# Quantifying organic carbon storage in temperate pondsediments.

**Authors**

Peter J. Gilbert[1], Scott Taylor[2], DavidA. Cooke[2], Michael E. Deary[2], Michael J. Jeffries[2]

[1]Environmental Research Institute (UHI), Castle Street, Thurso, KW14 7JD, United Kingdom.

[2]Department of Geography and Environmental Sciences, Northumbria University, Newcastle upon Tyne, UK

**Corresponding author**: michael.jeffries@northumbria.ac.uk.

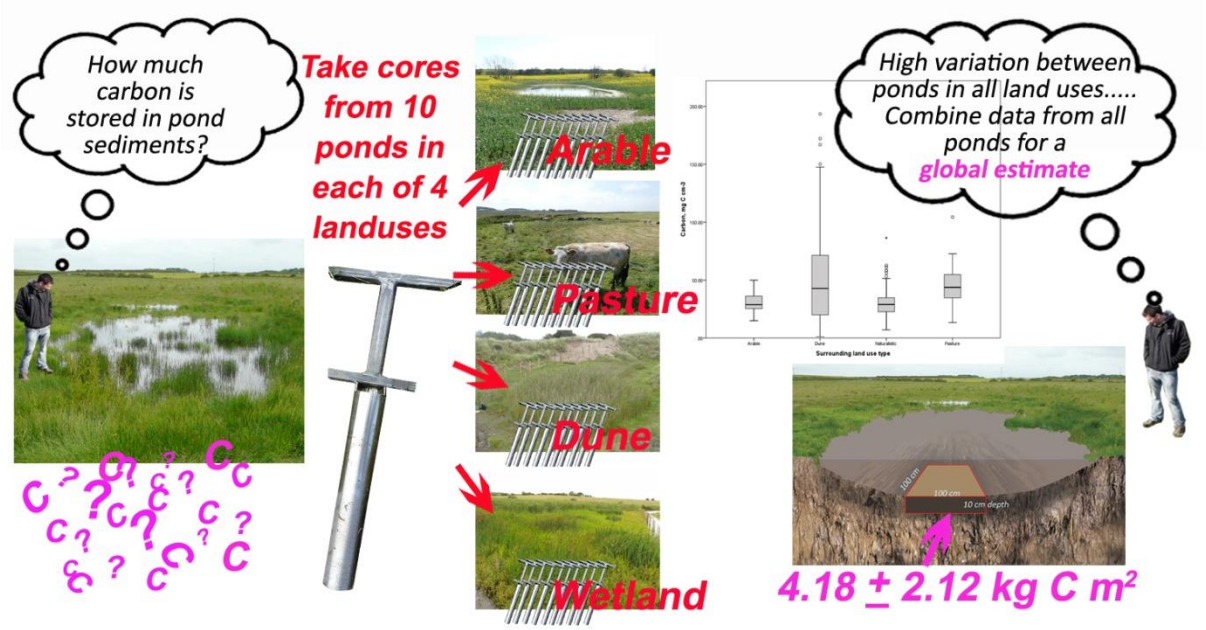
**Present address:** Mike Jeffries, Department of Geography and Environmental Sciences, Northumbria University, Newcastle upon Tyne, UK

**Orcid:**

**Michael Jeffries** 0000-0003-3454-1107

**Michael Deary** 0000-0002-2370-1243

**Graphic abstract**



**Abstract.**Ponds may hold significant stocks of organic carbon in their sedimentsand pond creation may offer a practical application for land managers to increase carbon storage. However, ponds are overlooked in global carbon budgets. Their potential significance is suggested by the abundance of ponds throughout terrestrial biomes and their high carbon burial rates, but we lack measures of sediment carbon stocks from typical ponds. We sampled sediment from lowland temperate ponds in north east England comparing carbon stocks from ponds categorised by surrounding land use, or dominant vegetation, or drying regime, along with measures of variation within ponds. Sediment carbon varied considerably between ponds. This variation was more important than any systematic variation between pond types grouped by land use, vegetation or drying, or any variation within an individual pond. Our estimates of pond sediment organic carbon give measures that are higher than from soils in widespread habitats such as temperate grassland and woodland, suggesting that ponds are significant for carbon budgets in their own right. Ponds are relatively easy to create, are ubiquitous throughout temperate biomes and can be fitted in amongst other land uses; our results show that pond creation would be a useful and practical application to boost carbon sequestration in temperate landscapes.

**Keywords**. Ponds, sediment carbon storage, land use, drying regime, plant communities

## 1. Introduction

Ponds are a part of theplumbing for the global carbon cycle, the freshwater ecosystems, from large rivers and lakes to small ponds and wetlands, responsible for transporting significant amounts of carbon (Cole et al., 2007). Tranvik et al.'s (2018) review of carbon in freshwaters noted the progress from studies of individual systems, to a holistic view of freshwaters as "collectors and reactors", active as transporters, sources and sinks. Much of the recent work on carbon fluxes in ponds was prompted by Downing's re-evaluation of their potential importance (Downing et al., 2006, 2008, Downing 2010). Downing combined evidence for the intensity of ponds' geochemical processing with data suggesting that we underestimated the number and area of small ponds, proposing that as a result, they play an "unexpectedly major role" in the global carbon cycle.The potential importance of small ponds in the carbon cycle was spotlighted by estimates of the numbers and areas of these habitats that suggested they made up a significant but unrecognised proportion of lentic habitats, notably Downing et al's. (2008) estimate of 277,400,000 ponds of <0.001 $km^2$. More recent data suggest numbers like this are over-estimates (Seekell et al. 2013, Verpoorter et al. 2014) but the contribution of small ponds could still be significant. For example Holgerson and Raymond (2016) estimated that ponds of <0.001 $km^2$have a global area of up to 861,578 $km^2$, 8.6% of the total area of lake and pond habitats, but could be responsible for 15.1% of $CO_2$ and 40% of diffused $CH_4$ emissions from these inland waters.

Contemporary studies have highlighted the important but variable role of ponds for carbon fluxes. Holgerson and Raymond (2016) suggest that ponds may be important sources of Carbon to the atmosphere, supported by evidence from boreal and arctic pools, (Abnizovaet al., 2012; Wik et al., 2016; Kuhn et al., 2018), a role likely to increase as climate change warms these higher latitudes (Wik et al, 2016). Conversely, Taylor et al. (2019) estimated carbon burial rates from temperate lowland ponds that were higher than other terrestrial habitats, although Gilbert et al. (2016), working on the same ponds, showed very rapid switches from being net sinks to net source as the ponds dried.Autochthonous and allochthonous inputs can be important in temperate, temporary ponds (Dalu et al., 2016), the precise balance likely to vary with pond hydrology (Abnizar et al., 2014) and succession (Taylor et al. 2019).

For exampleRubbo et al. (2016) demonstrated the importance of terrestrial inputs from leaf litter in a temporary forest pond, whilst Àvila et al. (2016) showed the dominance of autochthonous production in temporary ponds in south east Europe.

The evidence generally supports ponds' role as a source of C to the atmosphere (Torgersen and Branco, 2008), however, much remains uncertain for small, ephemeral systems (Marcé et al., 2019).

Whilst examples of flux measurements for ponds are increasing, the actual Sediment Carbon Stock (here-after SCS) currently stored in pond sediments remains largely unknown. Gilbert et al., (2014) foundhigher SCS in the sediments of lowland agricultural ponds compared to the surrounding soils, and other studies highlight ponds' high C burial rates (Mulholland and Elwood, 1982; Dean and Gorham, 1998; Downing, 2010; Taylor et al., 2019). Pondsare overlooked in land-use policiespromoted for climate change mitigation.This absence of pond SCS from landscape carbon budgets is due in part to an absence of data; ponds are an overlooked habitat (Céréghino et al., 2014; Biggs et al., 2017), despite their global ubiquity in terrestrial habitats (Jeffries 2012, 2016).Our aim was to quantify SCS in temperate ponds. We hypothesised that SCS in ponds would vary with three key variables that drive the variations inecosystem functions among ponds;(1) Surrounding land-use, (2) vegetation, and (3) permanence/drying regime. All three of these variables have the potential to be managed to maximise carbon stocks held by ponds but their importance is not understood.

Land-use has been identified as an important driver of variation in pond biodiversity in studies from around the world, both the nature of the landscape surrounding ponds and also direct impacts resulting from land-use taking place within the pond such as livestock access. For examplein Japan different land uses impactedfarm pond eutrophication (Usio et al.,2017), in Texan playa pools (seasonal ponds),decreasing invertebrate diversity was associated with landscape homogenisation (Hall et al., 2004) and in Belgian farm ponds plant community complexity declined with increasing trampling from livestock (DeClerck et al., 2006). Some pond types can survive in very intensive agricultural landscapes. For example, Bissels et al., (2005) and Altenfelder et al. (2016) highlight the shallow pools of arable fields in French and German lowlands, which may benefit from ploughing to maintain the disturbed ground inundation plant communities.

