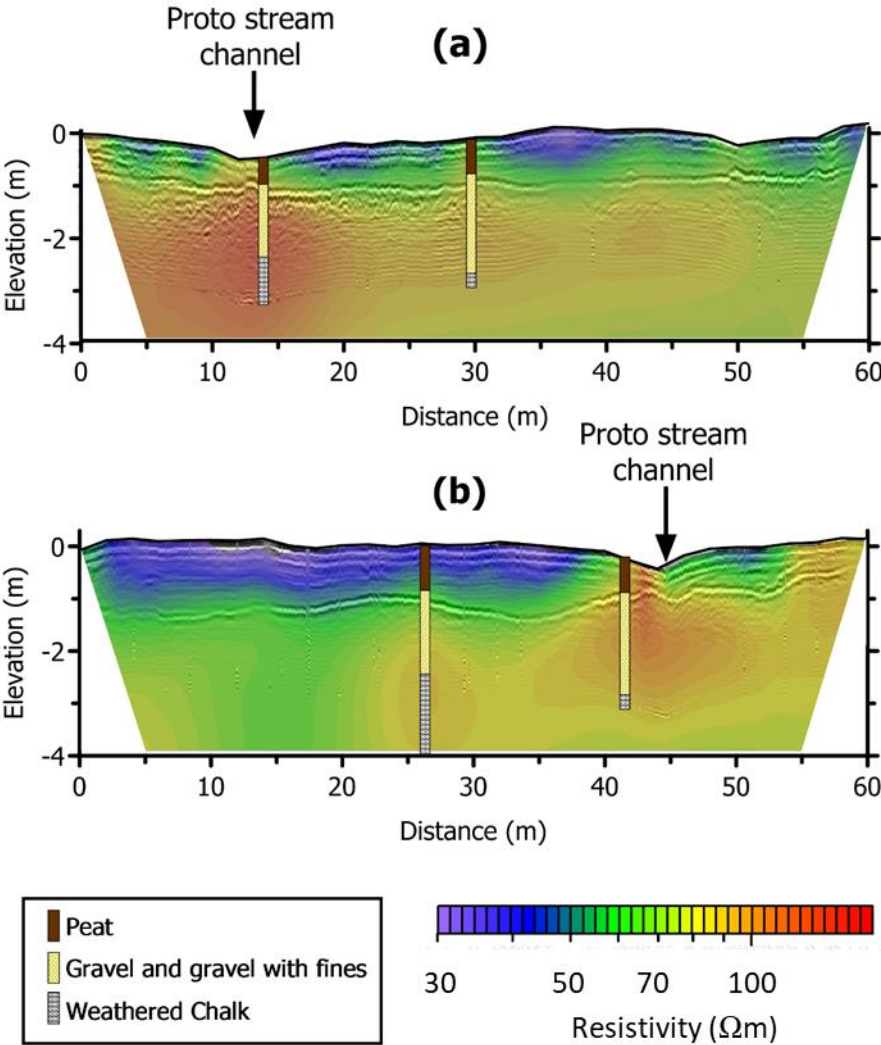
273   **Figure 4.** Electrical resistivity tomography (ERT) and GPR results along (a) Line 3 and (b) Line 8
274   (see Figure 3 for location of lines).   Also shown are soil core logs taken along the two transects.

275   Note that there is also a coincidence between the composition of the plant community distribution
276   and the ERT survey findings, with much lower resistivity occurring in the area to the NW of the site
277   supporting Tall Herb Fen (NVC M16) than in the SE where Tall Grass Washlands (NVC G18)
278   dominate. Both likely reflect the wetter conditions and thinner layer of alluvial soils in the area of
279   the wetland closest to the proto-stream channel. Thus the geophysical surveys provide a more
280   detailed discrimination of the surface structure and distribution of hydrological functional zones
281   across the wetland than the HGMU approach, and appear to reflect the variation of wetland
282   vegetation character across the site.

283   *3.4. Characterisation of the biogeochemical functional zones using nutrient speciation chemistry, major ion*
284   *geochemistry and isotope geochemistry approaches*

285   3.4.1.   Site instrumentation

286   The decision to continue to use the Boxford wetland for this study was made in part because the
287   wetland geophysical structure and vegetation community are fairly typical of tall herb wetlands in
288   lowland permeable UK catchments, and also because of the location of the wetland at the base of a
289   key dry valley feature. The wetland was instrumented with a 5 x 4 network of nested Teflon porous
290   cup samplers which were installed in the soil matrix at 20, 40 and 60 cm below the wetland surface.
291   Teflon porous cup samplers were selected as they are chemically inert for the purposes of N and P
292   hydrochemistry investigations. These were sampled weekly from 26-Sept-03 to 15-Jan-05,
293   augmenting earlier sampling and nutrient analysis at the site [5]. Adjacent to each sampler nest,
294   dipwells were installed to the upper surface of the gravels underlying the wetland and used to
295   monitor water level fluctuations across the wetland weekly over the same time period.

296   Further porous cup samplers were installed along the line of the proto-stream channel, and
297   piezometers were installed into the underlying gravel layer to a depth of 2.0 to 3.5m below the
298   wetland soil surface at 5 locations. Samples were collected from these additional samplers
299   fortnightly in the final year of study. Access was also arranged to the 30 m deep Boxford borehole
300   adjacent to the wetland (marked X, Figure 2), with samples collected approximately monthly over
301   the period of monitoring. Analyses conducted on these samples confirmed that the groundwater
302   feeding through the wetland was from the underlying Chalk aquifer. Finally, in the last 6 months of
303   the study, a fifth line of porous cup samplers were installed along the roadside margin of the site
304   (see Figure 2) and sampled weekly, following the discovery of phosphorus and ammonium
305   enriched water along this margin which might be derived from seepage from a Sewage Treatment
306   Works (STW) located in an adjacent quarry or alternatively from phosphatic rock (5% P_2O_5)
307   recorded in this same quarry in an earlier study [51].

308   All of the samplers were surveyed in, relative to Ordnance Datum (OD), so that the absolute
309   elevation of the sampler, the vegetation community with which the sampler was associated, and its
310   relative proximity to the River Lambourn, the proto-stream channel and the road were known.   A
311   summary of sampling undertaken at the site is given in Table 1.

312   3.4.2.   Samples analysis to determine N species and P fraction concentrations in porewaters

313   In order to provide a full assessment of the cycling of nitrogen (N) and phosphorus (P) within and
314   through the wetland, all water samples collected from the wetland soil water, gravels and Chalk
315   were analysed to determine the concentrations of dissolved inorganic N (NO_3-N, NO_2-N, NH_4-N),
316   dissolved organic N (DON), and soluble reactive P (SRP, measured as PO_4-P), dissolved organic P
317   (DOP), and dissolved organic C (DOC) as Non-Purgeable Organic Carbon (NPOC), using standard
318   operating procedures and quality assurance protocols [22,24]. Samples were collected over a period
319   of 4 hours at each sampler nest on a weekly basis and returned to the laboratory at 4 $^\circ$C in the dark.
320   All samples were analysed within 24 hours of collection using a Chemlab System 40 autoanalyser to

321

322   **Table 1.** Sampling programme details

Sampling programme	Dates
Weekly water sampling:	
Water level at dipwells	16-Sept-03 – 31-Jan-05
Soil porewaters in wetland alluvial soils, 23 nests, 20, 40, 60cm	23-Sept-03 – 31-Jan-05
Water levels in wetland alluvial soils, 23 dipwells,	23-Sept-03 – 31-Jan-05
Proto-stream surface water, 3 sites,	07-Jan-04 – 31-Jan-05
Boxford borehole, 30m depth	07-Jan-04 – 31-Jul-04
	01-Dec-04 – 31-Jan-05
Piezometers in gravels, 5 sites, 2m, 3m depth	01-Jul-04 – 31-Jan-05
Puncture tensiometers in wetland alluvial soils	01-Sept-04 – 31-Jan-05
Plant sampling	
Plant species list	April 2004, June 2004
Biomass sampling, single and mixed species stands, monthly	Monthly
Structural measurements, including canopy height, stem density per unit area, average stem diameter recorded monthly	Monthly
Plant health monitored monthly using fluorimeter	Monthly
Wetland soil porewater and source water geochemistry	
Soil porewaters, piezometers, river and groundwater sampled twice and analysed to determine geochemical signature.	July 2004, Dec 2004
Isotopic signatures of wetland biogeochemical function	
$^{15}N/^{14}N$ and $^{18}O/^{16}O$ determined on water samples collected from porous cup samplers along transects, piezometers, borehole, river	October 2004
$^{15}N/^{14}N$ determined on vegetation samples collected along transects	October 2004

323

324   determine total oxidised N (TON = NO_3-N + NO_2-N), total ammonium (NH_4-N + NH_3-N) and SRP
325   as orthophosphate (PO_4-P). A second run with modified manifold yielded NO_2-N concentrations
326   which, when subtracted from TON generated NO_3-N concentrations for each sample. Samples were
327   then digested using a modified method to determine total dissolved N (TDN) and P (TDP)
328   concentrations [22,52]. This procedure oxidises all N in the sample using potassium persulphate
329   ($K_2S_2O_8$) in a strongly alkaline environment under high temperature and pressure, yielding nitrate
330   with some residual ammonium. The by-product of this reaction simultaneously creates the acidic
331   conditions needed to support the oxidation of P in the presence of $K_2S_2O_8$ to yield orthophosphate
332   under conditions of high temperature and pressure. The technique uses a CEM MARS-Express
333   Microwave Digestion Unit to create high temperature and pressure under controlled conditions.
334   Sample digests were then analysed to determine TDN as TON and TDP as PO_4-P using standard
335   methods. DON was then calculated as TDN minus total inorganic N (TON + NH_4-N). DOP was
336   calculated as TDP minus SRP. All sample digests were completed within 48 hours of sample
337   collection to minimise sample degradation prior to analysis and analysed within 72 hours of
338   collection.

339

340   3.4.3.   Sample analysis to determine major element geochemistry

341   Major element geochemistry was determined on a subset of these samples. This included samples
342   collected from the soil matrix, gravels and Chalk underlying the wetland, and from the adjacent
343   river reach, borehole, and a second borehole array at Westbrook Farm, located 300 m upstream
344   from the wetland, the data from which have been reported in detail in a number of earlier
345   publications [29,36]. These data were collected to help determine, using similarity indices, the likely
346   origins of waters draining through the wetland. As Chalk groundwaters are dominantly of Ca-
347   HCO_3 type and subject to a greater degree of incongruent dissolution with residence time, greatest
348   separation of trends in sample geochemistry is often best achieved by examining the ratio of base
349   cations, in particular the strontium/calcium (Sr/Ca) and magnesium/calcium (Mg/Ca) ratios. Higher
350   ratios are likely to be indicative of relatively longer residence time of Chalk groundwater, whereas
351   lower ratios are more typical of surface water input or rainwater.

352   Sampling was carried out using a small submersible pump and included on-site measurements of
353   temperature, specific electrical conductance (SEC) and alkalinity (by titration) as well as pH,
354   dissolved oxygen (DO) and redox potential (Eh). The latter parameters were measured in a flow-
355   through cell to minimise contact with the atmosphere. To ensure a representative sample was taken,
356   a minimum of 2-3 well volumes was removed from the piezometers prior to sampling and samples
357   were collected after on-site readings of temperature, conductivity, pH, DO and Eh had stabilised.
358   Samples for major ion chemistry were collected in Nalgene bottles and filtered through 0.45 μm
359   membrane filters. Analysis of major cations and sulphate was carried out by ICP-OES and anions
360   were analysed by automated colorimetry.

361   3.4.4.   Sample analysis to determine isotope ratios in soil water, alluvial gravels and
362   wetland vegetation

363   Isotope geochemistry has a wide range of applications in the study of the natural environment, and
364   the study of natural abundance isotopic ratios has become a well- established technique for the
365   investigation of elemental cycling in a wide range of ecosystems. In the study of nutrient cycling
366   and flux in wetland systems, the isotopes commonly studied include the $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios
367   in nitrate, where the lighter isotopes are preferentially taken up in vegetation over the heavier
368   isotopes. It is also used as an indicator of the denitrification and ammonia volatilisation processes.
369   Because molecular bonds involving ^{14}N are weaker than identical bonds involving ^{15}N, reaction of
370   ^{14}N molecules is kinetically favoured relative to reaction of ^{15}N molecules. As a consequence, in
371   bacterially-mediated processes such as denitrification, the $\delta^{15}N$ value of the instantaneously-formed
372   product is lower than the value of the substrate. During denitrification, the residual nitrate becomes
373   enriched in both ^{15}N and ^{18}O, and combined $^{15}N/^{14}N$ and $^{18}O/^{16}O$ analysis of nitrate has been shown
374   to be a particularly effective way of identifying denitrification in riparian systems [53-55].

375   Samples of waters entering the wetland at the gravel-alluvial boundary, and accumulated within
376   the soil porewaters of the wetland alluvial soil can be collected as transient indicators of these
377   processes, but a longer term indication of the distribution of N metabolism across a wetland can
378   also be derived by examination of the isotope ratios in non-migratory resident organisms in a
379   wetland, such as the wetland plant community. In the latter case, variation in the stable isotope
380   composition of the plants across the wetland will reflect the dominant processes operating in the
381   subsurface zone. This approach was therefore adopted in this study, with leaf material of plants
382   collected for $^{15}N/^{14}N$ analysis from a number of sites on the wetland and River Lambourn. Sampling
383   concentrated on a single species, *Phalaris arundinacea*, to avoid inter-species variability. Water
384   samples were collected for $^{15}N/^{14}N$ and $^{18}O/^{16}O$ analysis of nitrate from the Boxford borehole, the
385   Westbrook borehole array, the proto-stream, and a few of the piezometers.

