The Power of Ponds?
Quantifying sediment carbon stocks within, and fluxes from, small ponds.

Peter J. Gilbert

Ph.D.

2016

The Power of Ponds?
Quantifying sediment carbon stocks within, and fluxes from, small ponds.

Peter J. Gilbert

A thesis submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of the University of Northumbria

Research undertaken in the Faculty of Engineering and Environment, University of Northumbria

May 2016

Abstract

The role of ponds within the terrestrial carbon cycle has been receiving increasing interest. Existing evidence suggests that they have substantial global coverage, with ecosystem function rates disproportionately intense for their size making them significant cyclers of atmospheric carbon. This project aims to: (1) provide a comprehensive survey and quantification of carbon stocks within lowland ponds from a diverse range of ecological pond types; (2) provide a comparison of carbon stocks from pond sediments across significantly different biogeographical regions across England; and (3) monitor the temporal and spatial variability of carbon fluxes from ponds.

Carbon stocks were surveyed in 40 ponds across Druridge Bay, Northumberland. These ponds were selected for their distinct plant communities and hydrological patterns that form four broad pond types: dune-slack ponds; arable field ponds; pasture field; and classically vegetated ponds. High measures of percentage carbon were found within the sediments, however, when quantified in terms of carbon (C) stock, (kg^{-1} C $m^{-2}_{< \text{upper 10 cm}}$), little difference was observed among classically vegetated, arable, and pasture pond types (means = 3.14, 3.17, 4.94 kg^{-1} C $m^{-2}_{< \text{upper 10 cm}}$ respectively); only sediment C stocks of dune-slack ponds (6.18 kg^{-1} C $m^{-2}_{< \text{upper 10 cm}}$) were significantly different from other pond types. Equally, the heterogeneity of C stocks among dune-slack ponds varied markedly, with ponds in arable fields being fairly consistent. No significant difference was observed between C stocks in the pond sediments compared to those in surrounding soil. This does not mean that they play a similar role in the carbon cycle, but highlights the importance of acquiring sediment burial rates within these systems in order to quantify their role as C stores.

To test if the patterns of C storage could be generalised beyond the Northumberland ponds to other regions in the England, 15 ponds were surveyed, 5 each from 3 separate regions of England with differing climatic influences and biogeographical characteristics: temporary ponds on the Lizard Peninsula, Cornwall, with Mediterranean climate; pingo ponds of Thomspon Common, Norfolk; and peat excavation ponds at Askham Bog, Yorkshire. Sediment C stocks of ponds sampled in Cornwall (mean = 2.6 kg^{-1} C $m^{-2}_{< \text{upper 10 cm}}$), were > 43 % lower compared to those in Yorkshire (6.0 kg^{-1} C $m^{-2}_{< \text{upper 10 cm}}$) and Norfolk (7.7 kg^{-1} C $m^{-2}_{< \text{upper 10 cm}}$). However, cumulatively, the variation observed among all sites was comparable to the high level of variation observed in the comprehensive survey of ponds at Druridge.

The absence of detailed C flux rates from small water bodies, especially from desiccated sediments during summer dry phases, is a key factor constraining their inclusion in terrestrial carbon budgets. Thus, CO_2 fluxes were monitored from 26 neighbouring experimental ponds of known age, history and ecology, focusing on short-term hydrological changes over two, two-week periods, comprising a drying phase and re-wetting phase. During the drying phase flux rates exhibited a 9-fold increase resulting in a shift from a net intake of CO_2 to a net site emission whilst the reverse was observed during the rewetting phase. Moreover, significant variability in fluxes of CO_2 were observed among ponds on individual sampling days; the highest range observed was -2154 to 10658 mg m^{-2} d^{-1}. The result is marked spatial variability in CO_2 processing.

The large degree of temporal and spatial heterogeneity repeatedly observed throughout this study, both in sediment carbon stocks and CO_2 fluxes, highlights the complexity of carbon processing within small aquatic systems such as ponds. This study specifically highlights the need for accurate measures of burial rates within pond systems in order to fully assess their carbon capture capability.

Table of Contents

List of Figures

List of Tables

List of Abbreviations and Terms

% RSD - % Relative Standard Deviation
A – Alpha diversity
ANOVA – Analysis of Variance
C – Carbon
C % - The percent concentration of carbon within the sediment
CH_4 – Methane
C:N ratio – Carbon to Nitrogen ratio
CO_2 – Carbon Dioxide
CO_3^{2-} – Carbonate
C Stock –The mass of carbon (kg C m^{-2}) stored within the upper 10 cm sediment
DBD – Dry Bulk Density
DIC – Dissolved Inorganic Carbon
DOC – Dissolved Organic Carbon
E-*F* CO_2 – Net ecosystem flux of CO_2
F CO_2 – Flux of CO_2
GHG – Green House Gases
GPP – Gross Primary Production
HCO_3^- – Bicarbonate
H_2CO_3 – Carbonic acid
IC – Inorganic Carbon
IGO – Inter-Governmental Organisation
LoD – Limit of Detection
LOI – Loss on Ignition
LoQ – Limit of Quantification
N – Nitrogen
NEP – Net Ecosystem Production
NGO – Non-Governmental Organisation
NO_3^- – Nitrate
NWT^1 – Northumberland Wildlife Trust
NWT^2 – Norfolk Wildlife Trust
OC – Organic Carbon
OM – Organic Matter
Pg – Peta grams
RIVPACS - River Invertebrate Prediction and Classification System
SD – Standard Deviation
SO_4^- – Sulphate
TEA – Total Elemental Analysis
Tg – Tera grams
TOC – Total Organic Carbon
TWINSPAN – Two-way Indicator Species Analysis
YWT – Yorkshire Wildlife Trust
γ – Gama diversity

Acknowledgements

Firstly, I would like to thank all three of my supervisors for all their years of teaching, guidance, advice, and support over the past decade, throughout both my undergraduate and postgraduate studies. Above all I would like to thank Dr Michael Jeffries who over my 10 years at Northumbria has never once failed to brighten my spirits or make me laugh when times were hard. I also thank Dr Dave Cooke for seeing the potential in me long before I did myself, and drawing it out by continually pushing me out of my comfort zone. And I thank Dr Michael Deary, who is one of the busiest academics I know yet has never failed to take the time to support me when needed. This PhD would not have been possible without their help, nor their influence on my life over the past 10 years, and I would never have progressed this far in academia, nor would I be where I am today.