Our second focus was the vegetation in ponds. The overall amount of plant biomass growing in a wetland is known to affect $CH_4$ fluxes although results vary, some showing a positive correlation (Christensen et al., 2003), others negative (Koelbener et al., 2010). Living plants may be an important route for emissions (Kelker and Chanton, 1997) or produce different rootexudates enhancing or diminishing carbon availability. Plant detritus may inhibit metabolising of carbon due, for example, to phenolics, or vary in robustness, for example,the substantial biomass of many reeds and rushes versus the slight and fragile remains of ephemeral herbs(Dunn et al., 2016).

Drying out is the third factor we investigated and can cause rapid changes to greenhouse gas (GHG) fluxes. Gilbert et al. (2016) measured $CO_2$ fluxes fromsmall ponds as they transitioned from wetted to dry, which resulted in a switch from being sinks to sources within days. Drying out increases $CO_2$ efflux as sediments are exposed to the air (Fromin et al., 2010; Catalan et al, 2014; Martinsen et al., 2019). Marcé et al.'s (2019) review of $CO_2$ and $CH_4$ emissions from dried out inland waters suggests that $CO_2$ efflux is generally increased by drying, whilst $CH_4$emissions are reduced, this reduction attributed to the reduced anoxia and lack of ebullition.

Another key factor impacting the inclusion of SCS in carbon budgets is the limited knowledge of how SCS varies within individual ponds. Ponds may contain localised focal points for sediment deposition, a trend often seen in impoundments(Pittman et al., 2013; Shotbolt et al., 2005; Vanni et al., 2011). Equally, given the seasonal nature in size or permanence of small ponds, it is likely that sediment that stays submerged for longer periods may be subject to longer periods of sediment anoxia, favourable for higher C preservation. Furthermore, shallowsystems are likely to be subject to high levels of disturbance from bioturbation or agricultural activities (e.g. grazing cattle or farm machinery). Given these likely variations, this study also comprises a sampling regime designed to investigate variations in SCS within individual ponds and explore optimal sampling densities for small ponds to inform future studies.

Understanding the inter-pond variation (differences of SCS *among a group* of ponds at a local scale: all the ponds are within 10km of each other in the same biogeographic unit, the South Northumberland Coastal Plain) and intra-pond variation (spatial differences in SCS *within an individual* pond) is crucialto enable upscaling studies to regional, national, and global estimates, their successful integration into carbon budgets and use for carbon mitigation. Our study specifically targets small, lowland ponds, a habitat found throughout the temperate biomes, to provide robust estimates of SCS and how these vary with land use, plant communities and drying regime to inform carbon budgets and the practical management of ponds for carbon sequestration. We measure SCS variation in sediment cores across 40 ponds, representing a range of land uses, plant communities and drying regimes. In addition, we measure the intra-pond variation of SCS by taking 10 cores from one pond in each different land-use category (arable, pasture, dune slack and naturalistic).

## 2. Materials and methods.

## 2.1 Study region.

The subject ponds were in north east England, on the coastal plain at Druridge Bay, Northumberland. The ponds in this region have beenthe focus of multiple studies, for example ontheir ecological importance, history, permanence and relationship to regional weather (Jeffries, 1998, 2012, 2016), and their geochemical processes and potential importance in the carbon cycle (Gilbert et al., 2014; Gilbert et al., 2016; Taylor et al., 2019).

There are over 130 ponds along Druridge Bay including a few farm ponds dating to at least the 1860s as well as ponds dug for nature conservation, subsidence ponds over coal seams, dune slacks and flooded World War 2 defences. The majority are <1000 $m^2$, and < 1m deep, their wetted areas fluctuating markedly with local rainfall. Jeffries (2012, 2016) characterises the Druridgepondscape in detail.

## 2.2 Pond selectionto explore inter-variations

This study sampled 40 ponds acrossDruridge Bay, selected to cover a range of land uses, vegetation, and permanence, and also depths and area. Their morphology, setting and wildlife are typical of lowland ponds in the UK, and of the temperate biomes in general (Jeffries et al., 2016).

Our primary purpose was to quantify the inter-variation in SCS among a range of pond types, using three approaches to divide the ponds into differing groups: (1)adjacent land-use, (2)plant communities and, (3)drying regime.A map of the ponds is given in Supplement 1 and a summary of each of the ponds samples is given in Supplement 2.

### 2.2.1Land use.

The four land-use groups in this study refer to the terrain immediately surrounding the ponds: arable fields used for commercial crops such as cereals, permanent pasture used for grazing sheep or cattle, sand dune slacks with some brackish influence, and the final type being deeper, mostly permanent ponds surrounded by a buffer of wetland vegetation and supporting plant communities typical of ponds in the region (hereafter arable, pasture, dune and naturalistic. Supplement 3details the characteristic plants from ponds in each land useSupplement4 shows a variety of ponds in situ). For example, ponds in the arable fields are routinely ploughed and planted with commercial crops most years, lack any surrounding buffer and usually dry out to leave exposed soil, whilst the naturalistic ponds are heavily vegetated and unmanaged. Species richness was similar in ponds from arable, dune and naturalistic land use ponds, but lower in pasture. The similarities may seem surprising but the arable ponds benefit from a combination of inundation species such *Juncus buffonius* combined with weeds of disturbed ground whilst the naturalistic ponds are often dominated by emergents and lacked submerged taxa perhaps because of their shallow, emergent-choked nature.

All 40 ponds rely primarily on rainfall, although the arable and pasture ponds are sensitive to changes in rainfall over 3-4 weeks, whilst the naturalistic and dune ponds are more buffered and respond, in terms of depth and extent, to variation over 4-5 months (Jeffries, 2016).

The 40 ponds compriseten each from the four land-uses, which had been unchanged for at least 40 years.

2.2.2 Plant communities.

All 40 ponds were included in botanical surveys of eighty ponds along Druridge Bay conducted between 2012 and 2015 (Jeffries, 2016).The survey followed the strategy of the UK National Pond Survey (Pond Action, 1998); all macrophyte plant within a pond's outer margin defined by the maximum winter water level were recordedbetween June and early September. Macrophyte abundance was recorded using the Domin scale, a 1-10 categorical scale each category representing a range of % cover used for the UK's National Vegetation Classification survey (Rodwell, 1995). Plants were identified using Stace (1997), including microscopic examination, for example of *Epilobium* seeds, except Starwort, *Callitriche* species, checked against Lansdown (2008). We also included bare ground as a category.

The ponds' plant communities were classified using TWINSPAN, run on CAP 3.1, taking this to four groups. The four groups coincidebroadly, though not exclusively, with land use, notably one set dominated by grassy pasture ponds and another by inundation weed communitieswhich contained all the arable ponds (Supplement 5). Whilst this is not surprising given the role of land-use in shaping pond biodiversity, it does mean that plant group and land-use type partly confound each other.

2.2.3 Drying regime.

All of the 40ponds had been monitored over several years to assess their vulnerability to drying out, thirty five of them as part of a specific study of their responses to local weather variation over three years (Jeffries, 2016). Drying regimeinformation was based on visits to the ponds carried out every six weeks from November 2010 to November 2013, fifteen visits in total: 2012 was an unusually wet summer so that sites did not dry in the summer. The remaining five ponds were visited regularly as part of other surveys over several years. Ponds were recorded as dried out if they had no water above the surface of the sediment. Ponds were allocated to one of three categories: never known to dry out ($n$ = 6), dried out occasionally over the survey period ($n$ = 13) or dry out annually ($n$ = 21). Whilst the assessment of drying regime of the ponds did not directly coincide with the period of the sediment sampling, it was of sufficient duration and closeness in time to be strongly indicative of the pond behaviour and, importantly, indicative of the geochemical conditions which the sediments were subjected to in the years prior to sampling.

2.3 Intra pond variations of SCS.

SCS sampling took place over the period April to December 2014. Four ponds were chosen, one from each land use type, to examine variations in SCS distribution within individual ponds. From each pond ten cores were collected in a systematic grid pattern across the full pond topography, depth profile, and susceptibility to drying edges; 10 cores gives 45 pair-wise comparisons of cores from within each pond, each of which were compared to test for significant variation of SCS within a pond.

214

215 2.4 Sediment coring.

216 Our study utilised a bespoke metal corer, designed specifically to allow extraction of cores from
217 ponds with markedly differing sediment consistencies. Constructed of a high polish chromium-
218 vanadium steel cylinder (core diameter 48 mm, length 500 mm), with a sharpened cutting edge
219 around the bottom rim, this corer gives a fine, clean cut with minimal micro-crevices to facilitate
220 extraction of the cored material.An internal plunger allowed the cored material to be extruded in
221 the field. Sediment depth in ponds is seldom reported but published figures of 8.9 cm (Gilbert et al.,
222 2014), 11 cm (Nicolet et al., 2004), 28 cm (DeClerck et al., 2006), 27 cm (Tsais et al., 2011), suggest
223 approximately 20 cm is a typical depth. For our study, for individual ponds, core depths ranged from
224 9.2 to 33cm (average, 16.9) for the inter-pond comparison cores and 10 to 33cm (average 18.3cm)
225 for the intra-pond comparison cores (Supplement 2).

226 Upon extrusion, the core was dissected into ~ 1 cm thick slices along the whole length of the
227 core,wrapped in tin foil, placed in a paper sample bag and stored in refrigeration prior to analysis.