386   Nitrate in waters was collected on anion exchange resins and processed to silver nitrate [56,57].
387   Plant samples, comprising the top 4-5 leaves of a plant were dried at 80 °C and homogenised.
388   $^{15}N/^{14}N$ ratios of the silver nitrate and plants were analysed by combustion in a Flash EA on-line to a
389   Delta Plus XL mass spectrometer (ThermoFinnigan, Bremen, Germany), with ratios calculated
390   as $\delta^{15}N$ values versus air (atmospheric N_2) by comparison with standards calibrated against IAEA N
391   1 and N 2. $^{18}O/^{16}O$ ratios of the silver nitrate were analysed by thermal conversion to CO gas at 1400
392   °C in a TC-EA on-line to a Delta Plus XL mass spectrometer (ThermoFinnigan, Bremen, Germany),
393   with ratios calculated as $\delta^{18}O$ values versus VSMOW (Vienna Standard Mean Ocean Water) by
394   comparison with IAEA-NO3.

395   Data generated from each of these methods are deposited in the Natural Environment Research
396   Centre (NERC) Environmental Information Data Centre (EIDC) LOCAR repository and are publicly
397   available along with subsequent monitoring at the Centre for Ecology & Hydrology (CEH) River
398   Lambourn Observatory at Boxford here: http://eidc.ceh.ac.uk/ and from from the lead author.

399   **3. Results**

400   *3.1 Characterising wetland hydrological function through hydrological monitoring and geochemical indices*

401   An indication of the variation in site hydrology over the sampling period is given in Figure 5.   Here,
402   water level variations at each sampler nest are presented for the period 16-Sept-03 to 16-Jan-05.
403   Along each SW-NE transect (A, B, C, D – see Figure 2) maximum water level is recorded in the period
404   March-April, in accordance with observations of water level in the adjacent reach of the Lambourn
405   [5,35].   Minimum water level occurs in the early autumn (September to October) in both years
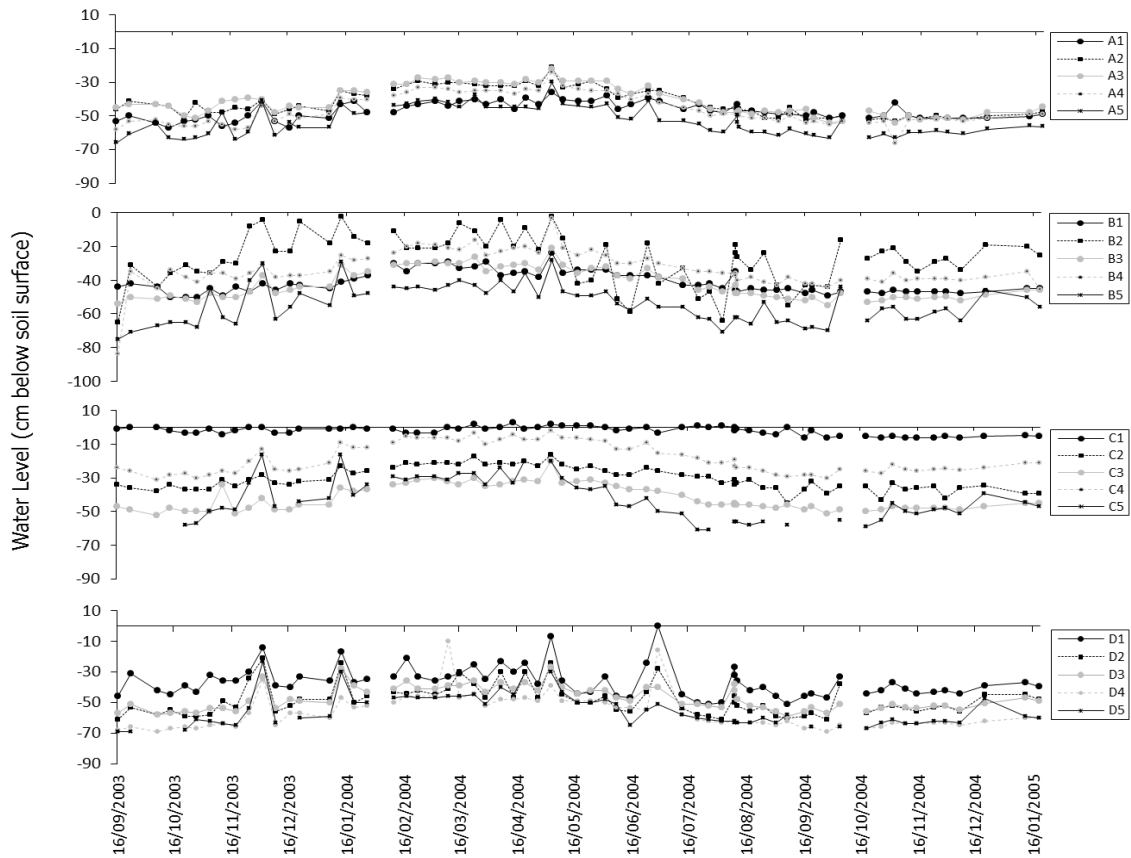406   studied.

407

411   **Figure 5.** Water level fluctuations across the Boxford wetland relative to soil surface (cm). Sampler
412   nest locations are shown in Figure 2.

413   Groundwater level was typically 30-60 cm below the wetland soil surface along transect A, the closest
414   to the river (Figure 2), indicating that sampler nests installed at 20cm depth at A1-A5 will have
415   consistently sampled the unsaturated zone of the soil. Samples collected at 20 cm depth along this
416   transect are likely, therefore, to include water stored in both micropores and macropores in the soil
417   matrix. At 40 cm depth the samples collected in the period January to May will have been taken from
418   the saturated soil zone, but for the remainder of the year these will have been taken from the
419   unsaturated soil zone. At 60 cm virtually all samples will have been taken from the saturated soil
420   zone. According to theories of nutrient hydrochemical function derived from the earlier work on the
421   Boxford wetland the unsaturated zone soil samples are likely to include water stored over a relatively
422   long period of time in the wetland, and therefore are likely to exhibit a significantly different
423   (processed) hydrochemistry than that of possible source waters (river water, groundwater) [5].
424   Conversely, water samples taken from the saturated zone are likely to include substantial
425   contributions of source water, and the chemistry of these waters is likely to show a greater similarity
426   to that of potential contributing source areas.
427
428   Water level along transect B, aside from that recorded at B2, is similar to that recorded along transect
429   A. B2 shows a more flashy response in terms of water table fluctuations, as does B5 to a lesser extent.
430   When piezometers were installed at B2, the coring evidence revealed the presence of putty chalk
431   below the wetland at this site, indicating poor connectivity between wetland soil porewaters and
432   groundwater, which is consistent with the geophysical survey findings for the site [49]. The data
433   collected for B2 are likely to show unsaturated zone chemistry typical of a longer hydraulic residence
434   time in the wetland. Of the remainder of the samplers, only those at B5 will have consistently been
435   taken from the saturated zone of the wetland and are therefore likely to show a chemistry consistent
436   with other areas of the wetland well-connected with potential source waters.
437
438   Water level along transect C which runs close and parallel to the proto-stream channel (Figure 6) is
439   variable along the transect. C1 is located immediately adjacent to the proto-stream channel and the
440   water level data indicate saturated conditions at this site for the whole of the sampling period and at
441   all three sampling depths. The hydrochemistry data for C1 are therefore likely to show similarity to
442   the data for proto-stream channel itself and other saturated sites across the wetland. Samplers at C4
443   are also very close to the proto-stream channel and the water level data indicate that here also the
444   samplers at all 3 depths are likely to have extracted samples from the saturated rather than the
445   unsaturated zone. The nutrient chemistry and geochemistry of these samples would be expected to
446   show similarities with that of the proto-stream channel and other source waters. At C2, C3 and C5
447   the sampler nests are further away from the proto-stream channel. In all three sites the samplers at
448   60 cm are likely to be sampling from the saturated zone, with samplers at 20 and 40 cm at C3 and C5
449   sampling from the unsaturated zone over the period July to February.
450
451   Water levels along transect D, adjacent to the road, indicate that few of the samples drawn at 20 cm
452   are likely to have been from the saturated zone. At 40 cm most of the samples collected from
453   September 03 to July 04 are likely to have been drawn from the saturated zone. At 60 cm all samples
454   are likely to have been drawn from the saturated wetland soils. This is again likely to be reflected in
455   the nutrient chemistry and geochemistry of the samples drawn from these sites and the extent to
456   which this is like that of potential source waters to the wetland.
457
458   These variations in the hydrological function, soil wetness and redox are picked up in the
459   geochemistry results produced for the site and presented in Figure 6. Sample transects are shown in
460   Figure 2. A total of 66 elements were determined on samples collected along these two transects
461   running from A2 to D2 and from C1 to C5. Samples were also collected from the proto-stream
462   channel, the Boxford borehole adjacent to the wetland (marked X on Figure 2), and the Westbrook
463   borehole array, 300 m upstream from the wetland. Good separation of trends in sample geochemistry
464   are evident in the Sr/Ca and the Mg/Ca ratios for these samples. Samples from the groundwater

465 boreholes, the proto-stream channel and the three porous cup samplers at C1 show a similar
466 geochemistry, with high Sr/Ca and Mg/Ca ratios. Water samples were collected from samplers in the
467 unsaturated zone of the wetland, at a greater distance from the groundwater, river and proto-stream
468 (A2, B2, C3 and C5) have more depleted Sr/Ca and Mg/Ca ratios, indicative of waters which, if
469 originally derived from groundwater sources, have been subject to modification (e.g. ion exchange)
470 while resident in the wetland soil matrix. The remaining samples, taken from C4, C2, and C2, which
471 are closer to the proto-stream channel than those at A2, B2, C3 and C5, but further from the channel
472 than those at C1, show an intermediate chemistry.
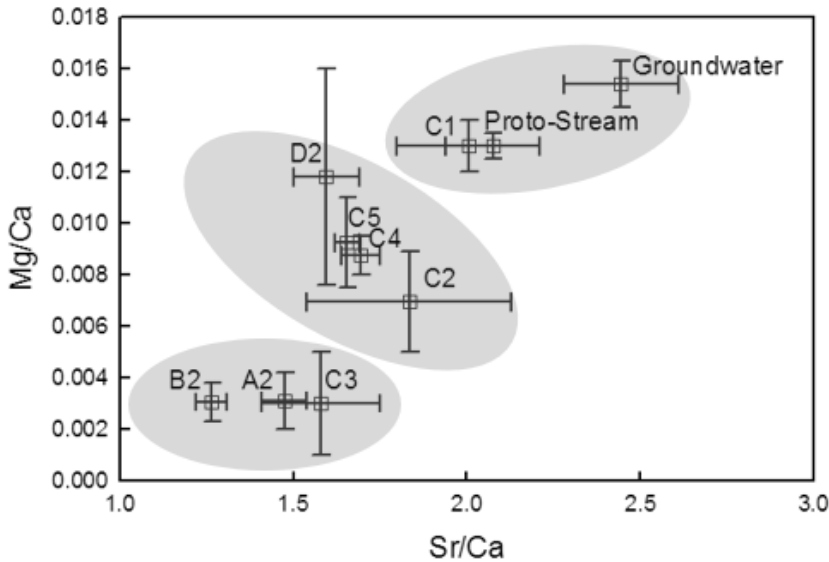
473
474 **Figure 6.** Wetland porewater and source water geochemical analyses. Mg and Ca measured in mg/L,
475 Sr measured in µg/L.

476
477 The geochemical survey of the wetland soil waters appears to provide a robust characterisation of
478 the spatial distribution of the major functional zones within this wetland, where there is a distinct
479 signature, providing a relatively simple technique for such systems. It requires far fewer samples in
480 both space and time than the detailed high-resolution sampling and analysis needed to characterise
481 the full range of nutrient species in wetland porewaters as these vary over space and time. Analytical
482 approaches to determine major ion chemistry are also more straightforward. This technique is likely
483 to work well in other calcareous wetlands fed by $CaCO_3$-rich groundwater. In wetlands receiving
484 water from complex sources, alternative approaches to characterise subsurface chemical transport
485 and wetland-stream-aquifer interactions under a wider range of conditions would use ^{222}Rn [58,59],
486 $^3H/^3He$ tracer techniques [60], or $^{87}Sr/^{88}Sr$ [61] while analysis for chlorofluorocarbons (CFCs) and
487 sulphur hexafluoride (SF_6) have also been shown a valuable and relatively inexpensive approach for
488 calculating the age of waters in the groundwater-dependent wetlands [62,63].