I would also like to thank the support staff within the Department of Geography at Northumbria University, notably David Thomas, and Lesley Dunlop for their consistent and patient support in laboratory analysis, and for making it a fun place to work.

My gratitude goes to those friends and family who have supported me over the past 3 years. Of my fellow Ph.D. students, with whom I have shared so many adventures and laughs, particular mention goes to: Scott for making field work unforgettable; Stephanie for all the motivation; and Tom for all the distractions. Unbelievable thanks goes to my family, especially my mum and dad, who have never stopped believing in me and have supported me beyond belief.

Thanks is also given to those people and organisations who permitted access and permission for sampling during fieldwork, including: Northumberland Wildlife Trust; Yorkshire Wildlife Trust; Norfolk Wildlife Trust; Natural England; the management of Blakemoor Farm and Riotinto Alcan and the Crown Estate.

Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Any ethical clearance for the research presented in this thesis has been approved. Approval has been sought and granted by the Faculty Ethics Committee on 30th June 2014

I declare that the word count of this thesis is 42,258.

Name: Peter J. Gilbert

Signature:

Date:

Publications and Communications

Specific to this thesis

Publications

Published

Gilbert, P., M. Jeffries, D. Cooke, M. Deary, S. Taylor. 2016. Quantifying rapid spatial and temporal variations of CO_2 fluxes from small, lowland freshwater ponds. *Hydrobiologia*. doi:10.1007/s10750-016-2855-y

Gilbert, P., S. Taylor, D. Cooke, M. Deary, M. Cooke, M. Jeffries. 2014. Variations in Sediment Organic Carbon Among Different Types of Small Natural Ponds Along Druridge Bay, Northumberland, UK. *Inland Waters*. 4(1)57-64.

To be Submitted

Gilbert, P., S. Taylor, D. Cooke, M. Deary, M. Jeffries. Pond Sediment Carbon Stocks of the UK. *To be submitted September 2016*.

Taylor S., P. Gilbert, M. Jeffries, M. Deary, D. Cooke. Ponds, Productivity and Probes: An Experimental Approach to Carbon Capture. *To be submitted June 2016.*

Reports

Natural England and the Northumberland, Yorkshire, and Norfolk Wildlife Trusts (15.03.16) A summary of works conducted on ponds across England and their sediment carbon stocks. *Report compiled for communication of works conducted with associated partners and to inform pond management practice.*

Northumberland Wildlife Trust (07.05.2014) The value of small aquatic systems beyond conservation and biodiversity. *Contributions to a report & presentation to estates manager of the trust on our research conducted on their sites over the previous 3 years.*

Conferences

Symposium of European Freshwater Sciences - Geneva - July 2015 - Oral presentation: *Quantifying the carbon stock of pond sediments across a low-land farmscape: The hidden 'crop'*

Society of Wetland Scientists / European Pond Conservation Network - Huesca, Spain - Sept 2014 - Oral presentation: *Capturing the aquatic breath: The impact of hydrology on CO_2 fluxes from seasonal aquatic systems in Druridge Bay, England*

British Organic Geochemistry Society - Plymouth - July 2013 - Oral & poster presentation: *The power of ponds - Sequestering organic carbon through 'the eyes of the landscape'* - **Award**: best student poster presentation

Northumbria University Research Conference - Newcastle - April 2013 - Poster Presentation: *Capturing carbon in seasonal aquatic environments*

External Article Reviewer for Inland Waters

Future projects

EuroRun project – A recently funded international collaboration among early career scientists aiming to assess CO_2 fluxes from European running waters. Founding member and played a key role in the project outline and grant application. For more information see: http://freshproject-eurorun.jimdo.com

EuroRun workshop - Lake Erken, Sweden - September 2016 – The first EuroRun workshop aiming to establish robust methods to be used for a simultaneous pan European gas fluxes study. Supported by all E.U. Freshwater Associations for the EuroRun collaboration.

Memberships

Freshwater Biological Association (FBA)
International Society of Limnology (SIL)

Collaborative research on arsenic – not related to thesis

Publications

Gilbert, P., D. Polya, D. Cooke. 2015. Arsenic Hazard in Cambodian Rice from a Market Based Survey and a Case Study of Preak Russey Village, Kandal Province. *Environmental Geochemistry & Health*. 37(4)757-766

Polya, D., M. Polizzotto, S. Fendorf, L. Rodriguez-Lado, A. Hegan, M. Lawson, H. Rowland, A. Giri, D. Mondal, C. Sovann, W. Al Lawati, B. van Dongen, P. Gilbert, A. Shantz. 2010. Arsenic in Groundwaters of Cambodia. *Water Resources and Development in South-East Asia.* SE Asia Centre, New York. 2010: eScholarID:111555

Reports

Society for Environmental Geochemistry & Health (08.09.2015) Arsenic hazard in rice from Kandal Province, Cambodia. *Article published on society website for public communication.*

Conferences

Society for Environmental Geochemistry & Health - Newcastle - July 2014 - Oral presentation: *Arsenic hazard in rice from Kandal Province, Cambodia* - **Award**: best student oral presentation.