228 All cores were taken by the same person to minimise any variation in technique and were taken
229 between April and December 2014, during which period all ponds held some standing water at the
230 time of sampling. For inter pond comparisons cores were taken in the centre of the pond within this
231 wetted area, or in some cases in slightly shallower water where the depth was too great to allow
232 safe wading access.

233

234 2.5 Sediment analysis

235 Data were recorded from each sample slicefromeach core. Firstly, the moisture content and Dry Bulk
236 Density (DBD) of each sample slice were measured, followed by % carbon analysis by total elemental
237 analysis (TEA). Moisture contentand DBDare inversely related: they give a measure of the density of
238 the sediment layer that has been laid down, allowing us to see how this varies with depth and
239 location. DBD and % carbon are both required to calculate the carbon density (mg C $cm^{-3}$) in a
240 sample, as detailed below. The carbon density can be scaled upto SCS (Kg C $m^{-2}$) for a specified depth
241 of sediment, typically 10cm, as used in this study.

242 For moisture content (%) all samples were weighed within 24 hours of coring then placed in a drying
243 cabinet for sevendays at ~ 40 $^{o}$C until a constant weight was achieved, to give the dry weight of each
244 sample. Soil moisture was calculatedusingequation 1.

245 $$moisture\ content\ (\%) = \frac{wet\ weight - dry\ weight}{wet\ weight} * 100 \qquad \text{Eqn 1}$$

246 Dry Bulk density (DBD; g $cm^{-3}$), was calculated using the dry weight and known volume for each
247 dissected 1 cm section using equation 2.

248 $$DBD = \frac{dry\ weight_{sediment\ section}}{Volume_{sediment\ section}} \qquad 2$$

249 Dried samples were ground and sieved and ~ 5 mg processed for total carbon analysis via
250 TEA(Thermo Scientific FLASH 2000 Series Organic Elemental Analyser; oven temperature = 980$^{o}$C;
251 run time = 360 seconds). Samples were placed in a carousel for automated analysis, with a program
252 that every tenth sample was run in triplicate to calculate the precision of analysis (% relative
253 standard deviation, RSD = 7.81 %),followed by a blank to monitor the Limit of Detection and Limit of
254 Quantification (0.46 % C and 1.43 % C respectively). These QC checks also allowed us to identify any
255 analysis sequences that had encountered instrumental issues or malfunctions. To calculate the mass
256 of carbon per 1 cm section (carbon density, mg C $cm^{-3}$) we combined the % C concentration from the

TEA with the DBD using equation 3. The same form of equation 3 was used to calculate the nitrogen density

Carbon density (mg C $cm^{-3}$) = $\frac{\%\ C}{100} \times DBD_{sediment\ section}\ (g\ cm^{-3}) \times 1000$ Eqn 3

2.6 Statistical analysis

2.6.1. Intra-pond differences

Variations in the density of C (mg C $cm^{-3}$)amongthe sediment cores were tested separately for each pond, using Linear Mixed Models. Each of the ten cores from a pond were included as factors, and as a random effect since individual cores may show different trends, along with depth as a covariate with a repeat measures design (AR1). Differences between individual cores were tested using Bonferonni post-hoc comparisons. Data were normalised using ln transformation. Differences in dry bulk density, % moisture and % carbon were also tested, DBD using the same mixed models design as the carbon density. The % moisture and % carbon were compared using the Kruskal-Wallace test as the data could not be effectively normalised.

2.6.2 Inter-pond differences.

Differences in the density of C (mg C $cm^{-3}$)and DBD between ponds were tested using Linear Mixed Models. Land-use, plant community and drying were included as fixed factors, with individual ponds as random effects and depth as a covariable with a repeat measures design (AR1) and ln transformed data. Because of the confounding of plant community within land-use categories, a full model with both could not be created. Plant communitiesand land use were tested in separate models in combination with drying regime and depth. Differences between individual factors were tested using Bonferonni post-hoc comparisons. Data were normalised using ln transformation.Differences of % moisture and % carbon between ponds were tested separately for land-use, plant community and drying regime using the Kruskal-Wallace test.

All statistics were run on SPSS 24, with the significance level at P = 0.05

2.6.3Decision Tree Analysis

To explore the interaction between plant community and wetting/drying cycles as explanatory factors for variation in carbon stocks, we used a Decision Tree Analysis (SPSS), which is a non-parametric multivariate statistical technique that has been used for a range of environmental applications (Baker et al. 2006; Elnaggar and Noller, 2010). The analysis was carried out on carbon density (mg C $cm^{-3}$) values from 616 samples, with permanence (always, sometimes or never dries) and plant community (diverse, ephemeral, grassy or reeds) as independent variables. The Chi-squared Automatic Interaction Detection (CHAID) branching method was used with 95% confidence levels.

3.Results and Discussion.

3.1 Intra-pond variation.

Our primary purpose was to quantify variations inSCS among a diversity of lowland ponds, testingintra-pond variation and, comparingcarbon density between ponds of different types defined by land use, plant communities and drying pattern, to inform carbon budget models and pond management for carbon capture.Variability in C distribution across small ponds is to be expected, perhaps due to localised focal points for sediment deposition(Pittman et al., 2013; Shotbolt et al., 2005; Vanni et al., 2011) or seasonal fluctuations to the wetted area creating gradients of anoxia resulting in differential C accumulation

Mean density of carbon (mg C $cm^{-3}$) for the ten sampled cores within each of the four pondsselected for the intra-pond comparison is shown in Figure 1 and Table 1

Table 1. Intra pond core summary, mean ± one standard deviation for carbon density, carbon %, bulk density of sediment and moisture % from the four ponds. "Intra core differences?" indicates if significant differences were found between some of the 10 cores from within a pond. The carbon densities (mean ± standard deviation) are shown for the significantly different cores from ponds 30 and 38. Different superscripts indicate the significant differences.

| | Pond: land use and pond ID | | | |
|---|---|---|---|---|
| | Pasture, 29c | Dune slack, CPP1 | Naturalistic, 30 | Arable, 38 |
| Carbon density (mg C $cm^{-3}$) | 23.00± 7.13 | 12.48 ± 6.47 | 48.59 ± 10.98 | 30.15 ± 6.21 |
| Carbon, % | 3.70 ± 3.17 | 1.09 ± 0.99 | 6.28 ± 3.68 | 3.14 ± 0.87 |
| Dry Bulk density, g $cm^{-3}$ | 0.86 ± 0.36 | 1.32 ± 0.34 | 0.95 ± 0.33 | 0.98 ± 0.12 |
| Moisture, % | 43.60± 15.6 | 27.13 ± 8.1 | 40.24 ± 13.04 | 29.99 ± 3.97 |
| Intra core differences? | ns | Ns | F = 8.09, df 1, 9, P<0.001 | F = 4.05, df 1,9, P<0.001 |
| Significant differences between cores | | | | |
| Pond 30, core numbers | 2, 6, 7 | 9 | | 1, 3, 4, 5, 8, 10 |
| Carbon density (mg C $cm^{-3}$) | 56.03 ± 10.89[a] | 48.78 b ±10.60[a,b] | | 44.31 ± 8.45[b] |
| Pond 38, core numbers | 1 | 2, 6, 9, 10 | | 3, 4, 5, 7, 8 |
| Carbon density (mg C $cm^{-3}$) | 26.41 ± 3.27[a] | 28.43 ± 6.86 [a,b] | | 32.19 ± 5.47[b] |

Figure 1. Within-pond carbon density variation. Carbon density from 10 cores taken from four ponds, with the carbon density reported as the mean mg C $cm^{-3}$ for the whole column length (column depth information is given in Supplement1; error bars represent ± 1 sd).The numbers along the x-axis refer to the ten cores from each pond. For comparison to Figure 2, the four ponds used for the intra pond sampling were 30 (naturalistic), 38 (arable), 29c (pasture) and CPP1 (dune slack). Carbon density is the mean concentration along the core