489
490 The geochemical analyses undertaken here appear to support the hypothesis that water stored for
491 longer periods of time in the wetland soil matrix will undergo greater transformation in terms of its
492 chemistry, and water which is more immediately derived from source waters is likely to share similar
493 geochemical characteristics to the source waters. This suggests that the nutrient chemistry and
494 geochemistry of waters within the wetland is likely to vary according to hydraulic residence time.
495 It can also be postulated that waters sampled from the rooting zone of the wetland (typically those
496 taken at 20 cm, with some rooting down to 40 cm in these tall herb fenlands) are likely to show greater
497 depletion in terms of their nutrient concentrations than those from samplers below the rooting zone,
498 and that depletion of inorganic nutrient chemistry and enrichment with dissolved organic nutrient
499 fractions is likely to be most dominant in the rooting zone. This pattern is evident in the data collected
500 from the porous cup samplers under baseflow conditions. An example of this pattern in the nutrient
501 hydrochemistry of the wetland is given for 3 August 2004 in Figures 7 (N) and 8 (P).
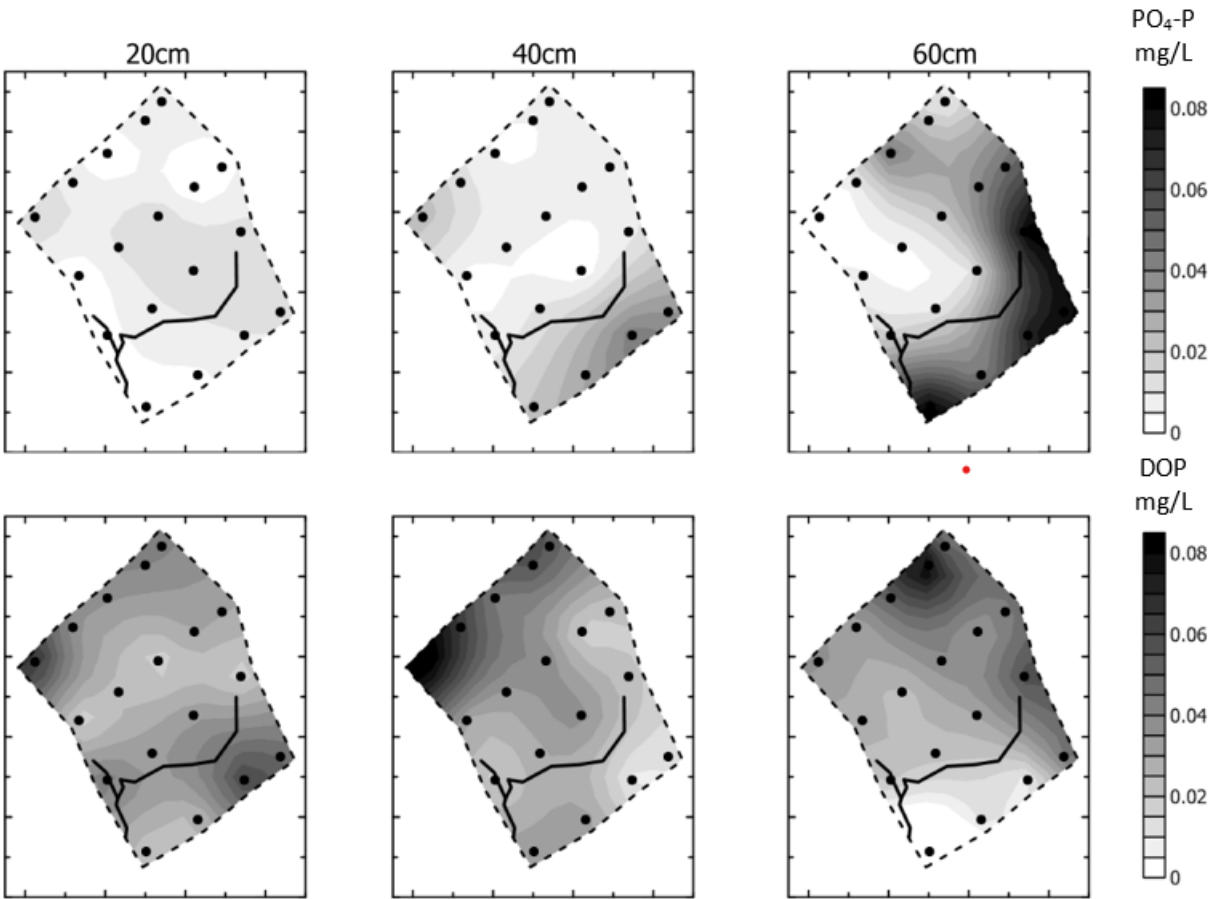
502
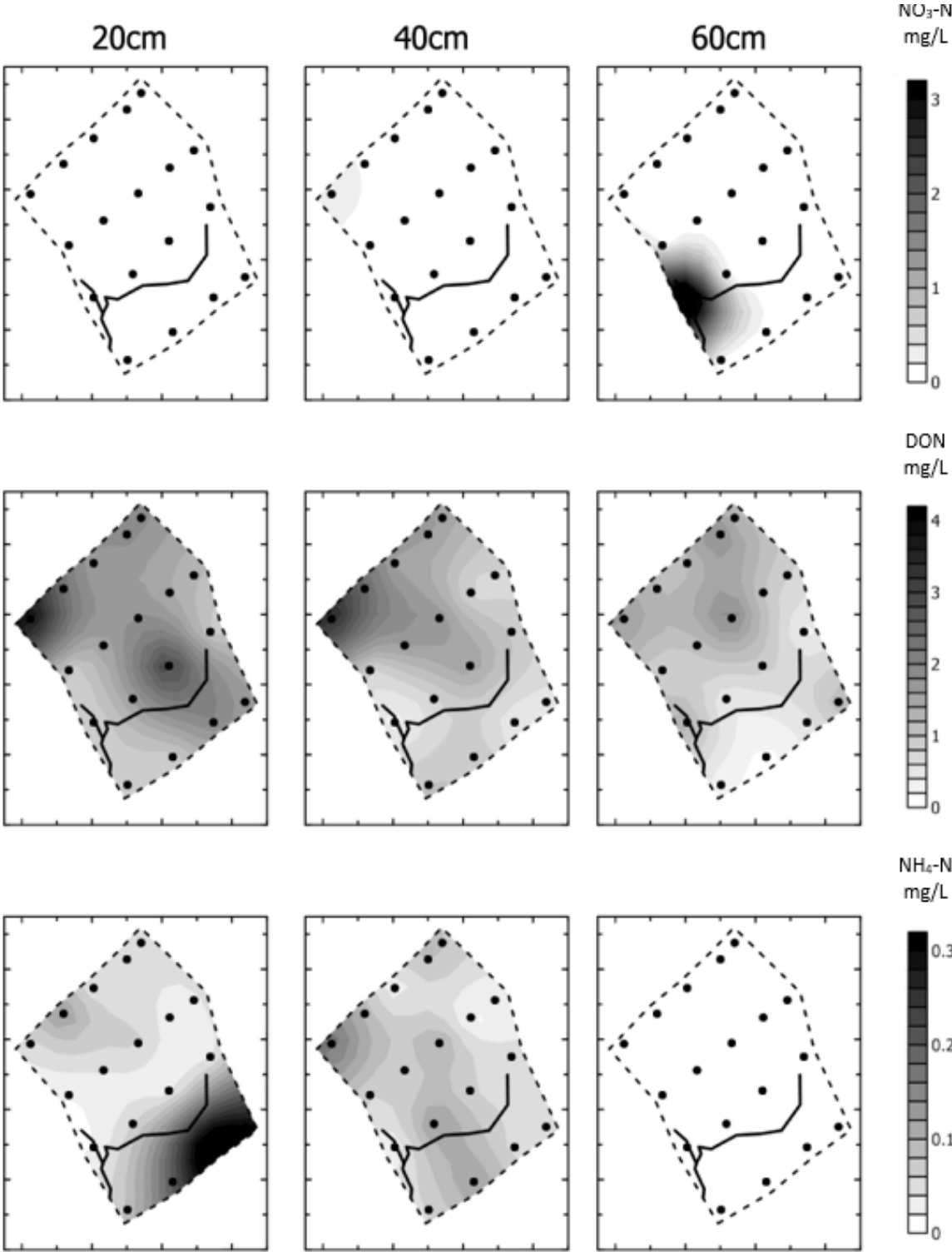
503
504
505   **Figure 7.**   Soil porewater N speciation under baseflow conditions (03-Aug-04). Sampler
506   locations shown as black dots, as detailed in Figure 2.
507
508

Figure 8. Soil porewater P fractionation under baseflow conditions (03-Aug-04).). Sampler locations shown as black dots, as detailed in Figure 2.

These data were collected in the growing season at the end of the summer period, when plant uptake was likely to be depleting inorganic nutrient concentrations in the rooting zone, and there had been plant uptake and microbial breakdown of dead organic matter in the surface soil layers of the wetland during the warm summer months. The nitrogen chemistry data reflect these controls, with nitrate present only at 60 cm depth, and only at sampler C1, close to the proto-stream channel, and reflecting continued input of nitrate rich groundwater to this part of the wetland. Across the remainder of the site there was a substantial build-up of DON, particularly in those areas of the wetland with the longest hydraulic residence time along transects A and B and at site C3. The greatest accumulation of DON occurred in the rooting zone (20 cm). There was some ammonium present in the wetland, notably along transect D, and the proximity of these samplers to potential leakage from the adjacent STW at this site, or the possibility of ammonium volatilisation from the STW and local deposition along this transect is the most likely cause of this pattern, though it could also be due to mineralization of DON in the rooting zone.

The P fractionation data collected on this day showed SRP enrichment along transect D at 60cm depth. The most likely cause is seepage of SRP rich waters from the adjacent STW, though it is possible that the phosphatic rock present at the top of this exposed Chalk section may also be responsible for part of the background P concentrations along this transect [51]. Generally, however, there is <0.02 mg/L PO_4-P in the wetland in the rooting zone, and there is some evidence of DOP build up at all depths across the site, with the highest concentrations in those areas of the wetland located furthest from the faster flushing proto-stream corridor, where water input from the groundwater aquifer and rainfall has the longest hydraulic residence time.

537
538 These patterns are clearly evident in the baseflow N chemistry data presented in Figure 9. For each
539 porous cup sampler, piezometer, the Boxford borehole and the River Lambourn adjacent to the
540 wetland, the mean annual nitrate, DON and ammonium concentrations are presented. The X axis is
541 labelled with samplers at 20 cm labelled 'a', 40 cm labelled 'b' and 60 cm labelled 'c'. Thus, the first 5
542 columns are A1 (20 cm), A2 (20 cm), A3 (20 cm), A4 (20 cm) and A5 (20 cm), where A1a refers to the
543 sampler at transect A, row 1, 20 cm depth.
544
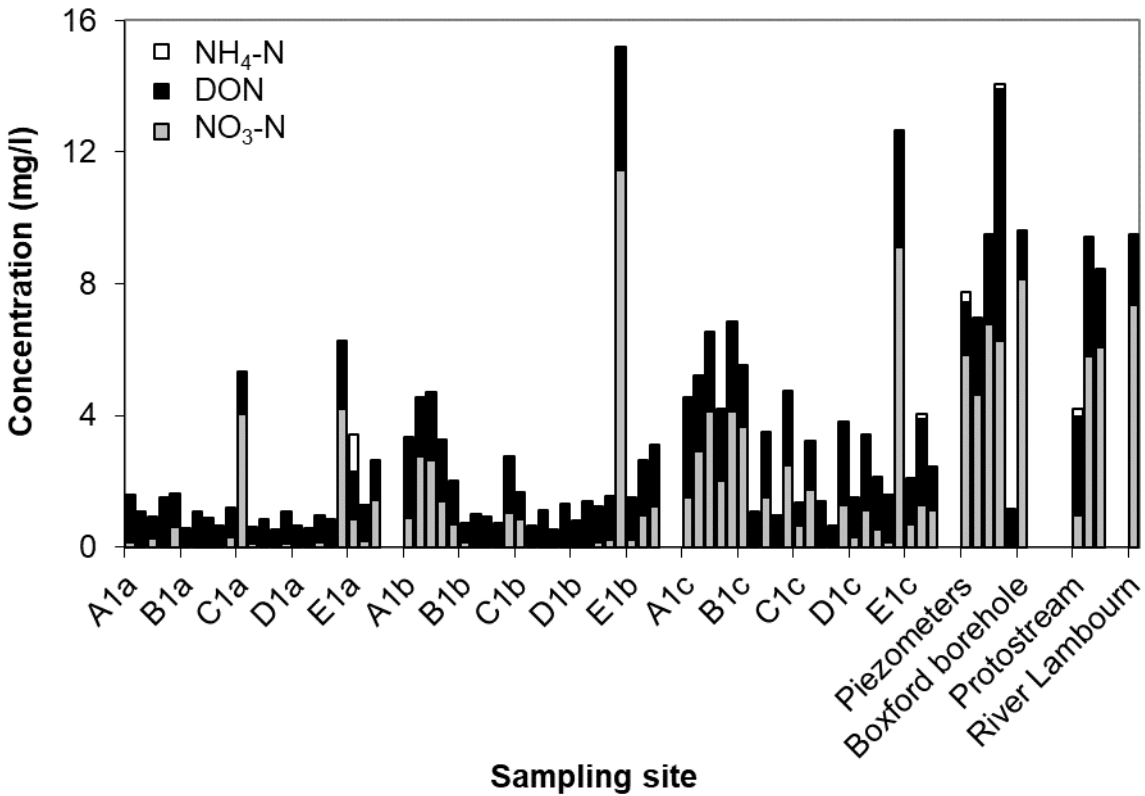
545
546
547 **Figure 9.** Baseflow N chemistry in the Boxford wetland, 16-Sep-03 to 16-Jan-05.
548
549 A number of trends are apparent in this figure:
550
551 • Groundwater nutrient chemistry is predominantly in the form of nitrate, with a mean
552   concentration of 9 mg/L nitrate (NO_3-N) and 1.5 mg/L DON in the Boxford borehole over the
553   period 16-Sept-03 to 16-Jan-05. Similar chemistry is evident in the proto-stream, the River
554   Lambourn and the piezometers at 2 - 3.5 m below the wetland. The data suggest that all of
555   these sites share a similar source, and it is likely that they are all recently derived from
556   groundwater sources.
557 • Mean DON concentrations in the River Lambourn, the piezometers and the proto-stream all
558   show some enrichment over the 1.5 mg/L DON recorded at the Boxford borehole adjacent to
559   the wetland, and this is likely to reflect mixing of groundwater with enriched soil porewaters
560   draining from the wetland under baseflow conditions, and/or biogeochemical cycling within
561   the River Lambourn [5, 35-37].
562 • Samplers within the wetland soil matrix which are very close to the proto-stream channel also
563   share this chemistry, notably those at C1 (20 cm), and at all three depths at D5. Sampler nest
564   D5 is very close (<5 m) to the Boxford borehole, and lies outside the wetland proper, in the line
565   of blackthorn scrub at the margins of the site. It may be that this indicates shared source water
566   with that sampled from the borehole.