Chapter I. Project Overview and Synopsis of Research into the Role of Small Aquatic Systems (Ponds) in the Global Carbon Cycle

1. Introduction

Until the 1980s ponds were largely disregarded as unimportant compared to larger lakes and rivers, not only in terms of their biodiversity value in the landscape, but also their cumulative global coverage and their biogeochemical processing power. This led to a gap in limnological research; the concept that they may have a significant role in global processes was unrecognised. Small shallow aquatic habitats such as ponds, were thought of as *"rather uninteresting features of the landscape"* (White, 1868), and even the study of pond ecology was undervalued as *"the activity of the amateur, who's humble pond hunting, if carried out systematically and carefully, may…contribut[e] to science"* (Clegg, 1952). However, over recent decades there has been a gradual increase in research focusing on small aquatic habitats. Biologists and ecologists have firmly cemented small temporary ponds as valuable ecosystems within the biome of freshwater habitats, and their ecological importance for biodiversity in the landscape is now well understood (Biggs et al., 2005; Ewald et al., 2012; Jeffries, 2008; Oertli, 2009; Williams et al., 2004).

While the benefits from small ponds have long been utilised in the localised landscape, such as nutrient retention and pollution buffers, their potential role in global biogeochemical cycles was wholly disregarded. Historically, the importance of any ecosystem in global cycles was based on its combined surface area, along with its processing rates, and until recently both of these factors were assumed to be minor in small ponds. However, this is rapidly changing. Over the last decade research has shown this assumption to be wrong and it is now thought that the smallest aquatic ecosystems combined potentially have global processing equal to the large lakes of the Earth (Downing, 2010). Of significant importance, and certainly relevance to this thesis, the role in which ponds and small wetlands play in the global carbon cycle is currently attracting widespread interest.

The frequency of high impact research demonstrating the global importance of ponds within the carbon cycle is increasing (Abril et al., 2014; Cole et al., 2007; Downing, 2010; Downing et al., 2008; Raymond et al., 2013). Evocative titles such as *'Little things mean a lot'*, *'Small is beautiful'* and *'Eyes of the landscape'* capture the power and beauty of small aquatic systems (Boix et al., 2012; Downing, 2010; Ewald et al., 2012). Research into the role that small inland waters play in the global carbon cycle is still in its infancy and subsequently the majority of research has focused on two primary areas: (1) quantifying the global distribution of small aquatic systems; (2) and quantifying their carbon stores and biogeochemical processes and rates. It is specifically on these two areas that this review of literature focuses to give an indication of the current state of research into the role of

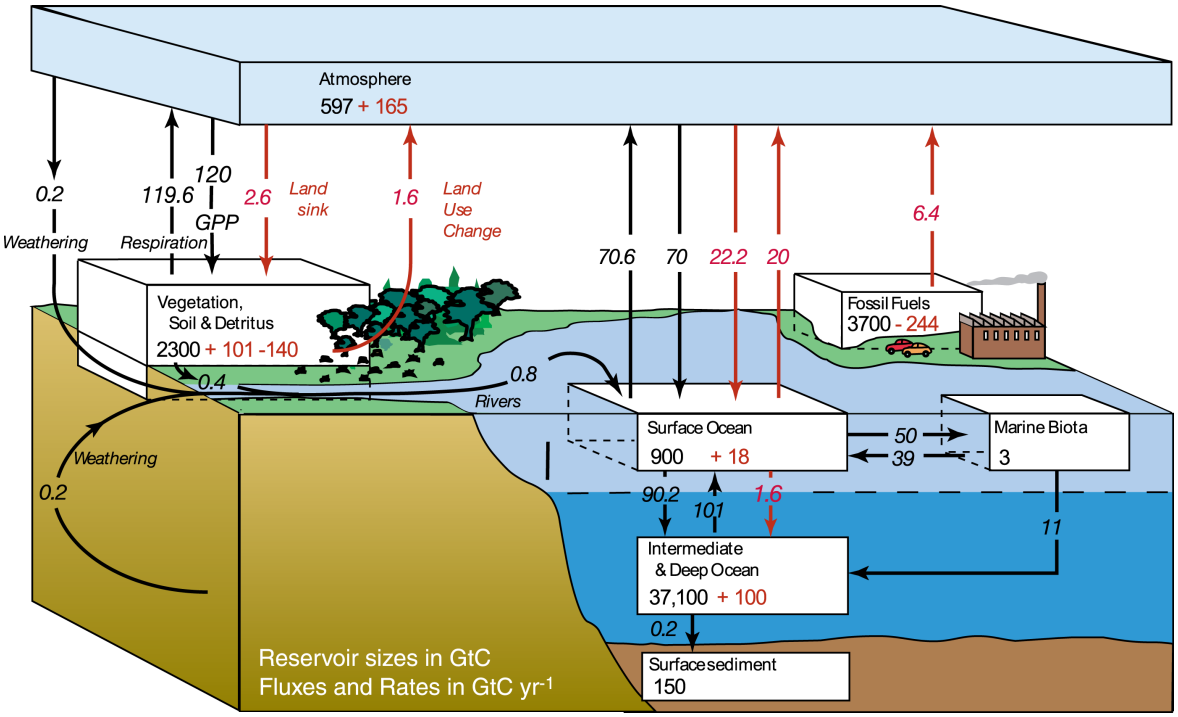
ponds in the global carbon cycle. However, first it is important to address the broader issue of why these systems need to be fully quantified in regards to the global carbon cycle.

2. The Global Carbon Cycle

The importance of carbon dioxide (CO_2) and methane (CH_4) as green-house gasses (GHG) in forcing climate change has long been understood, yet despite mitigation efforts global carbon emissions continue to increase (Friedlingstein et al., 2010). Irrespective of future GHG emissions, a mean surface temperature increase of 2 °C (over 1990 levels) is inevitable by 2100, with an increase of > 4 °C not unlikely (New et al., 2011; Prinn et al., 2011; IPCC 2001, 2007, 2014). However, there is considerable variation in projections stemming from uncertainties regarding climate feedback systems and the response of Earth surface systems to changes in atmospheric CO_2 concentrations and climate change (Brown and Wake, 2012; Knight and Harrison, 2013). Achieving a fully comprehensive and credible global carbon (C) budget, and fluxes between systems, is crucial to constraining climate change predictions and informing mitigation and policy.