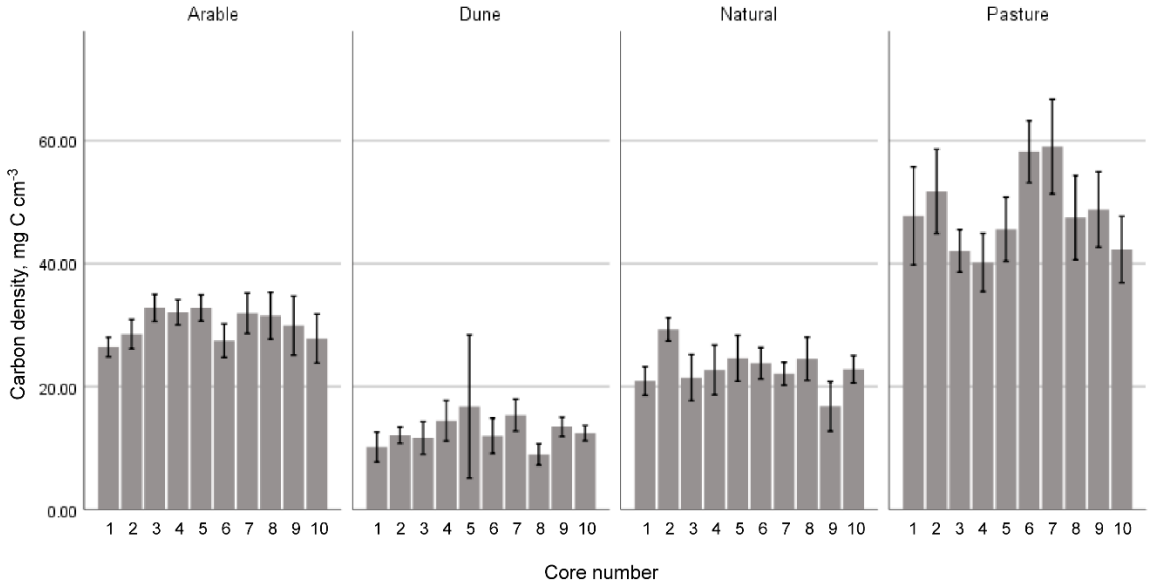
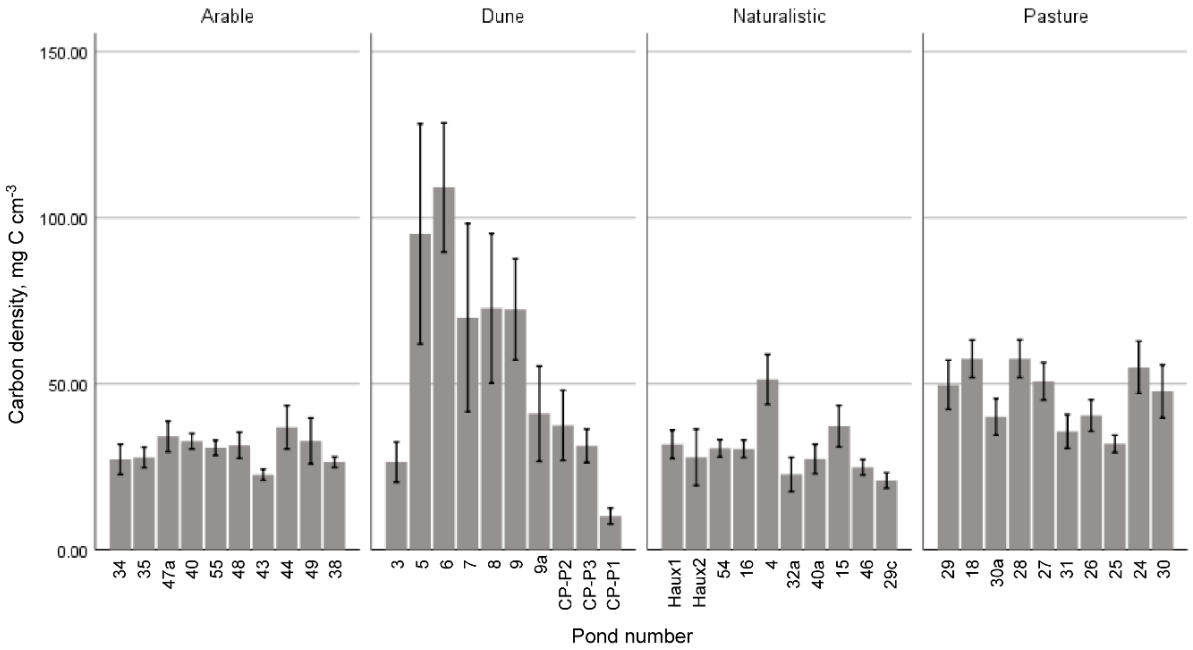


Figure 2. Between pond carbon density variation. Carbon density from 10 cores taken from forty ponds, ten ponds from each of four land uses , with the carbon density reported as the mean mg C $cm^{-3}$ for the whole column length (column depth information is given in Supplement1; error bars represent ± 1 sd). The names and numbers along the x-axis refer to the 40 individual ponds.



From the results of the Linear Mixed Model, among the ten replicate cores collected from the four individual ponds, significant differences in density of C(mg $cm^{-3}$) were observed between some of the cores in only two of the ponds. In the arable field pondone core was significantly different from five of the other nine. Inthe naturalistic pond out of the 45 possible pair-wise comparisons of cores there were 10 that gave significant differences resulting from three cores being significantly different to another group of six (Table 1 summarises these significant differences between individual cores).Thus, the results show some, but limited, intra pond variation in density of C.

The range of carbon density from the intra-pond replicates (10.5-59.0 mg C $cm^{-3}$) is broadly similar to the overall range observed from single cores in the 40 pond survey, (12-123 mg C $cm^{-3}$), only two of which had > 73 mg C $cm^{-3}$. At the 95 % CI, the margins of error among replicate cores were all < 11 % of the mean for each pond (mean = 8.4 %, range = 6.1 - 10.8 %) i.e., when calculating carbon stocks from individual sediment cores we can assume, with 95 % confidence, that the estimated C stock is representative of the sediment distribution across the pond within 8.4 %.

The low margins of error for replicate cores stated above indicate a good level of reproducibility in our sampling of these sediments. In an analysis of replicate cores from impoundments ~ 100,000 $m^2$, Pittman et al. (2013) found that a 25 % precision could be gained from 10 cores, and while the ponds in this study are ~ 1-2 orders of magnitude smaller, the mean % RSD was 16 %. Whilst the areas of the ponds were considerably varied (range = 366-6675 $m^2$) no relationship was observed between precision and sampling densities, suggesting that sediment distribution is equally varied among ponds of differing size.

Lack of detailed studies regarding the heterogeneity of sediment C distributions within systems is one of the major factors leading to poorly constrained C stock estimates within small water bodies such as ponds. This study highlights that whilst C concentrations (% C) may vary when compared among replicate cores from individual ponds, when calculating the carbon the margin of error in estimations is comparably low, with C density estimations from individual sediment cores being relatively representative of sediments across the pond. To include pond SCS in carbon budgets, it is more important to sample as many ponds as possible to capture the variation between ponds, rather thantake more samples from fewer ponds. These factors support the single core sampling strategy used in the 40 pond survey and the validity of single core, extensive surveys across as many ponds as possible for future work.

## 3.2 Inter-pond variation.

### *3.2.1 Relationship between carbon density and land use, plant community type and drying regime*

The data for all cores from the forty ponds,categorised by land use, are shown in Figure 2. The mean data for carbon density (mg C $cm^{-3}$) and C concentrations (%C), sediment DBD andmoisture % are summarised for the ponds categorised by surrounding land use (Table 2), drying regime (Table 3) and vegetation type (Table 4) and Figure 3.

Table 2. Summary, mean ± one standard deviation for carbon density, carbon %, bulk density of sediment and moisture % in the four categories of land use. Significant differences are indicated by different superscripts: carbon density and bulk density results from the linear mixed models; % moisture and % carbon from Kruskal Wallace test.

| | Land use | | | |
|---|---|---|---|---|
| | Naturalistic | Arable | Pasture | Dune |
| Carbon density (mg C | 29.92 ± 11.24 | 30.46 ± 7.24 | 44.82 ± 13.58 | 51.24 ± 39.19 |

| | | | | |
|---|---|---|---|---|
| $cm^{-3}$) | | | | |
| Carbon, % | 4.40 ± 4.46[a] | 2.89 ± 0.90[a] | 5.63 ± 3.27[b] | 9.12 ± 8.79[b] |
| Dry Bulk density,g $cm^{-3}$ | 0.99 ± 0.45 | 1.12 ± 0.33 | 0.96 ± 0.32 | 0.94 ± 0.48 |
| Moisture, % | 39.3 ± 18.7[a,b] | 33.1 ± 8.2[a] | 40.3 ± 13.0[b] | 42.7 ± 20.5[b] |

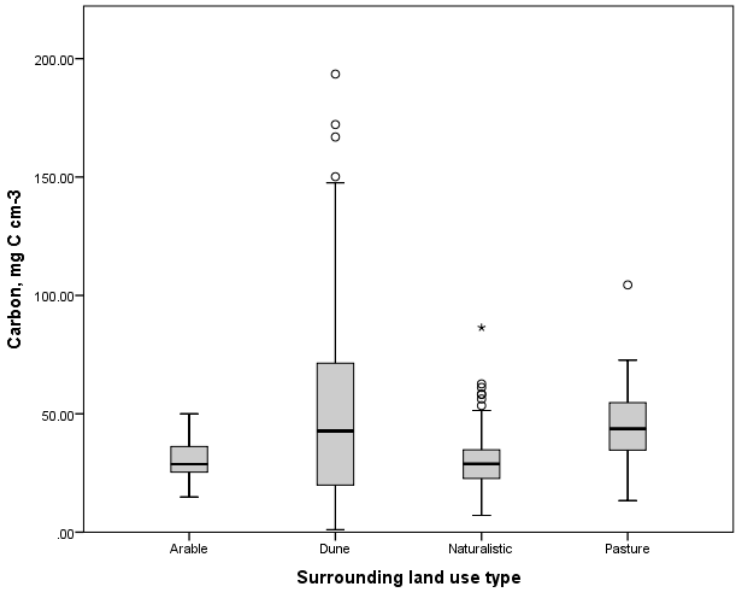
Mean carbon density varied with land use, from 29.92 mg C $cm^{-3}$ in the naturalistic ponds to 51.24mg C $cm^{-3}$in the dune sites (Figure 3a). Carbon density was higher in the sediments of ponds that dried out every year, 49.69 mg C $cm^{-3}$versus ponds that only dry in some years or never, at 32.52 mg C $cm^{-3}$ and 30.69 mg C $cm^{-3}$ respectively (Figure 3c). When the ponds were classified by vegetation types, the highest density was found in ponds with a diverse mixed sward of wetland flora, 48.8 mg C $cm^{-3}$ (Figure 3b)