567 • Samplers further from the proto-stream channel, with a longer hydraulic residence time, show
568 depletion of inorganic N under baseflow conditions, and this is likely to reflect both plant
569 uptake and gaseous N loss through denitrification.
570 • These samples also show enrichment with DON concentrations up to a maximum of 4 mg/L.
571 • Samples collected at 60 cm depth along transect A show a chemistry similar to that of the
572 adjacent River Lambourn, with a notable increase in nitrate concentrations from 20 to 60 cm
573 depth at all stations along this transect. This may reflect riverine incursion from the Lambourn.
574
575 *3.2 Identifying biogeochemical cycling hotspots using isotope geochemical analysis*
576
577 The extent to which these patterns could be explained by faster flushing through the proto-stream
578 channel corridor and denitrification in the wetter parts of the wetland was explored using isotope
579 geochemical analysis of wetland soil porewaters, river water and groundwater samples and plant
580 material collected from the site in October 2004. The $\delta^{15}N$ and $\delta^{18}O$ values for nitrate in water samples
581 are presented in Table 2 and Figure 10. There are no data for the porous cup samplers, where low
582 nitrate concentrations combined with low water volumes resulted in insufficient nitrate for analysis
583 by the silver nitrate method. Future studies employing these types of samples would benefit from
584 using the 'denitrifier' method of nitrate analysis, which requires much smaller samples [64].
585
586 **Table 2.** $^{15}N/^{14}N$ and $^{18}O/^{16}O$ composition of nitrate in water samples. Boxford borehole is marked X
587 on Figure 2. Westbrook array is 300m upstream from piezometer A5 on Figure 2.
588

	$\delta^{15}N$ (‰ vs. AIR)	$\delta^{18}O$ (‰ vs. SMOW)	NO$_3$-N mg/L
Boreholes			
Westbrook array			
PL26 D-1, SO4-01126	+3.9	+2	5.84
PL26 D-2, SO4-01127	+4.0	+6	6.29
PL26 N-4, SO4-01131	+4.3	+1	6.87
PL26 H-2, SO4-01134	+3.9	+5	6.09
PL26 E-2, SO4-01138	+4.1	+3	6.05
Boxford borehole (sampled 02-Oct-04)	+4.0	+3	
Boxford wetland (sampled 27-Oct-04)			
B2 'short' piezometer	+4.5	+2	5.76
C1 piezometer		+4	4.70
C1 proto-stream lower	+5.0	+3	5.58
C5 piezometer	+4.6	+3	6.67
D1 proto-stream main	+4.8	+1	5.60

589
590
591 Water samples from the Westbrook borehole array yielded nitrate with consistent isotope
592 composition averaging $\delta^{15}N$ = +4.0 ± 0.2 ‰ (1 SD) and $\delta^{18}O$ = +3 ± 2 ‰; the Boxford borehole adjacent
593 to the wetland, yielded groundwater of identical composition (Table 2). Waters sampled from three
594 piezometers and two positions on the proto-stream also exhibited a consistent isotope composition,
595 $\delta^{15}N$ = +4.7 ± 0.2 ‰ (1 SD) and $\delta^{18}O$ = +3 ± 1 ‰ (Table 2). Their $\delta^{15}N$ values are therefore only very
596 slightly higher than (and their $\delta^{18}O$ values unchanged from) those of the groundwater samples.
597

598 The $\delta^{15}N$ values for plants are shown in Figure 10. Leaves from three individual *Phalaris arundinacea*
599 plants were collected at 16 positions across the wetland close to porous cup nests, and at three sites
600 along the margins of the river. Values were again consistent, with no evidence of any spatial pattern
601 across the wetland (Figure 10), and no significant difference between the wetland (average $\delta^{15}N = +5.9$
602 ± 1.0 ‰ (1 SD)) and river margin (average $\delta^{15}N = +5.4 \pm 2.1$ ‰).
603
604
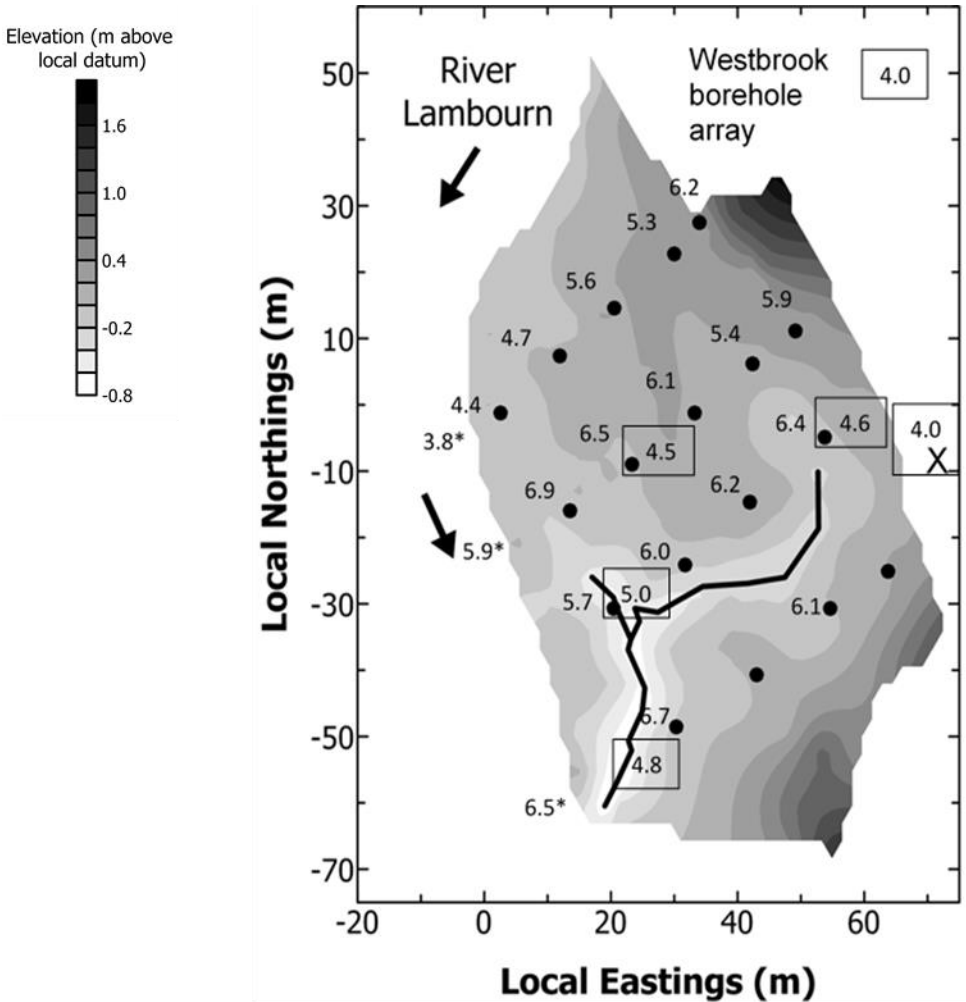
605
606
607 **Figure 10.** $\delta^{15}N$ values in the Boxford wetland. Data in boxes refer to $\delta^{15}N$ of nitrate in boreholes,
608 piezometers and the proto-stream. Data without boxes refer to average $\delta^{15}N$ of the top 4-5 leaves of
609 three individual *Phalaris arundinacea* plants. Numbers next to closed circles are for samples collected
610 close to ceramic cup samplers; numbers marked with an asterisk are for plants sampled from the
611 river bank.
612
613 The $\delta^{15}N$ values of the groundwater are within the range typical for non-denitrified nitrate in Chalk
614 aquifers [65, and Heaton, unpublished data]. The $\delta^{18}O$ values are also what would be expected for
615 unmodified nitrate originating from bacterial nitrification in the soils through which the waters
616 recharged [66]. Thus, if the stoichiometry of nitrate formation derives two thirds of the oxygen from
617 H_2O (average $\delta^{18}O$ value of water in the Westbrook borehole array = -7.2 ‰) and one third from
618 atmospheric O_2 ($\delta^{18}O = +24$ ‰ [67], we expect a nitrate $\delta^{18}O$ value of (⅔ • -7.2 ‰) + (⅓ • +24 ‰) =
619 +3 ‰; which is the same as the average measured value for groundwater nitrate in Table 2. Given the
620 consistency of the isotope composition of the groundwater nitrate samples in Table 2, and the
621 relatively homogeneous nature and long residence time of groundwater, we assume that $\delta^{15}N = +4.0$ ‰
622 and $\delta^{18}O = +3$ ‰ are representative of the values for groundwater nitrate input to the wetland over
623 the study period. The wetland piezometer and proto-stream waters analysed had essentially identical

624 $\delta^{18}O$ values, and $\delta^{15}N$ values only 0.7 ‰ higher, suggesting that these were derived from proximal
625 groundwater sources, rather than biogeochemically processed wetland or riverine waters.
626
627 During denitrification the reaction of nitrate molecules containing the lighter isotopes ^{14}N and ^{16}O, is
628 kinetically favoured, resulting in the remaining nitrate becoming enriched in the heavier isotopes.
629 In a well-mixed system, therefore, denitrification results in the $\delta^{15}N$ and $\delta^{18}O$ values of the remaining
630 nitrate increasing above the values of the initial nitrate, and such patterns have formed the basis for
631 identifying denitrification in a wide variety of studies [66, 68-69].
632
633 In vivo estimates of isotope fractionation for nitrogen ($\varepsilon_{N2\ NO3} \approx \delta^{15}N_{N2} - \delta^{15}N_{NO3}$) typically range from
634 -25 to -5‰ [70]. From the relationship:
635
636 $$\delta^{15}N_{NO3} = \delta 15N_0 + \varepsilon_{N2\ NO3} \bullet \ln(f)$$
637
638 where $\delta^{15}N_{NO3}$ and $\delta^{15}N_0$ are the $\delta^{15}N$ values of the remaining and initial nitrate, and f is the fraction
639 of remaining nitrate, it can be shown that loss of only 13% of nitrate by denitrification (i.e. f = 0.87)
640 would lead to an increase in the $\delta^{15}N$ of the remaining nitrate by +0.7‰ ($\varepsilon_{N2\ NO3}$ = -5‰) to +3.5‰ (ε_{N2}
641 $_{NO3}$ = -25‰). As $\varepsilon_{N2\ NO3}$ is very rarely less than -5‰ (in terms of its absolute value), the 0.7‰ difference
642 between the $\delta^{15}N$ value of nitrate in the groundwater and the wetland samples, if it was due to
643 denitrification, would imply that the amount of denitrification was not more than 13%.
644
645 Because the isotope data for wetland nitrate was based on relatively few samples, $\delta^{15}N$ analyses of
646 *Phalaris arundinacea* were used as a proxy indicator of the pattern of nitrate $\delta^{15}N$ over a wider area of
647 the wetland. While some isotope fractionation may occur during the uptake of nitrogen, it is probably
648 small in an environment where N is somewhat limited [71]. The uniformity of *Phalaris arundinacea*
649 $\delta^{15}N$ values across the wetland therefore suggests that the isotope composition of nitrate is also
650 relatively uniform, with no evidence for regions of higher $\delta^{15}N$ which might be indicative of
651 denitrification. It must of course be born in mind that these plant $\delta^{15}N$ values will reflect those of
652 the water at the rooting depth only and, especially important, the values are an integration of the $\delta^{15}N$
653 values of those waters over the period of growth of the plant's uppermost leaves, and are therefore
654 reflective of conditions in the rooting zone over the full growing season.   In this sense, they provide
655 a simpler and more integrated assessment of denitrification in the rooting zone than if samples had
656 been infrequently collected directly from the soil water pool.
657
658 Thus, the isotope characterisation approach indicated that $\delta^{15}N$ and $\delta^{18}O$ values of nitrate from a small
659 number of wetland water samples were very similar to those of the local groundwater, with $\delta^{15}N$
660 suggesting that any denitrification would have been minor (≤ 13%). This may be a function of short
661 residence times of oxidising groundwater as it discharges through the soil zone. Plant $\delta^{15}N$ also
662 showed no evidence for significant denitrification over the course of the growing season, providing
663 an integrated assessment of this process that was less time consuming, less costly and less invasive
664 than an analysis of high frequency samples collected from porous cup samplers across the wetland.
665 However, these conclusions must be tempered by realisation that episodic denitrification would not
666 have been detected using this approach.
667
668 *3.3 Developing a conceptual model of wetland biogeochemical function under baseflow vs stormflow*
669 *conditions*
670 A range of sources of information can be drawn together to interpret the trends in this analysis of
671 hydrochemical wetland function under baseflow conditions. These include the baseflow
672 geochemistry and nutrient hydrochemistry data, the known nutrient uptake behaviour of the
673 wetland plant community, the geophysical data providing a structural framework, and the

674 observations of water level fluctuations across the site. Taken together it is possible to generate a
675 conceptual model of the sources of water draining through the wetland under baseflow conditions.
676 This is outlined in Figure 11.