Carbon in the biosphere is distributed unevenly among three major reservoirs: terrestrial, oceanic and atmospheric. Typically, the marine coastal regions and land sinks are viewed as biologically active areas, connected with the atmosphere through gas exchanges. Figure I.1 shows the global carbon cycle, detailing the C stocks of, and exchanges between, the main reservoirs (IPCC, 2007). The increase of 165 Gt C to the atmosphere since pre-industrial times highlights the significance of anthropogenic emissions, of which during the 1990s approximately 20 % came from land use change

Figure I.1: The global carbon cycle for the 1990s. Taken from IPCC (2007) AR4WG1. Values show the main annual fluxes in Gt C yr^{-1}: pre-industrial 'natural' fluxes in black and 'anthropogenic' fluxes in red.



(1.6 Gt C) and the remaining 80% (6.4 Gt C) from the burning of fossil fuels (IPCC, 2007). Yet assessments of global C cycles are continually being updated, with differences among budgets not only representing discrepancies among models, but also a direct shift in the magnitude of fluxes among compartments. Estimated anthropogenic emission have increased from 8 Gt C yr^{-1} during the 1990s, to 9.1 Gt C yr^{-1} for the period of 2000 to 2006, of which 45 % (4.1 Gt C yr^{-1}) accumulated in the atmosphere, 24 % (2.2 Gt C yr^{-1}) was absorbed by marine sequestration, and 31 % (2.8 Gt C yr^{-1}) sequestered in the terrestrial biosphere (Battin et al., 2009). Evidence suggests that whilst atmospheric CO_2 concentrations have increased significantly since 1960, so too has the global net C uptake (Ballantyne et al., 2012). However, the capacity of terrestrial ecosystems to store C is limited, with current increase in sequestration and future potential largely reflecting past depletion from land use change (Mackey et al., 2013): some models even predict that respiration from the terrestrial C cycle may become a substantial source of atmospheric CO_2 (Heimann and Reichstein, 2008). While the broad range of predictions from different climate models demonstrates genuine differences in simulated climate change, it also indicates an overall poor understanding of processes of ecosystems and Earth system functions, especially climate feedback mechanisms in response to future climate change. A major challenge faced by climate scientists is linking the large-scale global models to the micro-scale processes (Lehner & Döll, 2004).

Creation of regional and global scale C balances is generally conducted from two approaches: (1) the top-down approach which uses inverse modelling techniques, working backwards from measurements of atmospheric CO_2 concentrations to determine the location and magnitude of C sinks/sources and rates of exchange; (2) and the bottom-up approach which up-scales C stores and fluxes from site-level observations of differing land-uses types (Battin et al., 2009; Raupach, 2011). However, the two techniques rarely match in their estimations. Extensive oceanographic and global C modelling in the 1990s identified a significant continental 'missing carbon sink' equivalent to roughly one-third of global fossil-fuel emissions (Aufdenkampe et al., 2011). One factor contributing to this gap in quantifications is that most inverse models are constrained to spatial resolutions too large to accurately distinguish between land use types at a regional scale, most notably, the presence of small inland waters which are consequently 'masked' as terrestrial environments (Battin et al., 2009). Equally, there is often an assumption that the net ecosystem production (NEP) is converted entirely to storage, without considering lateral export and remobilisation, in which inland waters play a major role (Cole et al., 2007; Battin et al., 2009; Aufdenkampe et al., 2011; Wehril, 2013).

In light of this, considerable research effort has focused on breaking down the terrestrial C sink into sub-compartments in an attempt to better understand and quantify the intricate interactions between systems. Emphasis has been placed on understanding how soil C stocks and processing will respond to changes in climate and vegetation (Manning and Renforth, 2013; Schmidt et al., 2011), and the

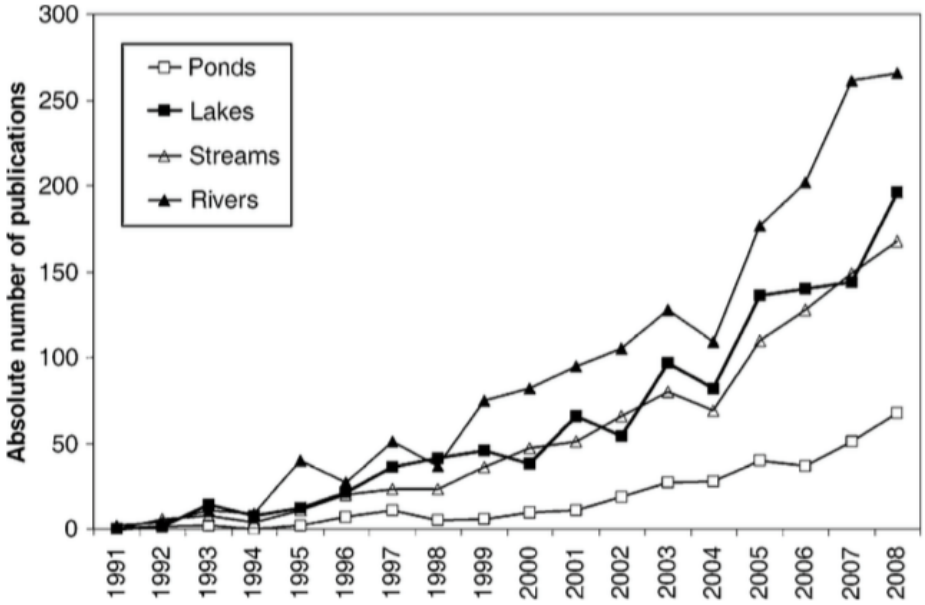
same importance is true for pond sediments. While increasing evidence is highlighting the importance of inland waters in the terrestrial C cycle, the role of the smallest aquatic environments is still largely omitted.