*3.2.2 Outcomes of the Linear Mixed Models*

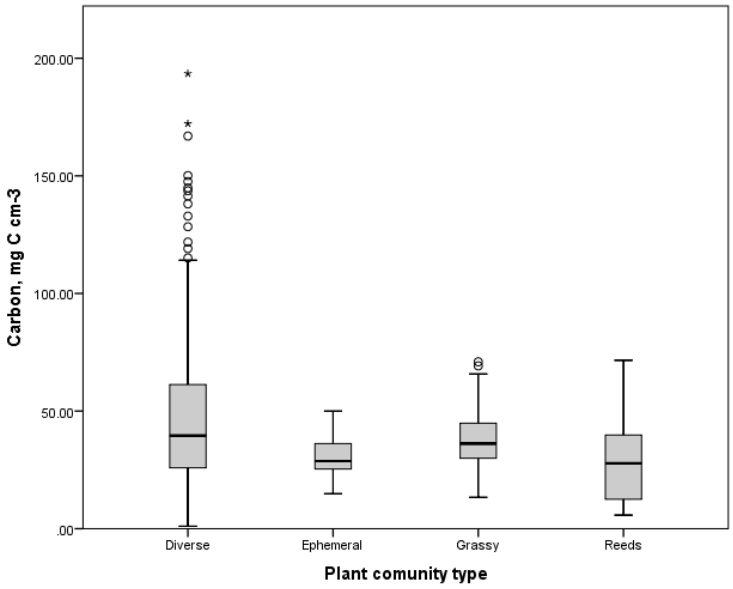
Summary outcomes for the models of carbon density are shown in Table 5. A combination of plant community type, drying regime and depth provide the best model based on Akaike's Information Criterion (AIC). Despite the differences in mean carbon density between ponds from the four different land uses, land use was not significant, unless used as the sole factor, because of marked variation of carbon density between ponds within each category Carbon density is significantly higher in the ponds that dry up every year compared to those that only dry some years or not at all (Figure 3b, Table 3). Carbon is also higher in those ponds with diverse wetland flora forming a dense cover (Figure 3c, Table 4).

Figure 3. Organic carbon density, mg C $cm^{-3}$, in pond sediments from (a) ponds in amongst four land-uses, (b) different plant communities dominated by reeds, diverse herbs and grasses, grass or ephemeral weed species,(c) ponds with different drying regimes. The thick horizontal bars show the median and the box, the inter-quartile range, IQR. Circles and ✶ are outliers 1.5 or 3 times the IQR beyond the upper quartile.

(a)




(b)




(c)

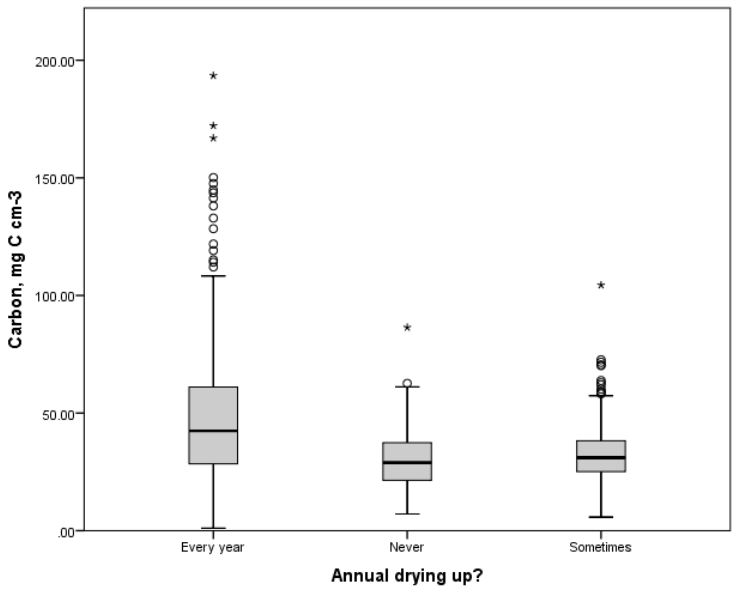

200.00
150.00
100.00
50.00
.00
Carbon, mg C cm-3
Every year
Never
Sometimes
Annual drying up?

Table 3. Summary, mean ± one standard deviation for carbon density, carbon %, bulk density of sediment and moisture % in the three categories of drying regime. Significant differences are indicated by different superscripts: carbon density and bulk density results from the linear mixed models; % moisture and % carbon from Kruskal Wallace test.

| | Dry period: do the ponds dry out annually? | | |
|---|---|---|---|
| | Never | Sometimes | Always |
| Carbon density (mg C $cm^{-3}$) | 30.69 ± 12.76[a] | 32.52 ± 14.27[a] | 49.69 ± 30.99[b] |
| Carbon, % | 4.10 ± 2.89[a] | 3.89 ± 3.80[a] | 7.88 ± 7.43[b] |
| Dry Bulk density $cm^{-3}$ | 0.96 ± 0.42 | 1.09 ± 0.38 | 0.94 ± 0.43 |
| Moisture, % | 39.16 ±17.01[a,b] | 35.88 ± 13.82[a] | 41.80 ± 17.98[b] |

Table 4. Summary, mean ± one standard deviation for carbon density, carbon %, bulk density of sediment and moisture % in the four categories of vegetation type. Significant differences are indicated by different superscripts: carbon density and bulk density results from the linear mixed models; % moisture and % carbon from Kruskal Wallace test.

| | Vegetation | | | |
|---|---|---|---|---|
| | 1, reeds | 2, diverse | 3, grassy | 4, ephemeral |
| Carbon density (mg C $cm^{-3}$) | 28.46 ± 16.70[a] | 48.80 ± 32.93[b] | 38.48 ±12.04[a,b] | 30.46 ± 7.27[a] |
| Carbon, % | 5.29 ±6.24[a,c] | 7.97 ± 7.41[b] | 4.42 ± 3.16[c] | 2.89 ±0.90[a,d] |
| Dry Bulk density $cm^{-3}$ | 1.02 ± 0.50 | 0.89 ± 0.44 | 1.06 ± 0.30 | 1.12 ± 0.32 |
| Moisture, % | 40.10 ± 21.75[a,c] | 43.65 ± 18.45[b] | 35.92 ±12.41[c,d] | 33. 07 ± 8.18[d] |

Table 5. Summary of General linear mixed models of carbon density, mg C $cm^{-3}$ ranked by AIC. The variablesincluded in each model are shown, along with their significance, in same order as the variables were included in the models and overall AIC and number of parameters.

| Variables included in model | Significant | AIC, total parameters |
|---|---|---|
| Plant community, permanence, depth | P<0.01, P<0.01, P<0.05 | 617.30, 10 |
| Land use, permanence | ns, P<0.01 | 627.30, 9 |
| Permanence | P<0.01, | 628.18, 6 |
| Land use, permanence, depth | ns, ns, P<0.01 | 629.40, 10 |
| Plant community, depth | P<0.05, P<0.01 | 630.48, 8 |
| Permanence, depth | P<0.05, P<0.05 | 630.57, 7 |
| Land use | P<0.05 | 630.94, 7 |
| Land use, depth | P<0.05, P<0.01 | 631.75, 8 |
| Depth | P<0.01 | 635.74, 5 |

The design of the inter-pond sampling anticipated finding marked differences among pond types. In particular, we hypothesised significant differences between all four land-uses given the striking differences in the morphology, biodiversity, and context of the landscapes, from arable field ponds which were ploughed and dried out every year to the naturalistic ponds which were unmanaged and sheltered within protective buffers of wetland and rank grassland. However, there were no systematic differences in carbon density between the ponds from the four different land uses. Our hypothesis that marked differences in land-use would result in very different carbon stocks is not supported. Instead, the carbon density in the sediments of individual ponds within each of the land use appears to be as heterogeneous as the well-established heterogeneity of pond wildlife at the landscape scale (Davies et al., 2008). In summary, pond sediments contain high densities of carbon

compared to many other land-uses such as grassland or forestry, and all types of ponds may have this potential.

However, the results of the linear mixed model do support the potential role of both plant community and wetting/drying cycles as explanatory factorsfor variation in carbon stocks, although it should be noted that plant community and land use could not be used together in models. Ponds with diverse plant communities and also ponds that dry out every year were associated with significantly higher carbon density than for other community types and ponds that either don't dry out, or do so sometimes. To explore further the interaction between these two factors, we used a SPSS decision tree classification analysis. The results in Figure 4 show that permanence is the most important factor in determining carbon density, with those ponds that dry out each year having 57% more carbon (49.7 vs 31.7 mg C $cm^{-3}$) than those that either dry out occasionally or not at all. In addition, of the ponds that always dry out, those withdiverse plant communities have higher carbon densities than other plant community types, with an average of 69.8 mg C $cm^{-3}$, or 76% higher than the average for all ponds.The next highest carbon density was in ponds with 'grassy' plant communities (47.3 mg C $cm^{-3}$, or 19.7% higher than the average for all ponds).Such information may be of use in designing landscape management strategies that maximise carbon densities in ponds that are constructed for the purpose of carbon sequestration. In the following sections we discuss the role of the rhizosphere in sediment carbon accumulation and the likely influence of community type and drying regime as an explanation for the results shown in Figure 4.