677
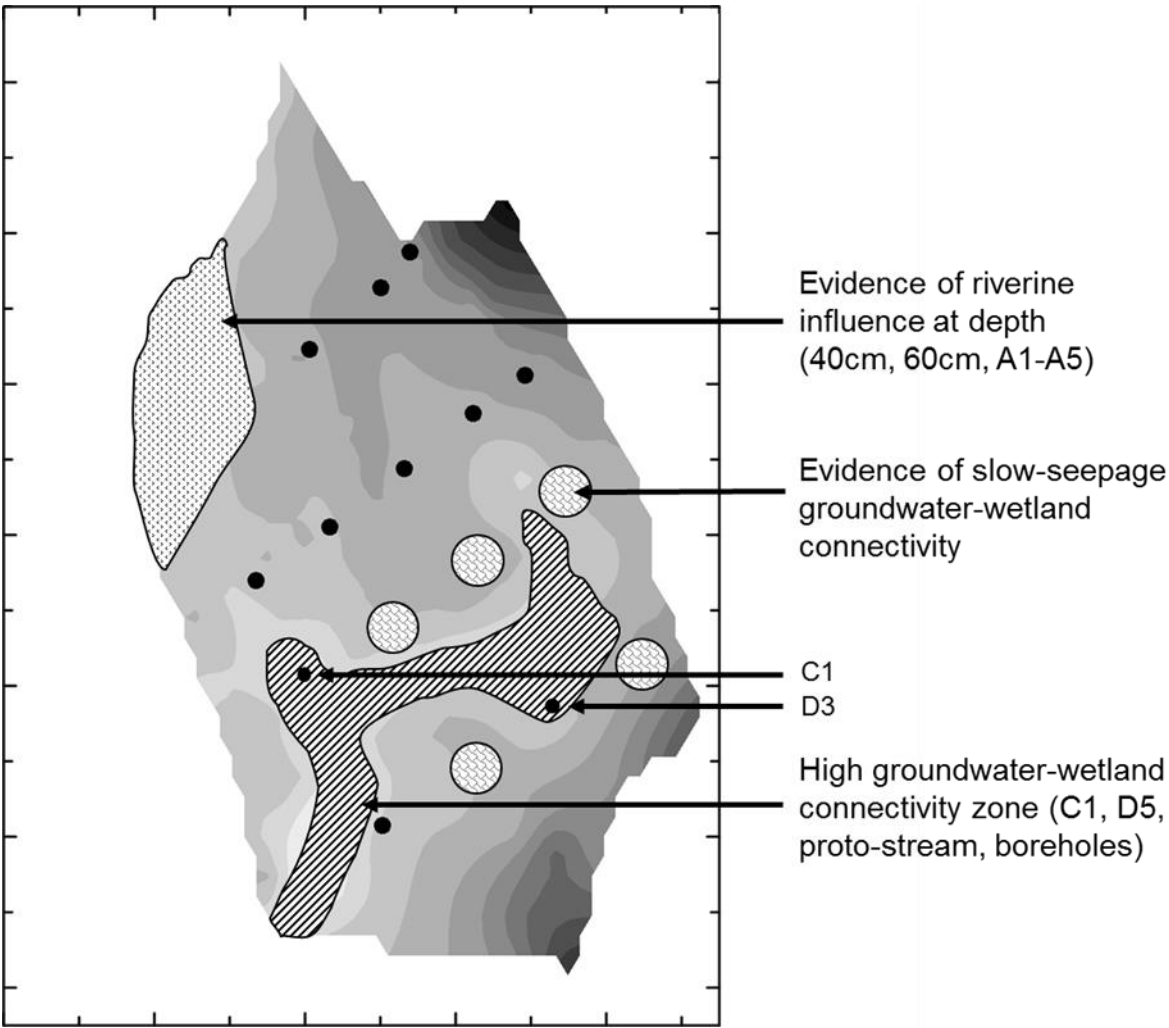
678 **Figure 11.** Conceptual model of baseflow wetland hydraulic connectivity at Boxford.

679

680 Under baseflow conditions this study suggests that the wetland is largely groundwater fed, with a
681 significant proportion of groundwater bypassing the biogeochemically active wetland soil matrix
682 and running NE to SW through the proto-stream channel to discharge to the River Lambourn. The
683 proto-stream channel chemistry and isotope geochemistry suggests that under baseflow conditions
684 there is only slight transformation of groundwater geochemistry and nutrient chemistry along this
685 preferential flow pathway, resulting from the drainage of DON and DOP rich porewaters from the
686 rest of the wetland site to this channel and/or the hyporheic zone beneath the wetland. This is most
687 likely to be due to the short residence time of groundwater in the soil as it discharges to the proto-
688 stream. This may also be enhanced by the large areas of unsaturated soils away from the proto-
689 stream where nitrate will remain stable in the presence of oxygen.

690 The porous cup sampler chemistry from sites with a higher hydraulic residence time indicate that
691 the wetland has the capacity to transform the inorganic nutrient chemistry and geochemistry of
692 source waters (groundwater), with depletion of inorganic N and SRP fractions. However, it is also
693 apparent that these fractions are not simply lost from the system. Rather, there is substantial
694 evidence of reduction in nitrate concentrations resulting from assimilation and uptake by the plant

695   community and release back to the porewaters under baseflow conditions in the form of DON
696   following microbial decomposition of DOM within the rooting zone. The SRP is similarly cycled,
697   transformed and stored within the micro and macropores in the form of DOP.

698   These conclusions can be drawn for the site under baseflow conditions. However, as site hydrology
699   is clearly important in influencing these trends, it can be postulated that as the site undergoes rapid
700   hydrological fluctuations under extreme flow conditions the proportion of water entering the
701   various wetland zones can be expected to vary, soil redox status is likely to change, and this is
702   likely in turn to affect the nutrient cycling processes operating within the wetland and the
703   speciation of the nutrient load, compromising the nutrient storage capacity of the wetland soil zone.
704   This is illustrated in Figures 12-13 which illustrate the rapid flushing of accumulated dissolved
705   organic-rich N and P from porewaters under high flow (storm) conditions, with riverine discharge
706   in the Lambourn increasing from 0.9 to over 1.5 m^3/s over the course of the event [5].

707   These data represent the nutrient flushing response of the wetland to an intense and prolonged
708   storm event sampled under wet and cold antecedent conditions, in the absence of active plant
709   uptake, at the end of the autumn period (from 16-Nov-96 to 23-Nov-96). The storm event drove an
710   increased flow into and through the wetland, with no over-bank inundation of the site, this being a
711   rare occurrence in permeable lowland catchments in the UK with a high baseflow index (BFI). The
712   River Lambourn had a BFI of 0.98 at Boxford during the period of study [35].

713   TDP concentrations (Figure 12) rose fourfold across the site from a maximum of 0.7 mg/L at the
714   start of the storm to over 3.0 mg/L at the height of the flushing event on 19-Nov-96. There is
715   evidence that the highest concentrations of TDP were observed moving along the line of the proto-
716   stream channel with the highest concentrations observed in the 20-40 cm samplers, largely in the
717   form of DOP (data not presented). Total Dissolved N (TDN) concentrations (Figure 13) showed a
718   clearer pattern, with concentrations rising fivefold from a maximum of 3 mg/L N to over 16 mg/L N
719   at the peak of the flushing event (19-Nov-96). Over 90% of the flux was in the form of DON (data
720   not presented). Note that the scale changes on the plots over the duration of the event. A clear
721   pattern emerges from these data, with nitrate rich water entering the site along the alignment of the
722   dry valley, and DON enriched water flushing from the site along the line of the proto-stream
723   channel.

724   These data, and other data collected from the site under a range of storm flow conditions in the
725   spring and autumn period, 2004 (not presented here) confirm the conclusions reached in the earlier
726   study at this site regarding the source/sink function of these lowland permeable wetlands [5].
727   Under baseflow conditions the wetland is groundwater fed, with most of the water bypassing the
728   nutrient transformation function of the site by flowing along preferential flow pathways including
729   the proto-stream channel. For groundwater entering the wetland soil matrix, there is evidence of
730   rapid transformation and storage of both nitrate and SRP in the plant biomass, in particulate form,
731   and in the form of DON and DOP in the wetland soil micropores and macropores following
732   microbial decomposition of dead organic matter from the plant and animal biomass in the wetland.
733   Some nitrate is 'lost' from the wetland through the process of denitrification, as proposed in other
734   studies on riparian wetlands, but by no means all of this load is 'lost'. Rather, the wetland acts to
735   attenuate the delivery of both N and P from the catchment to the river through transformation,
736   uptake and storage in the biomass, soil and porewaters, at least for the waters which drain through
737   the soil matrix. Under high flow events, however, particularly in the autumn and early spring, and
738   under wet antecedent conditions, the wetland flushes these accumulated nutrient stores from the
739   porewaters, and this is routed either vertically to the gravel layer, or laterally to the proto-stream
740   channel and thence to the Lambourn where it contributes to the increased N and P concentrations
741   instream reported previously [5,35-37].

742

743
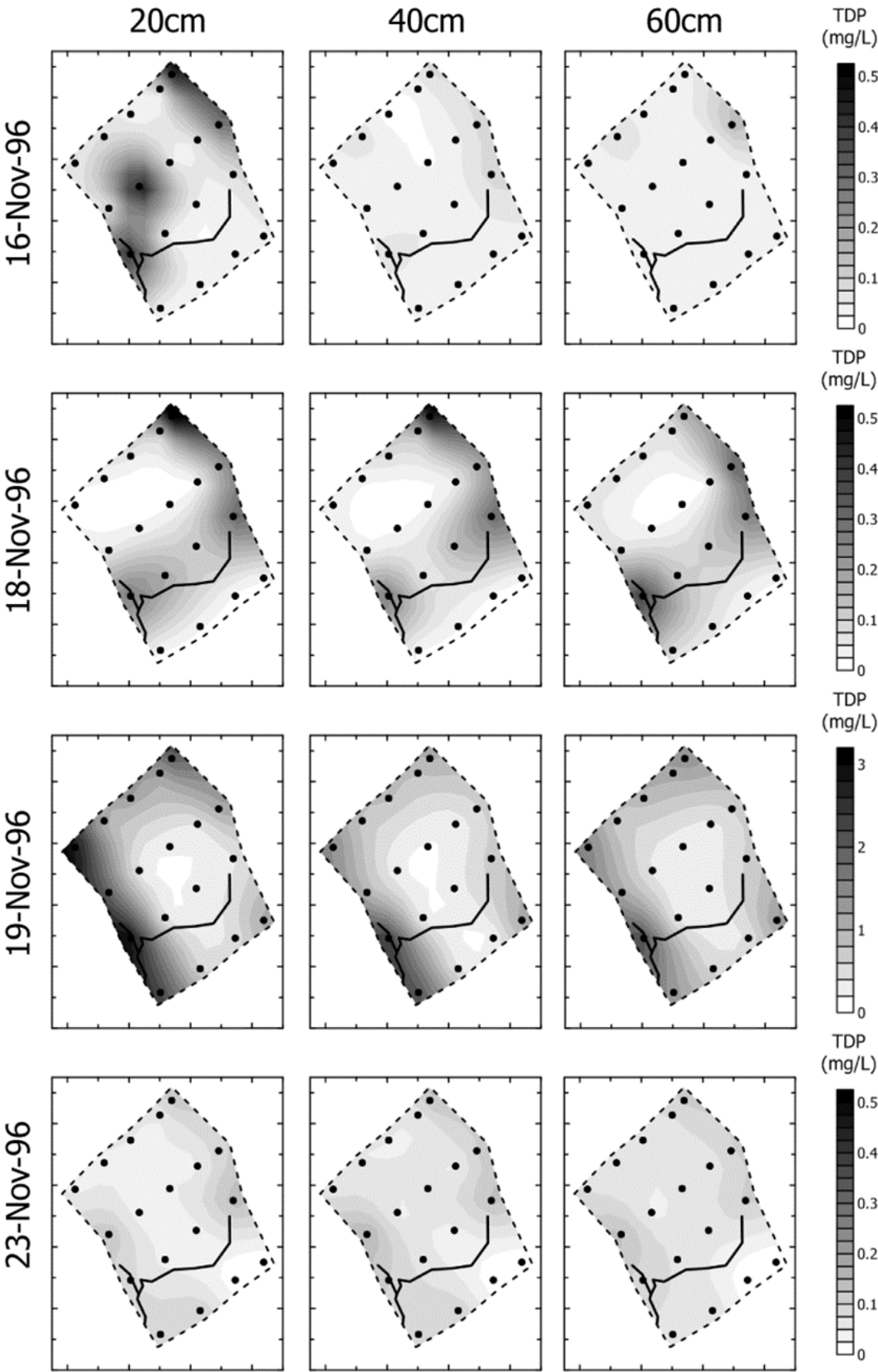
744   **Figure 12.** TDP dynamics under storm conditions, 16-Nov-96 to 23-Nov-96.   Note that the scale
745   varies the over course of the event