2.A. Processing by Small Lakes and Ponds is Mostly Ignored in Current Global Cycles

Since cataloguing of the world's lakes in the early 20th C (Halbfaß, 1922; Thienemann, 1925) it was assumed that large lakes cover the most area and subsequently played the largest role in aquatic processing by inland waters (Downing and Duarte, 2009; Downing, 2010; Downing et al., 2006). All but the largest continental water bodies were typically thought to be insignificant in global processes, simply being temporary reservoirs and conduits of water and materials before transportation to large lakes and the oceans, where it was assumed the dominant aquatic processing occurred. This preconceived idea led to a major gap in literature regarding the importance of small aquatic ecosystems (Lehner and Döll, 2004).

Biogeochemical processing in small ponds remained relatively understudied until the late 20th century (Downing, 2010), and even limnological studies on biodiversity show a bias towards streams, rivers, and lakes (Figure I.2; Oertli, 2009). The lack of studies on processes and rates within

Figure I.2: Increase in the number of peer-reviewed publications addressing together the topic 'biodiversity' and one of the four types of freshwater systems ('ponds', 'lakes', 'rivers' or 'streams') from 1991 to 2008.



Taken from Oertli (2009). The publications taken into consideration are those indexed in ISI Web of Knowledge database. The relative values (ratio to total number of publications indexed) present the same trends and are therefore not presented here.

small aquatic systems has unsurprisingly led to their absence from global cycle models. Of most concern is that of the global carbon cycle, where continental waters are frequently absent from not only global models, but also smaller national and regional carbon budgets alike (Downing, 2010). Table I.1 highlights just a few of the internationally respected reports that fail to account for ponds in their analysis of carbon cycles and budgets. Of concern is that this list contains significant international reports that focus on climate change mitigation; i.e., IPCC (2001; 2007; 2014). However, with a lack of scientific literature documenting the rates and processes within pond systems, as well as meaningful data that can be used in global scale models, their absence from policy making processes is to be expected.

Just as ponds were once overlooked as purely wildlife habitats, but are now known to be disproportionately rich in species and rarities compared to streams, rivers, and lakes (Davies et al., 2007; Williams et al., 2004), their potential significance for ecosystem services, such as carbon sequestration, is being increasingly recognised (Céréghino et al., 2013; Cole et al., 2007; Downing, 2010; Gilbert et al., 2014; Tranvik et al., 2009). The number of studies on carbon cycling within ponds is rapidly increasing, and within this overall biome of small ponds and wetlands, temporary systems are also receiving increasing interest (Catalán et al., 2014; Fromin et al., 2010; von Schiller et al., 2014; Torgersen and Branco, 2008). Simply put, the importance of any ecosystem in global

Table I.1: A selection of international and governmental reports, and academic literature, that omit the role of ponds or small lakes in carbon cycle or carbon budgets.

Reference	Title
International & Governmental reports	
IPCC 2001, 2007, 2014	International Panel on Climate Change Third, Fourth and Fifth Assessments Reports on Climate Change.
USCCSP, 2003	Strategic plan for the U.S. climate change science program: A report by the Climate Change Science Program and the Subcommittee on Global Change Research
Natural England Research Report NERR043	Carbon storage by habitat: Review of the evidence of the impacts of management decisions and condition of carbon stores and sources. (Alonso et al., 2012)
Academic Literature on global cycles	
Goody & Walker, 1972	Atmospheres
Bolin, 1983	The carbon cycle
Schimel et al., 1995	CO_2 and the carbon cycle
Mitra at al., 2005	An appraisal of global wetland area and its organic carbon stock
Zaehle et al., 2007	Projected changes in terrestrial carbon storage in Europe under climate and land-use change, 1990-2100
Heimann & Riechstein, 2008	Terrestrial ecosystem carbon dynamics and climate feedback
Saarino et al., 2009	Methane release from wetlands and water courses in Europe
Raymond et al., 2013	Global carbon dioxide emissions from inland waters

cycles is dependent upon: (1) their global distribution and cumulative coverage; (2) and their stores, and rates of processes and cycling. While previously assumed to be negligible for small aquatic systems, these two factors are now receiving increasing interest, and it is upon these two areas that the remainder of this chapter will focus.

3. The Global Distribution of Water Bodies

3.A. Size Matters: A Long Held Misconception

Recent inventories of inland waters, both regionally and globally, have shown that their spatial extent has long been underestimated. Moreover, it is the world's smallest lakes and wetlands that are proving to have been the most under-recorded, cumulatively covering a substantial portion of the terrestrial landscape (Lehner & Döll, 2004; Downing et al., 2006; Downing, 2010).

While recent work is elucidating the true distribution of inland waters, misconceptions about their sizes and aerial coverage held for the majority of the 20th C. Halbfaß (1922) published one of the first catalogues of the world's lakes, that was later expanded to include analysis of lakes within Europe (Thienemann, 1925). It was suggested that the global coverage of lakes and ponds was around 2.5 million km^2, or 1.8 % of the land surface. However, it was assumed that this coverage was dominated by a few very large lakes (Downing, 2009, 2010). For example, the great lakes of North America alone (i.e., Superior, Michigan, Huron, Erie, and Ontario) have a combined surface area of approximately 244,000 km^2, which equates to 10 % of Thienemann's (1925) global coverage prediction. That small ponds or lakes could possibly match the world's largest water bodies in aerial coverage or processing power was inconceivable (Herdendrof, 1984; Schuiling, 1977). This view remained relatively unchanged until the 2000s when research interest in small lakes and ponds began to grow.