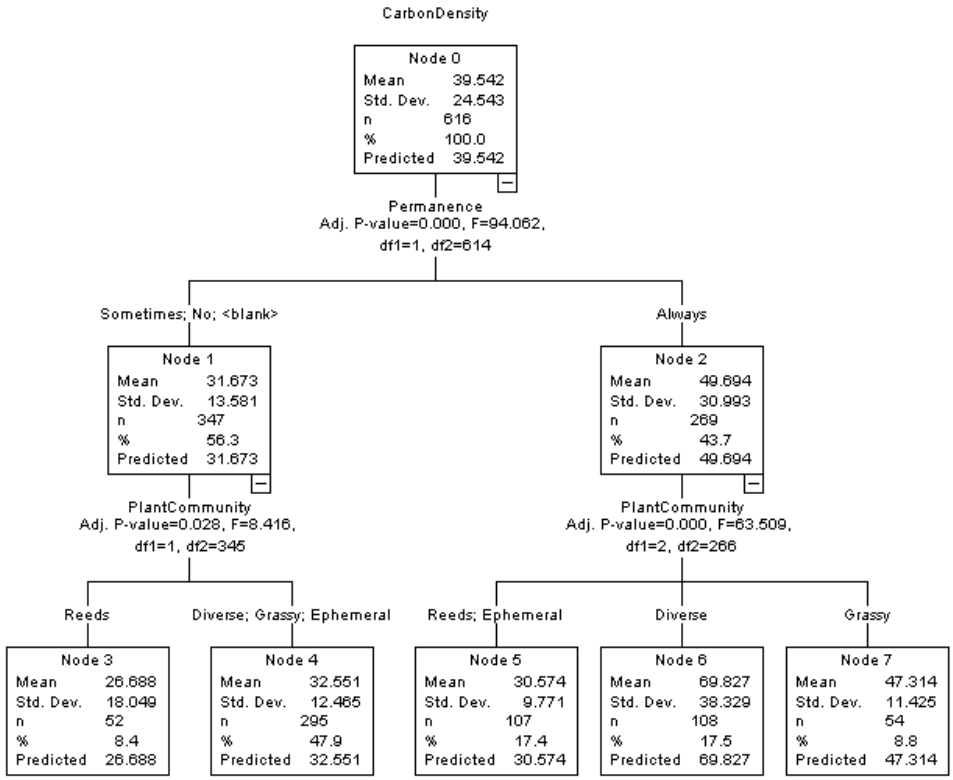



Figure 4. Decision tree analysis for carbon density (mg C $cm^{-3}$), based on permanence (always, sometimes or never dries) and plant community (diverse, ephemeral, grassy or reeds) as independent variables. The CHAID branching method was used with 95% confidence levels. Mean and predicted are the same in this model.

### *3.2.3 The significance of drying regime and plant community type*

The much higher carbon density observed in ponds that both dry out and have a diverse plant community can be explained by either greater carbon inputs, reduced carbon outputs or a combination of both, though the underlying ecological, microbiological and chemical factors are complex. Moreover, because ponds with highest carbon densities experience both wet and dry periods, processes that apply to both soil and submerged sediments should be considered when identifying the most likely contributing factors. The drying regime may have a direct impact on carbon storage, for example, there is evidence that cycles of wetting and drying, can stimulate the production of root exudates (Atere et al., 2017).

For the effect of plant communities on carbon storage, overall, we found that those communities associated with the highest carbon stocks were those with a thick sward, either of diverse herbs, grasses and a few larger rushes ("type 2, diverse") and sedges or predominantly grasses ("type 3, grassy"), with species such as flote grass (*Glyceriafluitans*), soft rush (*Juncus effusus*) or bistort (*Persicaria amphibia*). The ephemeral inundation weed community of community "type 4, ephemeral" included many annuals that rapidly decay away, such as cud weed (*Filago vulgaris*) and pineapple mayweed (*Matricariadiscoidea*), unlikely to leave substantial organic remains and the bare mud of these ponds is not visibly rich in plant debris. Carbon density was also lower in the "type 1, reeds" ponds, characterised by common reed (*Phragmites australis*).

To further investigate which aspects of the plant community might explain the higher C density shown from the decision tree analysis for "type 2, diverse" ponds that also dry out, we have identified, in Table 6, the plant species that were either uniquely associated with the 'diverse' plant communities or were present in a greater proportion of 'diverse' ponds, compared to the other community types (thespecies in Table 6 represented 30% by cover of all plant types present in 'diverse' ponds compared to only 6.5% in other community types). Of these, it is notable that several are members of genera that have been shown in the literature to have a high content of polyphenols, including *Ranunculus*(Neag et al., 2017), *Vicia*, (Orhan et al., 2009), *Equisetum*(Graefe and Veit, 1999) and*Epibolium*(Tóth et al., 2009). Plant biomass that is rich in polyphenols is known to show some resistance to microbial degradation, particularly bacterial degraders, which may be affected by the antimicrobial properties of some polyphenols (Yakimovich et al. 2018). Polyphenols can also leach into aquatic environments from plants in surrounding land (Cieślewicz 2014). In such circumstances, fungi, which are able to degrade polyphenols, may benefit from reduced competition. A microflora dominated by fungi will give rise to reduced $CO_2$ emissions compared to bacteria, which are considered to be the "drivers of more active decomposition" (Yakimovich et al. 2018). Thus, high polyphenol content in plant detritus is likely to contribute to the stabilisation of organic carbon in pond sediments.

Table 6. Species specifically associated with ponds that have 'diverse' plant communities, compared to ponds that have predominantly 'reeds', 'ephemeral' and 'grassy' plant communities. The comparison is made only for ponds that dry out each year.

| | |
|---|---|
| *Alisma plantago-aquatica* | *Ranunculus lingua* |
| *Capsella bursa-pastoris* | *Ranunculusscleratatus* |
| *Elytrigia repens* | *Rumex crispus* |
| *Epilobium hirsutum* | *Salix spp.* |
| *Equisitum fluviatile* | *Solanum dulcamara* |
| *Filipendulaulmara* | *Sonchus sp.* |
| *Iris pseudacorus* | *Sparganiumerectum* |

| | |
|---|---|
| *Juncus buffonius* | *Tripleurospermuminodorum* |
| *Juncus conglomeratus* | *Typha latifolia* |
| *Large unid sedge* | *Vicia cracca* |

Another major input of C into pond sediments, and one that likely to benefit from a diverse plant community type, is the production of root exudates, which is linked to the rhizosphere priming effect (Shahzad et al., 2015). The root zone of aquatic plants typically extends to 20 to 30cm (Bowden, 1987), and has the potential to transform the sediment environment through the supply of (labile) organic carbon as root exudates and root detritus, as well as oxygen (Bais et al. 2006; Kotas et al., 2019; Shahzad et al., 2015). The introduction of root exudates such as organic acids, sugars, amino acids and phenolics, as well, as polysaccharides and proteins (Bais et al. 2006), benefits the plant through the stimulation of the microflora to release nutrients from the stored organic carbon (Bais et al., 2006; Fontaine et al., 2011; Shahzad et al., 2015).Root exudates can also be directly incorporated into stable carbon stocks if deposited as aggregates with inorganic soil components (Atere et al., 2017). Cycles of wetting and drying, can stimulate the production of root exudates (Atere et al., 2017), which may be a factor in the increased carbon densities for ponds that dry out.

The effects of root exudate production on the microbial flora may be complex and vary with time. For example, in our recent work on some small ponds of exactly known age, also at Druridge Bay (Taylor et al., 2019), lower C burial rates were correlated with abundant *Juncus articulatus.* A possible explanation is that the labileroot exudates this species is known to promote microbial activity which decomposes organic matter (Dunn et al., 2016).Nevertheless, Kotas et al. (2019) demonstrated that for sedge wetlands, whilst bacteria were the initial beneficiaries of $^{13}C$ labelled exudates, fungi were the longer-term recipients. Fungi have been shown to have a major role in the decomposition of detrital matter, having the advantage of hyphal growth that can extend deep into the stored organic matter. Fungi also have the extracellular enzymes that can degrade the more recalcitrant organic matter fraction such as lignocellulose, though at slower rates than for bacterial degradation(Fontaine et al., 2011; Kotas et al., 2019).

### *3.2.4 The possible role ofnitrogen fixation*

For several of the plant species listed in Table 6, there are literature examples of either the same species or species within the same genera having rhizosphere associations with nitrogen-fixing bacteria. These include *Equisitum*(Andersson and Lundegårdh, 1999), *Iris*(Chung et al., 2015), *Juncus*(Tjepkema and Evans, 1976), *Sonchus*(Hong et al., 2009), *Typha*(Biesboer, 1984) and *Viccia*(Van Cauwenberghe et al., 2014). There is evidence from soils under trees whose rhizosphere was associated with nitrogen-fixing bacteria that the older (humified) carbon stocks are conserved in comparison to soil under non-nitrogen fixing trees (Binkley 2005; Resh et al. 2002). Whilst these are very different environments to the ponds in the present study (though these ponds do dry out), the effect may hold more generallyand could be an important factor in conserving sediment carbon.