746

747
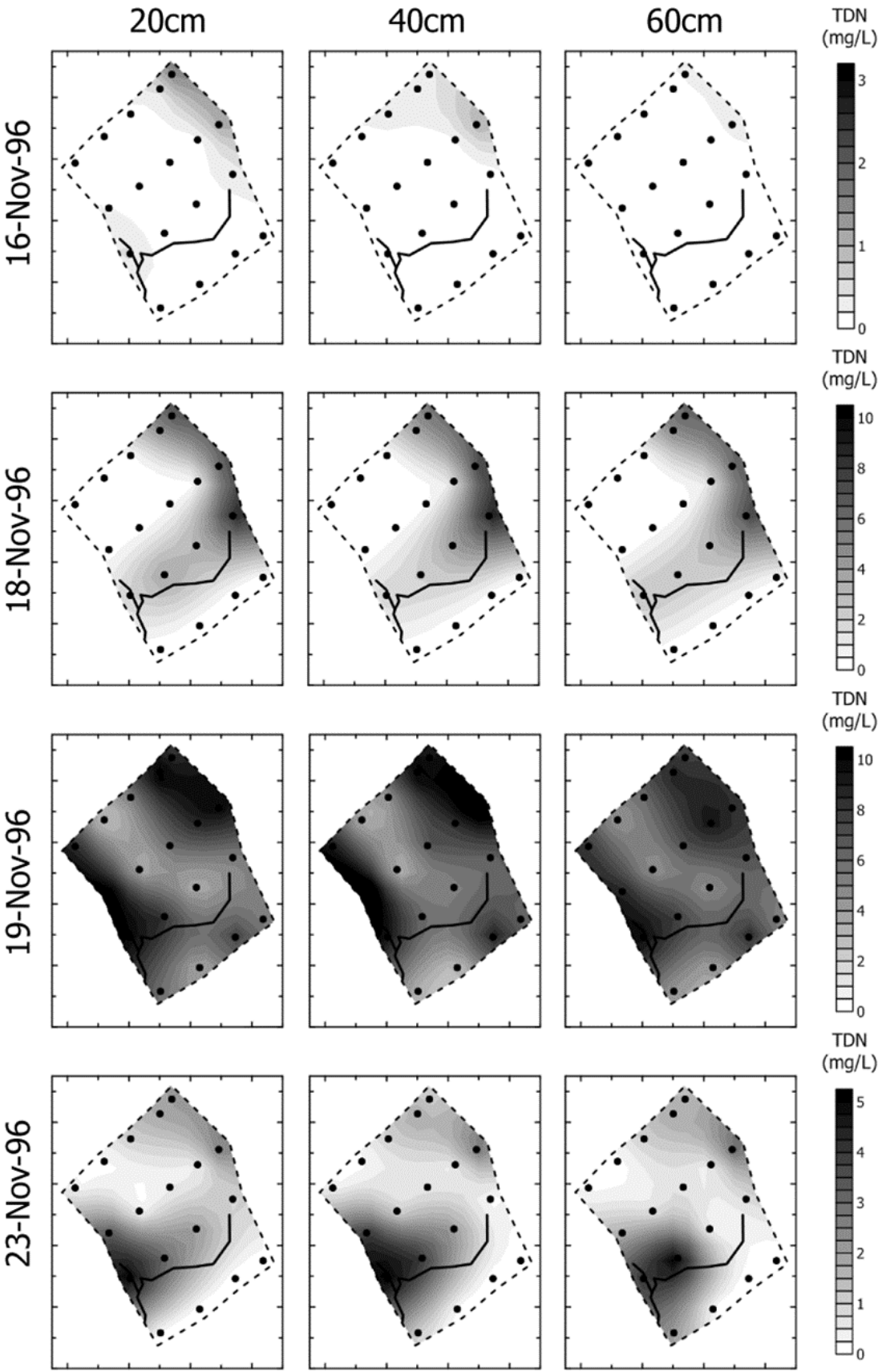
748   **Figure 13.** TDN dynamics under storm conditions, 16-Nov-96 to 23-Nov-96.   Note that the scale
749   varies over the course of the event

750   **4. Discussion**

751   Wetlands in lowland permeable catchments clearly act as biogeochemical cycling hotspots, zones of
752   pollutant and flow attenuation, and temporary stores for nutrients exported from diffuse
753   agricultural sources in the wider catchment. Placed at the base of the dry valleys and hillslopes as
754   they are within the Lambourn catchment (and elsewhere across permeable landscapes of NW
755   Europe), it is likely that they play an important role in regulating the rates, timing and speciation of
756   the nutrient load delivered to the River Lambourn from its catchment. This is pertinent in many
757   permeable catchments, where high nitrate concentrations in the unsaturated zone and groundwater
758   will provide a legacy of nitrate inputs to surface waters for some time to come. However, the
759   evidence from storm sampling of these systems must also be considered, notably the importance of
760   residence time and connectivity in regulating the amount of groundwater flow which actually
761   passes through these biogeochemical cycling hotspots. Similarly, the full range of nutrient species
762   studied here demonstrates the importance in assessing all forms of N and P in these
763   biogeochemically active systems. The evidence is clear that very little nitrate is actually 'lost' or
764   'removed' over the annual cycle.   Rather, once the dissolved organic nutrient fractions are factored
765   in, it is clear that the wetland acts to assimilate, breakdown and re-release inorganic nutrient
766   fractions in the form of DOM, which is then flushed out to adjacent waters during high flow events.
767   The process is one of transformation and attenuation, rather than removal or 'loss'.

768   In interpreting these findings, it is clear that the combination of geophysical survey, and low-
769   resolution sampling of porewater geochemistry, in combination with targeted isotope analysis of
770   plant material in the wetland was able to clearly distinguish the key functional zones within the
771   wetland. A separation of those wetter areas of the wetland likely to support denitrification, versus
772   those with a longer contact time between nutrient load and the plant and microbial communities
773   was clearly achieved. The findings map closely onto the key trends evident in the nutrient
774   speciation data collected for the site under baseflow conditions.   We argue, here, that this
775   combination of approaches was able to diagnose the likely nutrient cycling, retention and export
776   behaviour of the wetland, presenting a cost-effective approach for catchment managers
777   contemplating the use of riparian wetlands to ameliorate or transform nutrient flux from land to
778   water.

779   It is also worth noting that the placement of the wetland systems within the wider hydrogeological
780   framework of lowland permeable catchments must be born in mind when contemplating their
781   potential role in catchments. This is illustrated in Figure 14.

782
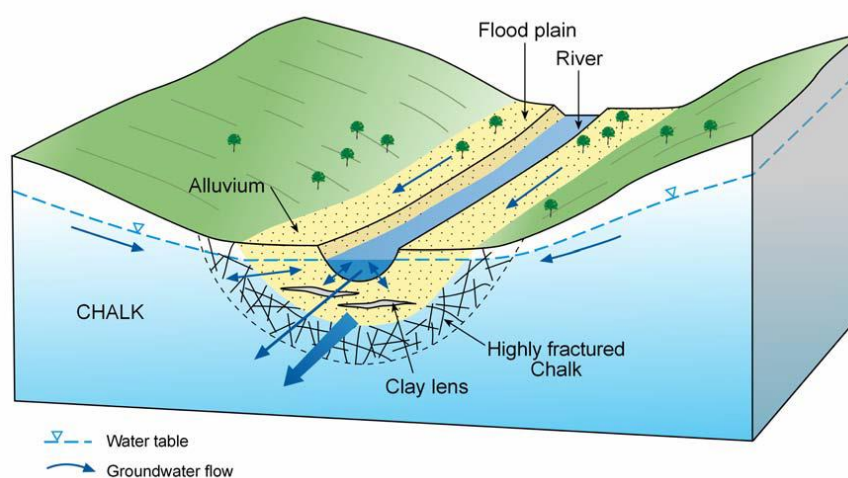
783   **Figure 14.** Wetland biogeochemical cycling hotspots: their role in lowland permeable catchment
784   hydrochemical function. Figure provided by British Geological Survey.

785   While it is clear that they have the capacity to transform inorganic nutrient inputs to dissolved
786   organic nutrient fractions stored within the wetland porewaters, this function only applies to that
787   water which passes through the wetland soil matrix, primarily that passing through the rooting
788   zone where assimilation is the dominant process. In reality, in permeable catchments much of the
789   flow from the saturated groundwater zone to the river is likely to bypass these wetland systems,
790   upwelling directly through the hyporheic zone to the bed of the river without passing through the
791   wetland soil matrix. If this is the case then wetlands, despite their potential to transform and
792   attenuate the nutrient chemistry and geochemistry signal from the catchment, may not realise this
793   potential and may therefore play little part in the modification of nutrient flux from land to stream
794   in lowland permeable catchments. The Boxford wetland is a good exemplar for wetlands typically
795   found in lowland permeable catchments, and the findings from this programme provide a useful
796   indication of the likely function of similar wetlands in other lowland permeable catchments.
797   However, further wetland research is needed at this scale in other geoclimatic regions to determine
798   the extent to which this sporadic and spatially confined functionality is common to all or some
799   wetland types.

800   The importance, then, of being able to identify subsurface architecture and its probable impact on
801   wetland hydrological function and, in particular, to have tools capable of identifying dominant
802   flushing behaviours through structures such as the proto-stream channel in the Boxford wetland is
803   key to providing the capacity to upscale knowledge acquired from such in-depth research to larger
804   tracts of wetland, or multiple wetlands within catchments. One approach to upscaling knowledge is
805   through the development of coupled hydrological and biogeochemical models at the catchment
806   scale, building the nutrient and flow attenuation properties of wetlands studied in detail into a
807   wider system scale model. Once this is complete, it will be possible to simulate the hydrological and
808   coupled hydrochemical function of these lowland permeable wetlands and to test their significance
809   by building them into catchment scale coupled surface-groundwater models.

810   An alternative approach is to evaluate the range of methods adopted in this study by comparing the
811   information they yield against information gained from the high frequency sampling programme
812   implemented at the site. Of the approaches tested, the HGMU method was the least sensitive,
813   lumping the whole wetland zone into a single functional unit. The NVC vegetation mapping was
814   more subtle, dividing the wetland proper into two zones representing plant communities with
815   differing affinities to water logging. However, this too did not distinguish those areas of the
816   wetland where soil redox status supported denitrification, from those where longer residence time
817   supported the uptake, storage, breakdown and release of inorganic nutrient loads export from the
818   wetland in the form of dissolved organic nutrient flux. The geophysical techniques adopted were
819   useful in identifying an anomaly at the B2 sampler nest, later confirmed as a lens of putty chalk
820   through the installation of a piezometer at that leading to a locally perched water table.   It also
821   identified the variable depth of the alluvial gravels across the site previously confirmed as an
822   important feature supporting lateral and downstream transfer of water and its associated chemical
823   load [38]. Perhaps more significantly, the geophysical survey methodology also confirmed the
824   existence of the proto-stream channel morphometry as a feature of the subsurface architecture
825   likely to promote preferential flow from land to stream through the wetland, assisting in the
826   interpretation of nutrient flux behaviours both in terms of spatial variations across the wetland
827   under baseflow conditions and flushing under storm flow conditions.

828   The geochemical characterisation of the site, in association with work on stable isotope ratios in
829   plant, river, groundwater and waters sampled from the gravels underlying the wetland provided
830   further detail. The determination of selected geochemical ratios helped to characterise the signature
831   of the differing source areas contributing water to the wetland soil matrix, confirming the
832   conceptual model of biogeochemical functional zones within the wetland derived from the high
833   frequency sampling and nutrient analysis programme, providing the potential to use a lower
834   intensity of nutrient sampling in combination with geochemical analyses to derive a robust

835   conceptual model of biogeochemical functioning for less well-resourced or multiple site
836   investigations into wetland biogeochemical function in future. Finally, the isotope work was
837   invaluable in providing, at a seasonally averaged scale, the evidence for the distribution of the
838   denitrification function across the wetland, and in identifying the limited spatial extent of this
839   function, at least for the period studied in this programme. Future studies, bringing together initial
840   site assessment using a combination of geophysical, geochemical and isotopic characterisation are
841   likely to be able to derive an initial conceptual model of biogeochemical and hydrological
842   functional zones with complex wetlands, significantly reducing the need for costly and invasive
843   high frequency nutrient flux monitoring.

844   Such studies must consider the wider environmental and historical context for any site. Historical
845   changes in land use may have impacted on the spatial extent of riparian wetlands along river
846   channels, affecting the catchments ability to attenuate and transform nutrient loads to rivers.
847   Climate variability and change may also reduce wetland area and limit these natural attenuation
848   processes, as demonstrated in this study in the differing functional behaviours of the wetland under
849   baseflow versus stormflow conditions, and in the earlier work on the differing stormflow responses
850   generated under wet versus dry, and cold versus warm antecedent conditions [5]. Certainly, there
851   is no one dominant wetland biogeochemical function across whole wetland systems and this varies
852   from wet to dry year, over the seasons, in relation to the history of nutrient enrichment of the site,
853   and from year to year in response to climatic and groundwater flow variations. Careful
854   consideration should be given to management options that consider rehabilitation of wetland areas,
855   particularly considering the legacy of historical anthropogenic inputs of nutrients, much of which is
856   still present in the unsaturated zone of much of the Chalk aquifer.