3.B. Global Estimates of Coverage

With the rapid development of satellite imagery and increasing resolution there is now great potential for the use of remote sensing to map inland waters, especially over large scales and in remote areas. There are many databases documenting lake sizes and distributions, with estimates of global lake coverage having ranged from 2-2.8 x 10^6 km^2 (Downing et al., 2006; Kalff, 2001; Lehner and Döll, 2004; Meybeck, 1995; Shiklomanov and Rodda, 2004), roughly 1.3-1.8 % of the Earth's non-oceanic area (Downing et al., 2006). One database that remained the most comprehensive for nearly a decade was the Global Wetlands and Lakes Database (GWLD; Lehner & Döll, 2004): a database of inland waters greater than 0.1 km^2 in area, created from a combination of satellite imaging, varying sources for lakes and wetlands, and the use of Global Information Systems (GIS), which estimated a global coverage of lakes and reservoirs to be 2.7 x 10^6 km^2. With improvements in satellite imagery resolution and computer processing power, estimates of global coverage are becoming even more accurate. One of the most recent estimates, the GLObal WAter BOdies database (GLOWABO; Verpoorter et al., 2014), used high resolution Landsat imagery with pixel resolution of 14.25 m^2, to map ponds and lakes greater than 0.002 km^2 in area (i.e., 2000 m^2, based on a cut-off

point of 9 pixels), estimating their global coverage to be 5 x 10^6 km^2 (3.7 % of the Earth's non-glaciated land surface) and that this is dominated by large and intermediate sized lakes (Verpoorter et al., 2014). This estimate is at least double those of previous ones.

The main factor leading to variations in estimates is the image resolution of aerial imagery and the precise methods of remote sensing. While the minimum size thresholds detectable for small water bodies has been considerably improved over the past decade (≥ 1 km^2, Lehner & Döll, 2004; ≥ 0.005 km^2, Jones et al., 2009; ≥ 0.004 km^2, Pitt et al., 2011; ≥ 0.002 km^2, Verpoorter et al., 2014), it is still often greater than many small ponds (Jeffries, 2015). Equally, given that most inland water databases are created from singular snapshot images (as apposed to a series of time-based images) it is likely that temporary features in the landscape are highly under-recorded or altogether missed (Jeffries, 2015).

Quantifying the total land surface occupied by ponds and lakes is crucial to elucidate their role in the global carbon cycle. Many variables, such as species richness, primary productivity, and methane concentrations, increase relative to a decrease in lake size (Downing, 2010), meaning that smaller systems are disproportionately more productive per unit area when compared to larger systems. Attempts were made by Downing et al., (2006) to explore the size dependence of the abundance of water bodies in order to formulate scaling-laws for estimating the global extent and size distribution of lakes. The data for lake-size distributions down to 0.001 km^2 corresponded well to that of a Pareto distribution (Downing et al., 2006); a power law that estimates the probability of distribution. Results suggested there are around 304 million ponds and lakes > 0.001 km^2 in the world covering approximately 4.2 million km^2, or 2.8 % of the land surface area (Downing et al., 2006). This figure was nearly twice those assumed by several others (Schlesinger, 1997; Kalff, 2001; Wetzel, 2001; Shiklomanov & Rodda, 2004). Yet while lake size and area relationship estimates made by the Pareto distribution appear accurate for water bodies > 0.001 km^2, the same is not true of ponds in the size range of 0.0001-0.001 km^2 (100-1000 m^2). Calculations made by Downing (2010) from the Pareto distribution estimate ponds in this size range to have a combined global area of 0.8 billion km^2. Given that the surface area of the Earth is only 0.149 billion km^2 there is clearly inaccuracy in these estimates; the result reflects a tendency for the Pareto distribution to over estimate categories in the lower tail of the distribution (Jeffries, 2015; Seekell and Pace, 2011). More recent models have challenged the estimations made by Downing et al., (2006; Downing, 2010), suggesting that whilst small lakes and ponds are traditionally underestimated, they are not as abundant as suggested by the Pareto distribution (Seekell and Pace, 2011; Verpoorter et al., 2014).

Given the size restraints placed on water bodies included in global inventories (typically only including those ≥ 2000 m^2) it is likely that they underestimate small water bodies, requiring more

qualitative data on their occurrence and distribution. Ground surveys provide an exceptionally robust method for documenting the numbers and size distribution of small water bodies generally resulting in an increase in recorded numbers, especially of temporary ponds and wetlands, and within areas where the ground visibility of aerial images is restricted; i.e., woodlands (Calhoun et al., 2003; Jeffries, 2015; Pitt et al., 2011). Certainly, many of the smallest aquatic features are often temporary in nature (Jeffries, 2015), yet identifying them and justifying their inclusion in geochemical cycles is far from simple.

4. What is a Pond?

At first glance this may seem like one of the most simplistic questions. Ponds are a very familiar habitat to most people, part of the natural landscape as well as our urban environment and culture. Yet the familiarity with something so small and common place can lead to many misconceptions. If asked to imagine in one's mind *what is a pond* the response will vary greatly depending on the individuals experience with wetland environments. Each description will vary in their appearance (e.g., size, depth, vegetation coverage), and while it may not be obvious, so too will their biogeochemical processes and rates. Typically it is the aesthetic criteria that are used to separate water bodies into categories, typically by size, depth, or macrophyte coverage, yet this creates a complication for small ponds as the aesthetic criteria often overlap whilst their processes and functions may be wholly different.

When does a puddle become a pond and when does a pond become a lake? By their inherent nature most people can tell the difference. A 2 m^2 pool of water at the side of the road that regularly occurs after rainfall but is absent in dry periods is surely a puddle; a 2 m^2 pool of water in a field that regularly occurs after rainfall but absent in dry periods and houses aquatic plant and animal species that thrive on this periodical inundation is surely a pond. While their hydrological regimes may be similar, their ecological functions are extremely different. Yet, defining the exact boundaries between systems is nearly impossible. Combined with the rigorous confines and labels that government and academic practices like to place on systems, pinning down a universal description becomes difficult. Various criteria have been used to classify ponds such as the occurrence of rooted macrophyte communities, surface area and potential wave action, or depth and subsequent light penetration. A review of the definitions of the term *'pond'* (Table I.2) found in books, reports and journals, by Biggs et al. (2005) highlighted four broad categories of definition:

1) it is difficult (if not impossible) to define a pond
2) ponds are small and shallow
3) ponds are shallow enough for rooted plants to grow throughout
4) miscellany of other physical characteristics

Many of these definitions within these categories border on the overly simplistic, such as a pond is *"a smaller version of a lake"*; it is well known that their ecosystem processes and functions are considerably different. For the purpose of this research, the most appropriate definition is that given by Biggs et al. (2005) as developed from Pond Conservation's 15 year assessment:

Table I.2: Definitions of the term 'pond' given in books, reports and journals. Taken from Biggs et al. (2005). Definitions fall into four broad categories, reflecting the main concepts most frequently repeated.