The discussion in this and the previous section highlights the importance of factors that affect the ecological balance of the soil microflora and the role this has on nutrient cycling and decomposition rates of organic matter (Fontaine et al. 2011). Therich and varied rhizosphere that arises from diverse plant communities will have an important function in determining this ecological balance, and therefore on carbon storage.

Our results suggest that the rhizosphere may be a key but overlooked driver of carbon storage in pond sediment, in need of investigation to maximise the effectiveness of pond creation and management for carbon storage.

### 3.2.5 Pond age

The age of the pond might also affect carbon accumulation. The precise age of the study ponds is not known accurately enough for all ponds to include it in the models. Most of the ponds were at least 40 years old, created by subsidence over old coal mines (Jeffries, 2012), although probably even older. One of the natural ponds is known to be over 100 years old and another, the youngest, at 19 years when sampled. New ponds may show a lag time of two or three years before carbon accumulation becomes substantive (Taylor et al., 2019), but all the ponds in this study are considerably older. The results do not suggest that the amount of time ponds have had to accumulate carbon may be a confounding factor in our data.

## 3.3 Overall carbon stocks

Our results highlight the need to quantify carbon density rather than just a percentage. The high % C in dune ponds was compensated by low bulk density so that the overall carbon density was lowest in these sites. Conversely, the low % of carbon the arable field ponds, which when dry would be exposed, baked and cracked mud, barely different from the surrounding soil that had not been inundated, was compensated by a high bulk density so that overall carbon density was relatively high.

In Table 7, we present the scaled-up sediment carbon stock for each land use type and for all ponds.The ponds' mean sediment carbon (4.18 ± 2.21 kg C $m^{-2}_{< 10\ cm}$) is in the midrange of values reported for habitats of the UK (range = 2.9-5.9 kg C $m^{-2}$; calculated as < 10 cm from values reported in Countryside Survey, 2007), being higher than those of coastal margins, agricultural land, grassland, and woodland, yet lower than wetlands, bogs, and fens, marshes and swamps. Given the relative youth of the ponds, the amount of SCS compared to many other habitats and the ease of pond creation within heterogeneous landscapes, the results show that ponds have the potential to be an important tool in the mitigation of C emissions.

Table 7. Scaling up pond sediment carbon stocks (SCS) to Kg C $m^{-2}$. The core data have been used to estimate the carbon stock in a 1 $m^2$ area of sediment over a depth of 10 cm, including a global figure for all ponds combined.

| | Pond type | | | | |
|---|---|---|---|---|---|
| | Naturalistic | Arable | Pasture | Dune | All ponds |
| SCS, mean ± SD, Kg C $m^{-2}$ | 3.01 ± 0.77 | 3.04 ± 0.48 | 4.74 ± 0.70 | 5.92 ± 3.25 | 4.18 ± 2.12 |
| Min, Max, Kg C $m^{-2}$ | 2.14, 4.90 | 2.26, 3.54 | 3.43, 5.98 | 1.12, 11.81 | 1.12, 11.81* |

## 5. Conclusions.

The carbon buried in pond sediments is higher, volume for volume, than many other terrestrial habitats. The striking outcome of the survey is that individual ponds show considerable variation in carbon stocks, sufficient to obscure many systematic differences that might be expected due to land use, vegetation, or drying out. However, this result also suggests that a global estimate combining the data from all ponds is useful regardless of categorisations such as land use. The combined measure of sediment carbon stocks is 4.18 ± 2.12 kg C $m^{-2}$,over the top 10cm, a first estimate for typical lowland temperate ponds. Variation within individual ponds is less than between ponds: future studies should maximise the number of ponds sampled to capture inter-pond variation, one core per pond.

The results suggest that the drying regime and vegetation of ponds deserve more detailed investigation as potential drivers of carbon accumulation. Such considerations will be important if

ponds are constructed to capture and hold carbon to maximise their effectiveness as carbon sinks. The relative ease of pond creation suggests their potential as an application to help maximise carbon sequestration at the landscape scale. Recent proposals for landscape rewilding have been explicitly included ponds as a means of carbon sequestration (Rewilding Britain, 2019), and our study shows that ponds can indeed play a significant role.

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**Appendix A: Supplementary data**

S1. Map of ponds

S2. Details forr each pond, e.g. area, drying.

S3. Detail on the four land use categories.

S4Photographs of a range of the ponds in situ.

S5 Details of plant TWINSPAN classification.

**Data repository**:

The data set is lodged with the Mendeley repository:

Jeffries, Michael; Gilbert, Pete; Deary, Mike; Taylor, Scott; Cooke, Dave (2019), "Druridge Bay (Northumberland, UK) pond sediment carbon core data for 40 ponds", Mendeley Data, V1, doi: 10.17632/wmbhzhdr6b.1

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[Dataset] Jeffries, Michael; Gilbert, Pete; Deary, Mike; Taylor, Scott (2019), "Druridge Bay (Northumberland, UK) pond sediment carbon core data for 40 ponds", Mendeley Data, V1, doi: 10.17632/wmbhzhdr6b.1

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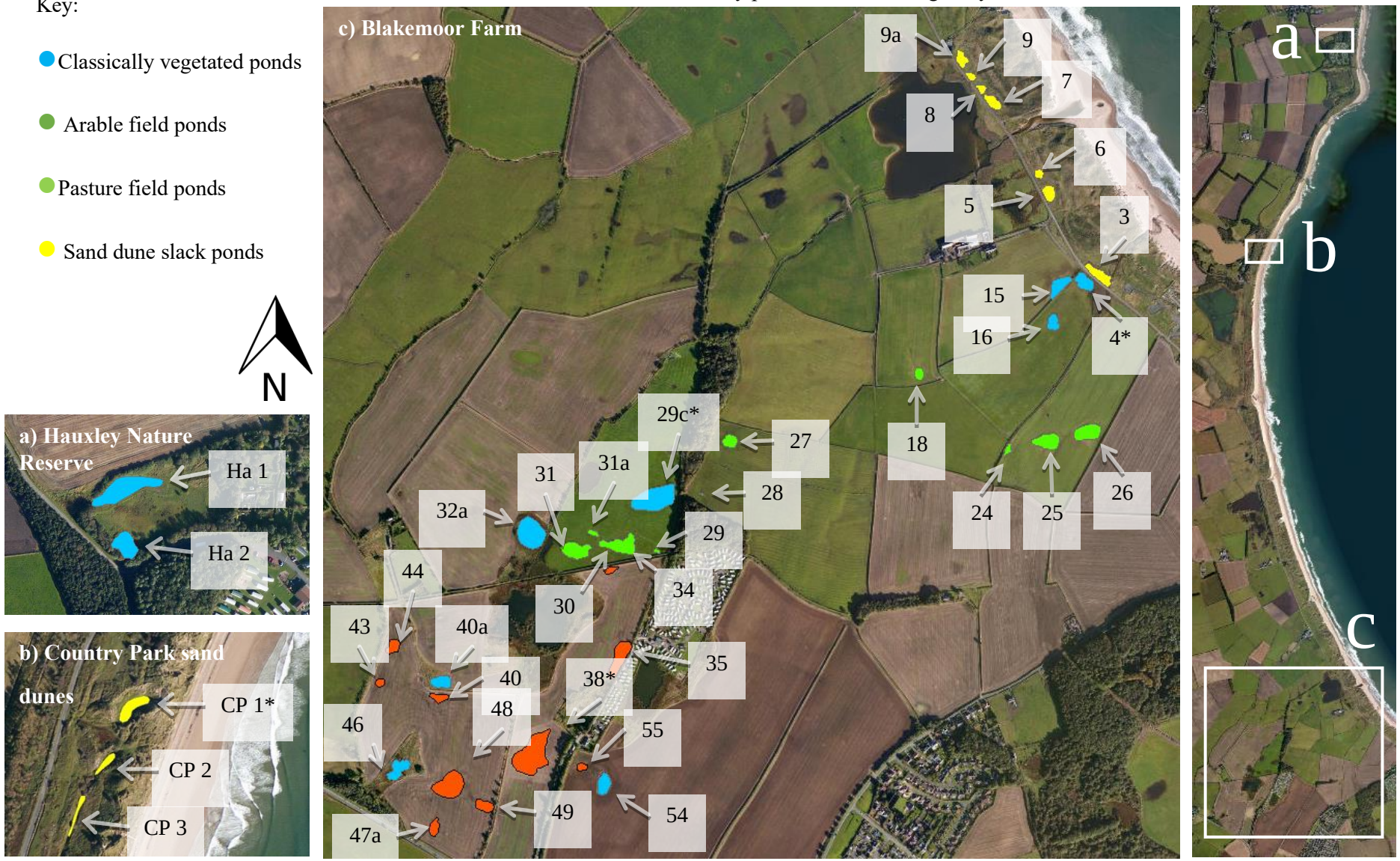
Suplementary