857   **5. Conclusions**

858   The combination of approaches adopted in this investigation of wetland biogeochemical function
859   allowed an unusually detailed assessment for the Boxford Experimental Wetland.   Key conclusions
860   are as follows:

861   • Attenuation of pollutant delivery from land to stream appears to occur both within the
862   hyporheic zone and the adjacent riparian wetland ecosystem.

863   • The primary pathway for modification of the nutrient speciation of inflowing waters is through
864   plant uptake of inorganic nutrient species and microbiological breakdown of DOM to release
865   DON and DOP compounds.

866   • The reciprocity in inorganic and organic nutrient fraction concentrations across the wetland
867   suggests no net storage of nutrients within the soil porewaters, nor substantial export of
868   nitrogen to atmospheric sources through denitrification.

869   • The primary mechanism for the export of nutrients accumulated in soil porewaters appears to
870   be flushing of the macropores and micropores during storm events, with nutrient rich waters
871   exported primarily via the proto-stream channel, but also through lateral flow to the Lambourn
872   and vertical exchange with groundwater through the alluvial gravels.

873   • The findings from the geochemical analyses of soil porewaters and source waters suggest that
874   the wetland is groundwater fed, with flows likely to be delivered from the major dry valley
875   feature which appears, topographically, to be aligned upslope with the line of the wetland
876   proto-stream channel.

877   • The extent to which nutrient chemistry of inflowing waters is modified by wetland
878   biogeochemical cycling depends on the residence time for the water within the wetland

879   • Well-drained areas of the wetland with a short hydraulic residence time exhibit soil porewater
880   nutrient chemistry and major and trace element geochemistry comparable to Chalk

881   groundwater, suggesting little transformation of the nutrient load moving along these flow
882   pathways.

883   •   Surface and subsurface features identified through the site topographic and geophysical
884   surveys, supplemented by soil coring, such as existence and extent of the proto-stream channel,
885   the depth of the alluvial gravels, and the presence of putty chalk lenses, are also critical in
886   defining the hydrological function of wetland systems and the likely role any wetland may
887   play in the transport and transformation of nutrient loads exported from land to stream.

888   •   Geophysical methods, in association with limited site characterisation through geochemical,
889   isotope and nutrient hydrochemical techniques may be useful in future studies to indicate the
890   likely role played by wetlands in the nutrient hydrochemical function of lowland permeable
891   catchments, allowing them to be built into catchment scale models of biogeochemical function.

892   •   This combination of techniques, building on recent advances in geophysical, geochemical and
893   isotope characterisation provides a novel approach for improved understanding of
894   biogeochemical function in permeable wetlands. Perhaps more importantly, no one
895   technique on its own would have given an unambiguous representation of the hydrological
896   and biogeochemical function of the wetland, and a toolkit comprising a range of
897   complementary approaches will provide a more complete and robust indication of wetland
898   function at a process scale.

899   Overall, the multi-proxy approach adopted in this study has highlighted the important role that
900 wetlands play in modifying the nutrient chemistry of waters draining through them in lowland
901 permeable catchments, and their role in the transient storage of waters under baseflow conditions, in
902 the transformation of nutrient chemistry during these periods, and in the flushing of these
903 accumulated nutrient stores in the form of DON and DOP under high flow conditions. However,
904 the wetland is rarely 'wet', a common characteristic in lowland permeable wetlands, and apart from
905 the zone of the proto-stream channel, water level rarely reaches 20 cm below the wetland surface,
906 suggesting that the majority of groundwater flow does not pass through these functions. At
907 catchment scale, their significance in terms of attenuation or modification of the nutrient enrichment
908 of surface waters from land-based sources in their catchments may be very limited under present
909 land use and climate scenarios.

910

911   **Author Contributions:** PJ was PI on both grants and designed and executed the nutrient hydrochemistry
912 sampling, analysis and interpretation with HP and MK; AB led, designed and conducted the geophysical survey
913 component with support from Heather Musgrave, in collaboration with PJ and MPK;, PS and DG designed and
914 executed the wetland geochemistry sampling and analysis in collaboration with PJJ and provided access to
915 British Geological Survey data and borehole assets, THEH collaborated with PJ and MPK to design and execute
916 the sample collection and isotope analysis component. All authors contributed to the writing of the manuscript,
917 preparation of data for presentation, and data analysis. The project was conducted in collaboration with a wide
918 range of other researchers funded under the £10m NERC LOCAR programme.

919

925

929   **Conflicts of Interest:** The authors declare no conflict of interest

References

1. Peterjohn, W.T.; Correll, D.L. Nutrient dynamics in an agricultural watershed: observations on the role of a riparian forest. Ecology 1984, 65, 5, 1466-1475. doi.org/10.2307/1939127

2. Seitzinger, S.P. Linkages between organic matter mineralization and denitrification in 8 riparian wetlands. Biogeochemistry 1994, 25, 19-39. doi.org/10.1007/BF00000510

3. Hill, A.R. Nitrate removal in stream riparian zones. Journal of Environmental Quality 1996, 25, 4, 743-655. doi.org/10.2134/jeq1996.00472425002500040014x

4. Kuusemets, V.; Mander, U.; Lohmus, K.; Ivask, M. Nitrogen and phosphorus variation in shallow groundwater and assimilation in plants in complex riparian buffer zones. Water Science and Technology 2001, 44, 615-622. doi.org/10.2166/wst.2001.0888

5. Prior, H.; Johnes, P.J. Regulation of surface water quality in a Chalk catchment, UK: an assessment of the relative importance of instream and wetland processes. Science of the Total Environment 2002, 282/283, 159-174. doi.org/10.1016/S0048-9697(01)00950-0

6. Revsbech, N.P.; Jacobsen, J.P.; Nielsen, L.P. Nitrogen transformations in microenvironments of river beds and riparian zones. Ecological Engineering 2005, 24, 447-455. doi.org/10.1016/j.ecoleng.2005.02.002

7. Burgin, A.J.; Groffman, P.M. Soil O2 controls denitrification rates and N2O yield in a riparian wetland. Journal of Geophysical Research 2012, 117, G01010, 10 pp. doi.org/10.1029/2011JG001799

8. Arheimer, B.; Pers, B.C. Lessons learned? Effects of nutrient reductions from constructing wetlands in 1996–2006 across Sweden. Ecological Engineering 2017, 103, Part B, 404-414. doi.org/10.1016/j.ecoleng.2016.01.088

9. Mander, U.; Tournebize, J.; Sauvage, S.; Sanchez-Perez, J.M. Wetlands and buffer zones in watershed management. Ecological Engineering 2017, 103, 289–295. Doi.org/10.1016/j.ecoleng.2016.12.005

10. Neilen, A.D.; Chen, C.R.; Parker, B.M.; Faggotter, S.J.; Burford, M.A. Differences in nitrate and phosphorus export between wooded and grassed riparian zones from farmland to receiving waterways under varying rainfall conditions. Sci. Tot. Env. 2017, 598, 188–197. doi.org/10.1016/j.scitotenv.2017.04.075.

11. Bernard-Jannin, L; Sun, X.; Teissier, S.; Sauvage, S.; Sánchez-Pérez, J.M. Spatio-temporal analysis of factors controlling nitrate dynamics and potential denitrification hot spots and hot moments in groundwater of an alluvial floodplain. Ecological Engineering 2017, 103, 272-284. doi.org/10.1016/j.ecoleng.2015.12.031.

12. Peralta-Maraver, I.; Reiss, J.; Robertson, A.L. Interplay of hydrology, community ecology and pollutant attenuation in the hyporheic zone. Science of the Total Environment 2018, 610–611, 267–275. doi.org/10.1016/j.scitotenv.2017.08.036.

13. Scheliga, B.; Tetzlaff, D.; Nuetzmann, G.; Soulsby C. Groundwater dynamics at the hillslope–riparian interface in a year with extreme winter rainfall. Journal of Hydrology 2018, 564, 509–528. doi.org/10.1016/j.jhydrol.2018.06.082.

14. Old, GH.; Naden, P.S.; Rameshwaran, P.; Acreman, M.C.; Baker, S.; Edwards, F.K.; Sorensen, J.P.R.; Mountford, O.; Gooddy, D.C.; Stratford, C.J.; Scarlett, P.M.; Newman, J.R.; Neal, M. Instream and riparian implications of weed cutting in a chalk river. Ecological Engineering 2014, 71, 290-300. doi.org/10.1016/j.ecoleng.2014.07.006.

15. Shabaga, J.A.; Hill, A.R. Groundwater-fed surface flow path hydrodynamics and nitrate removal in three riparian zones in southern Ontario, Canada. Journal of Hydrology 2010, 388, 52–64. doi.org/10.1016/j.jhydrol.2010.04.028.

16. Balestrini, R.; Sacchi, E.; Tidilia, D.; Deconte, C.A., Buffagni, A. Factors affecting agricultural nitrogen removal in riparian strips: Examples from groundwater-dependent ecosystems of the Po Valley (Northern Italy). Agriculture, Ecosystems and Environment 2016, 221, 132–144. doi.org/10.1016/j.agee.2016.01.034

17. Pinay, G.; Decamps, H. The role of riparian woods in regulating nitrogen fluxes between the alluvial aquifer and surface water: a conceptual model. Regulated Rivers: Research and Management 1988, 2, 507-516. doi.org/10.1002/rrr.3450020404

18. Hefting, M.M.; Bobbink, R.; de Caluwe, H. Nitrous oxide emission and denitrification in chronically nitrate-loaded riparian buffer zones. Journal of Environmental Quality 2003, 32, 4, 1194-1203. doi.org/10.2134/jeq2003.1194.

19. Weyer, C.; Peiffer, S.; Lischeid, G. Stream water quality affected by interacting hydrological and biogeochemical processes in a riparian wetland. Journal of Hydrology, 2018, 563, 260–272. doi.org/10.1016/j.jhydrol.2018.05.067.

20. Frei, S.; Peiffer, S. Exposure times rather than residence times control redox transformation efficiencies in riparian wetlands. Journal of Hydrology 2016, 543 (2016) 182–196. doi.org/10.1016/j.jhydrol.2016.02.001

984   21. De Sosa, L.L.; Glanville, H.C., Marshall, M.R.; Williams, P.; Abadie, M.; Clark, I.M., Blaud, A.; Jones, D.L.
985   Spatial zoning of microbial functions and plant-soil nitrogen dynamics across a riparian area in an
986   extensively grazed livestock system. Soil Biology and Biochemistry 2018, 120, 153–164.
987   doi.org/10.1016/j.soilbio.2018.02.004.

988   22. Yates, C.A.; Johnes, P.J.; Spencer, R.G.M. Assessing the controls on dissolved organic matter export from two
989   contrasting lowland catchments. Science of the Total Environment 2016, 569–570, 1330–1340.
990   doi.org/10.1016/j.scitotenv.2016.06.211

991   23. Lloyd, C.E.M.; Johnes, P.J.; Freer, J.E.; Carswell, A.; Jones, J.I.; Stirling, M.W.; Hodgkinson, R.A., Richmond,
992   C.; Collins, A.C. Determining the origins of nutrient flux to waters in catchments: examining the nutrient
993   speciation balance to inform the targeting of mitigation measures.   Science of the Total Environment 2019,
994   648, 1179-1200. doi.org/10.1016/j.scitotenv.2018.08.190.

995   24. Yates, C.A.; Johnes, P.J.; Owen, A.T.; Brailsford, F.L.; Glanville, H.C.; Evans, C.D.; Marshall, M.R.; Jones, D.L.;
996   Lloyd, C.E.M.; Jickells, T.; Evershed, R.P. (2019) Variation in dissolved organic matter (DOM) stoichiometry
997   in freshwaters: assessing the influence of land cover and soil C:N ratio on DOM composition.   Limnology
998   and Oceanography 2019, 9999, 1-13. doi.org/10.1002/lno.11186.

999   25. Mander, U.; Kuusemets, V.; Ivask, M. Nutrient dynamics of riparian ecotones – a case study from the Porijogi
1000   River catchment, Estonia. Landscape and Urban Planning 1995, 31, 1-3, 333-348. doi.org/10.1016/0169-
1001   2046(94)01061-C

1002   26. Kull, A.; Kull A.; Jaagus, J.; Kuusemets, V.; Mander, U. The effects of fluctuating climatic conditions and
1003   weather events on nutrient dynamics in a narrow mosaic riparian peatland.   Boreal Environment Research
1004   2008, 13, 243-263.

1005   27. Newell, A.J.; Vane, C.H.; Sorensen, J.P.R.; Moss-Hayes, V.; Gooddy, D.C. Long-term Holocene groundwater
1006   fluctuations in a chalk catchment: evidence from Rock-Eval pyrolysis of riparian peats. Hydrological
1007   Processes 2016, 30, 4556-4567. doi.org/10.1002/hyp.10903

1008   28. Brinson, M.M. A Hydrogeomorphic Classification for Wetlands. US Army Corps of Engineers Waterways
1009   Experiment Station, USA, 1993, Wetlands Research Program Technical Report WRP-DE-4.