(i) It is difficult to describe a pond	
'… in general, no scientific distinction can be made [between ponds and lakes].'	Macan and Worthington, 1972
'There is no satisfactory definition of a pond for the term covers a wide variety of freshwater habitats.'	Clegg, 1974
'No firm boundaries exist between the various sorts of standing water…'	Williams, 1983
There is no point at which a definitive line can be drawn between a pond and a lake or even between a puddle and a pond.'	Fitter and Manuel, 1986
'… it is impossible to provide a precise, technical difference.'	Jeffries and Mills, 1990
'… it is probably better to think of ponds as a special class of lakes than as something separate.'	Ashworth, 1991
'The discrimination between large lakes and small lakes or ponds is difficult to establish as the lake size gradient comprises an environmental continuum without any clear delimitation.'	Søndergaard et al., 2005
(ii) Ponds are small and shallow	
'… lakes of slight depth.'	Forel, 1892; in Horne and Goldman, 1994
'A body of standing water that is smaller than a lake.'	Ashworth, 1991
'… bodies of water small enough that a rainstorm will significantly change the water chemistry'	Ashworth, 1991
'A small body of still water of artificial formation, its bed being either hollowed out of the soil or formed by embanking and damming up a natural hollow.'	Simpson and Weiner, 1989
'A fairly small body of still water formed naturally or by hollowing or embanking.'	Allen, 1990
A smaller version of lakes.	Moss, 1988
'A pond is a small freshwater lake.'	Porter, 1988
'… ponds are shallow enough to allow light to penetrate to most of their depths.'	Porter, 1988
(iii) Ponds are shallow enough for rooted plants to grow throughout	
'… a body of water which is so shallow that rooted plants can grow all the way across it.'	Morgan, 1930
'… very small, shallow bodies of standing water in which the relatively quiet water and extensive plant occupancy are common characteristics.'	Welch, 1952
'A pond can be described as a body of still water which is sufficiently shallow to enable attached water plants to grow all over it. This cannot hold true for all ponds …'	Brown, 1971
'… they are small bodies of shallow, stagnant water, usually well supplied with aquatic plants.'	Clegg, 1974
'… small bodies of freshwater, shallow enough for vegetation to grow across the whole surface area.'	Sterry, 1982
'Ponds are of many kinds but typically are small bodies of shallow stagnant water in which rooted plants can grow even in the deepest parts.'	Clegg, 1974
'A pond, then is likely to be a small body of water, shallow enough for plants rooted on the bottom to grow all over it (though this also depends on the clarity of the water) and to ensure a fairly even temperature throughout.'	Fitter and Manuel, 1986
'… shallow, but often thermally stratified waters, with abundant growths of rooted and floating macrophytes.'	Horne and Goldman, 1994
(iv) A miscellany of other physical characteristics	
'… a typical pond is virtually a self-contained system, a closed biotope, a world within itself '	Coker, 1968
'Ponds are much less stable than lakes. Heavy rain may change completely the water in a pond. In dry weather it may disappear.'	Macan, 1973
Small pond: between the size of a tree-hole and 20 sq. yards (17 sq. m.) Pond: < 1 acre (0.4 hectares)	Elton and Miller, 1954
Water bodies up to a size of about 2000 m^2.	MAFF, 1985
'… still-waters no deeper than 3 metres and ranging in size from a few square metres to 0.405 hectares.'	Probert, 1989
'… a pond [is] anything less than 50 m (165 feet) or so across …'	Beebee, 1991
'Ponds' includes water bodies up to 0.5 hectares. Water bodies of 1.5 hectares are called 'large' by Fryer. No upper or lower size limits are defined.	Fryer, 1993

"water bodies between 1 m^2 and 2 ha (20,000 m^2) in area which may be permanent or seasonal including both man made and natural water bodies."

While at first this may again seem simplistic, it is justifiably an overly inclusive definition that is now widely recognised. Many definitions restrict pond size (see Table I.2; ≤ 2000 m^2, MAFF, 1985; ≤ 5000 m^2, Elton & Miller, 1954; Probert, 1989; Fryer, 1993; ≤ 50 m across, Beebee, 1991) yet ponds can have extremely large surface areas, with their shallow nature meaning they hold aquatic plant species that are not found in larger lake systems. Equally, the definition does not restrict on depth, and whilst usually correlated with surface area, ponds with small areas can have depths greater than several metres; definitions based on restricting depth are nonsensical. The above definition is also inclusive of temporary ponds; those that exhibit seasonal desiccation of their sediments. Temporary features are often dismissed yet they hold many unique species not found in permanent systems, and as shall be discussed further (Chapter IV), they are proving to have extremely complex geochemical processing rates that need detailed examination.

Many definitions of ponds state the absence of direct inflow and outflow, being solely rainfall dependant. While systems with direct inflow will no doubt have very different organic material dynamics to those that do not, to call one a pond and another a lake on this approach would be irrational; the two systems may be the same sizes, have similar flora and fauna, and have comparable geochemical processing rates. Finally, the inclusion of both natural and human constructed ponds in the definition provided by Biggs et al. (2005) is important, as not only do ponds form during natural geomorphological processes, many are created through both direct and indirect human activity (Table I.3). Whilst some may be heavily managed (e.g., fish farm aquaculture ponds), many are created for wildlife conservation or their previous use has been forgotten rendering them virtually indistinguishable from their naturally occurring counterparts.