Plate…: Location of the forty ponds across Druridge Bay



Supplementary Table 1. Details of the ponds analysed in this study. Pond IDs are numbers/names used in previous studies and kept here for consistency, and to match the Mendeley data deposition.Those with an asterisk indicate ponds that were sampled as part of the intra-pond comparison of SCS, with all 10 replicate core depths listed, the one used in the inter-pond comparisons first, the remaining 9 used in the intra-pond comparison in brackets.

| Pond ID | Land use | Twinspan vegetation classification | Area (max) / $m^2$ | Dries out | Core depth analysed / cm |
|---|---|---|---|---|---|
| PHaux1 | Naturalistic | Reeds | 1077 | Sometimes | 14 |
| PHaux2 | Naturalistic | Reeds | 487 | Sometimes | 10 |
| P54 | Naturalistic | Grassy | 1472 | Sometimes | 17.3 |
| P16 | Naturalistic | Grassy | 996 | Sometimes | 18 |
| P4 | Naturalistic | Diverse | 1517 | No | 16 |
| P32a | Naturalistic | Diverse | 4417 | No | 12.5 |
| P40a | Naturalistic | Diverse | 1401 | Sometimes | 16 |
| P15 | Naturalistic | Diverse | 1612 | Sometimes | 13.8 |
| P46 | Naturalistic | Diverse | 1835 | No | 27 |
| P29c* | Naturalistic | Diverse | 5513 | No | 24.5 (26.0, 23.8, 20.5, 26.0, 23.5, 33.0, 10.2, 18.5, 33.0) |
| P34 | Arable | Ephemeral | 403 | Annually | 9 |
| P35 | Arable | Ephemeral | 2766 | Annually | 11.3 |
| P47a | Arable | Ephemeral | 845 | Annually | 13 |
| P40 | Arable | Ephemeral | 795 | Sometimes | 21.5 |
| P55 | Arable | Ephemeral | 435 | Annually | 22.4 |
| P48 | Arable | Ephemeral | 3902 | Sometimes | 14.5 |
| P43 | Arable | Ephemeral | 407 | Annually | 11 |
| P44 | Arable | Ephemeral | 987 | Annually | 12 |
| P49 | Arable | Ephemeral | 1161 | Sometimes | 14.5 |
| P38* | Arable | Ephemeral | 6675 | Sometimes | 20.5 (16.0, 17.0, 18.0, 18.0, 17.5, 18.0, 19.0, 17.5, 17.0) |
| P29 | Pasture | Diverse | 151 | Annually | 10 |
| P18 | Pasture | Grassy | 618 | Annually | 11 |
| P30a | Pasture | Grassy | 344 | Annually | 13 |
| P28 | Pasture | Diverse | 49 | Annually | 11 |
| P27 | Pasture | Diverse | 1008 | Sometimes | 30.5 |
| P31 | Pasture | Grassy | 2446 | No | 28 |
| P26 | Pasture | Grassy | 2350 | Annually | 18 |
| P25 | Pasture | Grassy | 2124 | No | 20.5 |
| P24 | Pasture | Grassy | 3111 | Annually | 9.2 |
| P30* | Pasture | Grassy | 3603 | Annually | 11 (14.0, 14.0, 12.0, 10.0, 15.0, 11.0, 12.5, 15.5, 12.0) |
| P3 | Dune | Diverse | 1721 | Sometimes | 20 |
| P5 | Dune | Diverse | 1110 | Annually | 12 |
| P6 | Dune | Diverse | 466 | Annually | 13.5 |
| P7 | Dune | Diverse | 1055 | Annually | 14.3 |
| P8 | Dune | Diverse | 463 | Annually | 18.5 |

| | | | | | |
|---|---|---|---|---|---|
| P9 | Dune | Diverse | 401 | Annually | 21 |
| P9a | Dune | Diverse | 984 | Annually | 20 |
| CP-P2 | Dune | Reeds | 141 | Sometimes | 22 |
| CP-P3 | Dune | Reeds | 142 | Annually | 33 |
| CP-P1* | Dune | Reeds | 366 | Sometimes | 16 (19.4, 18.0, 16.0, 16.0, 17.5, 18.0, 22.0, 22.0, 21.5) |

**<u>Gilbert et al, Organic carbon storage in temperate pond sediments: testing intra and inter pond variation</u>**. Photographs to show examples of the pond types within the four land-uses.

Ponds in arable fields. In amongst crops, no buffer, routinely ploughed and planted. The ponds are nonetheless recurrent features every year

824

Naturalistic ponds, typically surrounded by wetland vegetation or damp grassland, with diverse, structurally complex vegetation typical of lowland ponds

Ponds in pasture fields. Dominated by a few grass species and bare ground, no buffer, accessed by cattle and sheep

Ponds in dune slacks. Often well vegetated but prone to drying most years. Some species are indicators of brackish influence in some

836 Supplementary Table 2. The four pond types defined by land use, their characteristic macrophyte plant communities, species richness ($T_s$ mean $\pm$ 1 SD),
837 Conductivity (mean $\pm$ 1 SD), dry-phase history.

838

| Type | Description | Characteristic plants |
|---|---|---|
| Arable | Ponds in arable fields, cropped for cereals and oil seed rape. No buffer zone between wetland and crops, bare ground within pond margins extensive. All ploughed at least once during survey period; nine ponds dry every summer except summer of 2012.<br>$T_s$ 14.4 $\pm$ 4.9. Cond 375.6 $\pm$ 121.2 $\mu Scm^{-1}$. | Extensive bare ground widespread with sparse cover of inundation, disturbed ground weeds, e.g. *Matricariamatricarioides, Atriplexprostrata, Polygonum avicularia, Poa annua* akin to NVC communities OV18, OV29-OV31 (Rodwell, 1995) |
| Pasture | All ponds in permanent pasture, with sheep or cattle access every year. No buffer between pond and grazing. Some bare ground in six of ten ponds, nine of which dry most years, except summer of 2012.<br>$T_s$ 9.3 $\pm$ 3.3. Cond. 431.4 $\pm$ 107.7 $\mu Scm^{-1}$ | Dominated by grasses *Alopecurus geniculatus, Eleocharis palustrius, Glyceriafluitans*and some bare ground. Very few herbs<br><br>Species richness significantly lower than the other three pond types (ANOVA, $P<0.05$) |
| Sand dune | Wetlands on landward side of dunes with markedly sandy soil. Surrounded by extensive natural dune grassland. Occasional cattle access to a few ponds seven of which dry every year, except in summer 2012. Occasional brackish flooding into eight sites.<br>$T_s$ 14.4 $\pm$ 3.2. Cond. 1599.8 $\pm$ 1692 $\mu Scm^{-1}$. | *Agrostis stolonifera, Potentilla anserine, Eleocharis palustris* widespread.<br><br>Occasional brackish tolerant species present, e.g. *Triglochinmaritimum, Ranunculusscleratatus.*<br><br>Very little bare ground |

| | | |
|---|---|---|
| Naturalistic | Amongst natural wetland or wet grassland complexes or along edges of pasture but adjacent to wetland with distinct buffers of natural vegetation. Only two have dried out on just one occasion<br>$T_s$ 14.1 ± 6.1. Cond. 670.4 ± 155 $\mu Scm^{-1}$ | Wetland species widespread, e.g. *Eleocharis palustris, Agrostis stolonifera, Juncus articulatus, Typha latifolia* and *Sparganiumerectum* and some submerged taxa or floating taxa, e.g. *Lemna minor*, *Callitriche*sp.<br><br>Very little bare ground. |

Supplement 4. TWINSPANclassification of the ponds. The classification was run for two rounds of division to create four end groups. The indicators species at each of the two rounds of division are shown along with a broad description of the ponds in each end group and how many from each land use type there are in the groups.

| | | | | |
|---|---|---|---|---|
| | 40 ponds | | | |
| *TWINSPAN division 1 Indicator species* | *Agrostis stolonifera* | | *Matricaria matricariodes, Polygonum aviculare, Alopecurus geniculatus*, bare ground | |
| *TWINSPAN division 2 Indicator species* | *Phragmites australis, Iris pseudacorus,*mosses | (noindicators) | *Alopecurus geniculatus* | *P. aviculare, Orache* spp. |
| Number of ponds in each group | 12 ponds | 33 ponds | 13 ponds | 22 ponds |
| Group name | 1, reeds | 2, diverse | 3, grassy | 4, ephemeral |
| General type of pond | Ponds primarily from dunes<br><br>Extensively vegetated<br><br>Dominated by reeds, with rushes and sedges.<br><br>Rarely dry out | A mix of dune slacks, pasture and natural wetland ponds.<br><br>Diverse flora of wetland herbs and monocotyledons but not dominated by tall monocotyledons | Predominantly pasture ponds dominated by low growing grasses.<br><br>Usually dry out but remain covered by grass sward with little exposed substrate | Ponds in arable fields, usually ploughed over and planted with crop species.<br><br>Most dry out except in wettest years. Dominated by ephemeral disturbed ground and inundation species |
| Numbers of ponds from the four land use types sampled for carbon | Dune 3,<br>Naturalistic 1 | Dune 7, Pasture 3<br>Naturalistic 7 | Pasture 7<br>Naturalistic 2 | Arable 10 |