1010   29. Allen, D.J.; Darling, W.G.; Gooddy, D.C; Lapworth, D.J.; Newell, A.J.; Williams, A.T.; Allen, D.; Abesser, C.
1011   Interaction between groundwater, surface water and the hyporheic zone in a chalk stream. Hydrogeology
1012   Journal 2010, 18, 5, 1125–1141. doi.org/10.1007/s10040-010-0592-2

1013   30. Binley, A.; Hubbard, S.S., Huisman, J.A.; Revil, A.; Robinson, D.A.; Singha, K.; Slater, L.D., The emergence
1014   of hydrogeophysics for improved understanding of subsurface processes over multiple scales, Water Resour.
1015   Res. 2015, 51, 3837–3866. doi.org/10.1002/ 2015WR017016.

1016   31. Uhlemann, S.S.; Sorensen, J.P.R.; House, A.R.; Wilkinson, P.B.; Roberts, C.; Gooddy, D.C.; Binley, A.;
1017   Chambers, J.E. Integrated time-lapse geoelectrical imaging of wetland hydrological processes. Water
1018   Resources Research 2016, 52, 3, 1607-1625. doi.org/10.1002/2015WR017932.

1019   32. House, A.R.; Sorensen, J.P.R.; Gooddy, D.C.; Newell, A.J.; Marchant, B.; Mountford, J.O.; Scarlett, P.; Williams,
1020   P.J.; Old, G.H. Discrete wetland groundwater discharges revealed with a three-dimensional temperature
1021   model and botanical indicators (Boxford, UK). Hydrogeology Journal 2015, 23, 4, 775-787.
1022   doi.org/10.1007/s10040-015-1242-5

1023   33. Wheater H.S.; Peach D. Developing interdisciplinary science for integrated catchment management: the UK
1024   Lowland Catchment Research (LOCAR) programme. International Journal of Water Resources Development
1025   2004, 20, 3, 369–385. doi.org/10.1080/0790062042000248565

1026   34. Wheater, H.; Peach, D.; Binley, A. Characterising groundwater-dominated lowland catchments : the UK
1027   Lowland Catchment Research Programme (LOCAR). Hydrology and Earth System Sciences 2007, 11, 1, 108-
1028   124. www.hydrol-earth-syst-sci.net/11/108/2007/

1029   35. Evans D.J; Johnes P.J.; Lawrence D.S. Sediment transport in lowland streams – storm integrated monitoring.
1030   IAHS 2003, Publication 283, 103-110.

1031   36. Evans, D.J; Johnes, P.J. Physico-chemical controls on phosphorus cycling in two lowland streams: I – Water
1032   column. Science of the Total Environment 2004, 329, 145-163. doi.org/10.1016/j.scitotenv.2004.02.018

1033   37. Evans, D.J.; Johnes, P.J.; Lawrence, D. Physico-chemical controls on phosphorus cycling in two lowland
1034   streams: II – Bed sediment. Science of the Total Environment 2004, 329, 165-182. doi.org/
1035   10.1016/j.scitotenv.2004.02.023

1036   38. Lapworth, D.J.; Gooddy, D.C.; Allen, D.; Old, G.H. Understanding groundwater, surface water, and
1037   hyporheic zone biogeochemical processes in a Chalk catchment using fluorescence properties of dissolved

and colloidal organic matter. Journal of Geophysical Research 2009, 114, G00F02, doi.org/10.1029/2009JG000921.

39. Grapes T.R.; Bradley C.; Petts G. E. Dynamics of river-aquifer interaction along a Chalk stream: the River Lambourn, UK. Hydrological Processes 2005, 19, 2035–2053. doi.org/10.1002/hyp.5665

40. Griffiths, J.; Binley, A.; Crook, N.; Nutter, J.; Young, A.; Fletcher, S. Streamflow generation in the Pang and Lambourn catchments, Berkshire, UK. Journal of Hydrology 2006, 330, 71–83. doi.org/10.1016/j.jhydrol.2006.04.044

41. Newell, A.J.; Sorensen, J.P.R.; Chambers, J.E.; Wilkinson, P.B.; Uhleman, S.; Roberts, C.; Gooddy, D.C.; Vane, C.H.; Binley, A. Fluvial response to Late Pleistocene and Holocene environmental change in a Thames chalkland headwater: the Lambourn of southern England. Proceedings of the Geologists' Association 2015, 126, 683-697. DOI 10.1016/j.pgeola.2015.08.008.

42. Pretty J.L.; Hildrew A.G.; Trimmer M. Nutrient dynamics in relation to surface–subsurface hydrological exchange in a groundwater fed Chalk stream. Journal of Hydrology 2006, 330, 84–100. doi.org/10.1016/j.jhydrol.2006.04.013

43. Lapworth, D.J.; Gooddy, D.C.; Jarvie, H.P. Understanding phosphorus mobility and bioavailability in the hyporheic zone of a chalk stream. Water, Air, & Soil Pollution 2011, 218, 1-4, 213-226. doi.org/10.1007/s11270-010-0636-1

44. Aldiss, D.T.; Royse, K.T. The geology of the Pang-Lambourn catchment, Berkshire, British Geological Survey 2002, Comm. Rep., CR/20/289N, 38 pp.

45. Rodwell, J.S. National Vegetation Classification: Users' Handbook. Joint Nature Conservation Committee, UK, 2006.

46. Kettridge, N.; Comas, X.; Baird, A.; Slater, L.; Strack, M.; Thompson, D.; Jol, H.; Binley, A. Ecohydrologically important subsurface structures in peatlands revealed by ground-penetrating radar and complex conductivity surveys. J. Geophys. Res. 2008, 113, G04030. doi.org/10.1029/2008JG000787.

47. Robinson, D.A.; Binley, A.; Crook, N.; Day-Lewis, F.; Ferré, P.T.; Grauch, V.J.S.; Knight, R.; Knoll, M.; Lakshmi, V.; Miller, R.; Nyquist, J.; Pellerin, L.; Singha, K.; Slater, L. Advancing process-based watershed hydrological research using near-surface geophysics : a vision for, and review of, electrical and magnetic geophysical methods. Hydrological Processes 2008, 22, 18, 3604-3635. doi.org/10.1002/hyp.6963

48. McLachlan, P.J.; Chambers, J.E.; Uhlemann, S.S.; Binley, A. Geophysical characterisation of the groundwater–surface water interface. Advances in Water Resources 2017, 109, 302–319. doi.org/10.1016/j.advwatres.2017.09.016.

49. Musgrave, H.; Binley, A. Revealing the temporal dynamics of subsurface temperature in a wetland using time-lapse geophysics. Journal of Hydrology 2011, 396, 3-4, 258–266. doi.org/10.1016/j.jhydrol.2010.11.008

50. Binley, A.; Kemna, A. DC Resistivity and Induced Polarization Methods. In Hydrogeophysics; Rubin, Y., Hubbard, S.S., Eds.; Springer, New York, USA, 2005, 129-156.

51. Jarvis, I.; Woodruff, P. The phosphatic chalks and hardgrounds of Boxford and Winterbourne, Berkshire - two tectonically controlled facies in the late Coniacian to early Campanian (Cretaceous) of southern England. Geological Magazine 1981, 118, 2, 175-187. doi.org/10.1017/S0016756800034373

52. Johnes, P.J.; Heathwaite, A.L. A procedure for the simultaneous determination of total nitrogen and total phosphorus in freshwater samples using persulphate microwave digestion. Water Research 1992, 26, 10, 1281-1287. doi.org/10.1016/0043-1354(92)90122-K

53. Cey, E.E.; Rudolph, D.L.; Aravena, R.; Parkin, G. Role of the riparian zone in controlling the distribution and fate of agricultural nitrogen near a small stream in southern Ontario. Journal of Contaminant Hydrology 1999, 37, 45-67. doi.org/10.1016/S0169-7722(98)00162-4

54. Mengis, M.; Schiff, S.L.; Haris, M.; English, M.C.; Aravena, R.; Elgood, R.J.; MacLean, A. Multiple geochemical and isotopic approaches for assessing ground water NO_3^- elimination in a riparian zone. Groundwater 1999, 37, 448-457. doi.org/10.1111/j.1745-6584.1999.tb01124.x

55. Devito, K.J.; Fitzgerald, D.; Hill, A.R.; Aravena, R. Nitrate dynamics in relation to lithology and hydrologic flow path in a river riparian zone. Journal of Environmental Quality 2000, 29, 1075–1084. doi.org/10.2134/jeq2000.00472425002900040007x

56. Chang, C.C.Y.; Langston, J.; Riggs, M.; Campbell, D.H.; Silva, S.R.; Kendall, C. A method for nitrate collection for $\delta^{15}N$ and $\delta^{18}O$ analysis from waters with low nitrate concentrations. Canadian Journal of Fisheries and Aquatic Science 1999, 56, 1856-1864. doi.org/10.1139/f99-126

57. Heaton, T.H.E.; Wynn, P.; Tye, A. Low $^{15}N/^{14}N$ ratios for nitrate in snow in the High Arctic (79 °N). Atmospheric Environment 2004, 38, 5611-5621. doi.org/10.1016/j.atmosenv.2004.06.028

58. Mullinger, N.J.; Pates, J.M.; Binley, A.; Crook, N.P. Controls on the spatial and temporal variability of ^{222}Rn in riparian groundwater in a lowland Chalk catchment. Journal of Hydrology 2009, 376, 1-2, 58-69. doi.org/10.1016/j.jhydrol.2009.07.015

59. Cook, P.G.; Wood, C.; White, T.; Simmons, C.T.; Fass, T.; Brunner, P. Groundwater inflow to a shallow, poorly mixed wetland estimated from a mass balance of radon. Journal of Hydrology 2008, 354, 1-4, 213-226. doi.org/10.1016/j.jhydrol.2008.03.016

60. Massmann, G.; Sultenfuss, J.; Dunnbier, U.; Knappe, A.; Taute, T.; Pekdeger, A. Investigation of groundwater residence times during bank filtration in Berlin: a multi-tracer approach. Hydrological Processes 2008, 22, 6, 788-801. doi.org/10.1002/hyp.6649

61. Christensen, J.N.; Dafflon, B.; Shiel, A.E.; Tokunaga, T.K.; Wan, J.; Faybishenko, B.; Dong, W.; Williams, K.H.; Hobson, C.; Brown, S.T.; Hubbard, S.S. Using strontium isotopes to evaluate the spatial variation of groundwater recharge. Science of the Total Environment 2018, 637–638, 672–685. doi.org/10.1016/j.scitotenv.2018.05.019.

62. Gooddy, D.C.; Darling, W.G.; Abesser, C.; Lapworth, D.J. Using chlorofluorocarbons (CFCs) and sulphur hexafluoride (SF6) to characterise groundwater movement and residence time in a lowland chalk catchment. Journal of Hydrology 2006, 330, 44-52. doi.org/10.1016/j.jhydrol.2006.04.011.

63. Chambers, D.; Gooddy, D.C.; Binley, A. Use and application of CFC-11, CFC-12, CFC-113 and SF6 as environmental tracers of groundwater residence time: a review. Geoscience Frontiers 2019, 10, 5, 1643-1652. doi.org/10.1016/j.gsf.2018.02.017.

64. Sigman, D.M.; Casciotti, K.L.; Andreani, M.; Barford, C.; Galanter, M.; Böhlke, J.K. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Analytical Chemistry 2001, 73, 4145-4153. doi.org/10.1021/ac010088e

65. Feast, N.A.; Hiscock, K.M.; Dennis, P.F.; Andrews, J.N. Nitrogen isotope hydrochemistry and denitrification within the chalk aquifer system of north Norfolk, UK. Journal of Hydrology 1998, 211, 233-252. doi.org/10.1016/S0022-1694(98)00245-5

66. Kendall, C.; Elliott, E.M.; Wankel, S.D. Tracing anthropogenic inputs of nitrogen to ecosystems. In Stable Isotopes in Ecology and Environmental Science, R. Michener, R., Lajtha, K. Eds.; Blackwell Publishing, Oxford, UK, 2007, pp. 375-449.

67. Luz, B.; Barkan, E. The isotopic composition of atmospheric oxygen. Global Biogeochemical Cycles 2011, 25, GB4006. doi.org/10.1029/2010GB003883

68. Chen, D.J.Z.; Macquarrie, K.T.B. Correlation of $\delta^{15}N$ and $\delta^{18}O$ in NO_3^- during denitrification in groundwater. Journal of Environmental Engineering and Science 2005, 4, 3, 221–226. doi.org/10.1139/s05-002

69. Heaton, T.H.E.; Stuart, M.E.; Sapiano, M.; Micallef Sultana, M. An isotope study of the sources of nitrate in Malta's groundwater. Journal of Hydrology 2012, 414–415, 244–254. doi.org/10.1016/j.jhydrol.2011.10.037

70. Green, C.T.; Böhlke, J.K.; Bekins, B.A.; Phillips, S.P. Mixing effects on apparent reaction rates and isotope fractionation during denitrification in a heterogeneous aquifer. Water Resources Res. 2010, 46, W08525. doi.org/10.1029/2009WR008903

71. Evans, R.D. Physiological mechanisms influencing plant nitrogen isotope composition. Trends in Plant Science 2001, 6, 122-126. doi.org/10.1016/S1360-1385(01)01889-1