One downfall of using such an inclusive definition for the term *'pond'*, such as that given by Biggs et al. (2005), is that it will inevitably lead to a classification of systems that are so diverse in both their ecology and geochemical processes and rates that regional quantification and upscaling of their ecosystem functions will result in estimations that are considerably variable. Furthermore, because small ponds are so varied across the landscape, it is difficult to accurately map and distinguish between different pond types in a view to upscaling processes to regional models. A few rare studies have utilised remote sensing of aerial images to evaluate broad trophic status and ecological characteristics of small ponds (López-Blanco and Zambrano, 2002). Identifying the trophic status of large numbers of ponds could aid in estimating GHG fluxes from ponds, as this plays a major role

Table I.3: Examples of the processes by which ponds are created, comprising natural processes, human construction, and indirect creation as a result of human activity.

Naturally occurring ponds	Man-made constructed ponds	Indirect human creation
Post glacial retreat or permafrost thawing, i.e., pingo ponds or pulsars	Agricultural: farm ponds, or water supply for livestock or irrigation	Military: pond formation in anti-tank block removal or anti-tank ditches.
Creation of new depressions: landslides, land-subsidence, erosional processes.	Agricultural: direct farming practices, i.e., rice paddies	Military: flooding of bomb craters
Stagnation of flowing water courses: i.e., oxbow lakes or seasonal drying of water courses	Aquaculture: commercial fish impoundments:	Indirect raising of the water table from damning can result in saturation of low lying depressions in the catchment
Seasonal inundation of depressions: flooding of riverine flood plains or rise in water table leading to flooding of existing depressions.	Conservation: protection of existing or creation of new ponds specifically for conservation.	Industry: soil or extraction
Sand dune depressions	Recreation: swimming, fishing, ice skating or curling ponds.	Industry: flooding of open cast mining
Created ponds by wildlife: i.e., Beaver ponds.	Aesthetic enhancement: garden ponds, golf course ponds, village duck ponds	Industry: land subsidence from mining activity
Depressions left in place of root systems of fallen trees	Industry: water retention for fire protection	
	Industry: ice harvesting	
	Industry: Waste water filtration systems	

in determining the processing rates of a system, yet the accuracy of such techniques is as yet limited. Equally, while broad ecological characterisations can be achieved (e.g., total macrophyte coverage) they lack the ability to determine macrophyte assemblages; currently the only way to accurately categorise macrophyte assemblages is by ground surveying, which is not feasible for large scale modelling (i.e., national carbon budgets). This is not to say that one should ignore these differences in types of pond as studies that focus on both ecological and geochemical variations among pond types show interesting results (Gilbert et al., 2014; Jeffries, 2015). Moreover, as stated in a review of UK carbon storage by habitat, "differentiation…between England's distinct wetland types and their individual contribution to the UKs carbon balance" is a key knowledge gap underpinning their full inclusion in C budgets (Alonso et al., 2012). Rather, for the purpose of defining the term *'pond'*, it is important to be inclusive of all small aquatic features (that are not classed as rivers or streams) so that their presence in the landscape and processing rates are not omitted from regional and national inventories or process cycles.

4.A. Temporary Ponds

It is worth highlighting the temporary nature of many of the smallest ponds that occur across terrestrial landscapes. Contrary to belief, temporary ponds are ecologically distinct and valuable habitats, with predictable hydrological patterns (Biggs et al., 1994). The key feature that unifies such systems is that they exhibit seasonal changes in their hydrological regimes resulting in periodic dry phases, exposure of the base substrate, and often desiccation of sediment layers. This may occur annually, less frequently, or even several times within one season, depending on regional weather patterns and hydrological regimes of the individual location. Typically, in temperate climates, recharge is rainfall dependent, and as such this change in hydro-period is dependent on the balance between evaporation rates and net rainfall over short periods. In the UK unreliable summer rainfall (Fowler and Kilsby, 2002) often results in several drying and re-wetting cycles over short periods of time, with rainfall variations from year to year further complicating the quantification and modelling of their ecosystem processes. This problem is compounded by the likely increase in localised climate variability caused by global climate change. A particular uncertainty arises from new extremes of rainfall and temperature that will subject wetlands and their wildlife to novel stresses, and may alter existing geochemical processing rates and species distributions (Jeffries, 2010, 2015; Jones, 2013).

There is however a lack of uniformity in the classifications given to temporary ponds that have become known by a range of diverse regional names or technical definitions: seasonal, ephemeral, playa or vernal (Keeley and Zedler, 1998). They are ubiquitous in all climatic zones across the globe from: thaw ponds in Arctic Tundra (Gallagher and Huissteden, 2011); temporary pools in Mediterranean and desert biomes (Catalán et al., 2014); constructed rice paddies in equatorial tropics (Jonai and Takeuchi, 2014); melt pools in Antarctica (Allende and Mataloni, 2013). They are also typical of temperate biomes such as: South American grasslands (e.g., mallines; Kutschker et al., 2014); prairie potholes and woodland vernal ponds in North America (Batzer et al., 2005; Gala and Melesse, 2012); across the riverine plains of Europe (e.g., tributaries of the Danube in Hungary; Boven et al., 2008); through into the Asian steppes (Mozley, 1937); an unusual example of a rare early appreciation of their value. Temporary habitats can also be historically long-lived features in the landscape, such as the pingo wetlands of East England which date approximately 11,000 years to the last (Devensian) ice age (Foster, 1993; Williams et al., 2001), so that their geochemical impact will also play out over many years. However, their presence is frequently overlooked both in natural landscapes such as grassland or temperate forest, and in intensively modified landscapes such as arable or grazing agriculture (Williams et al., 2001).

4.B. Ponds are of Significant Ecological Importance

Examples of early scientific experimentation in pond management can be found as far back as the 18th C; e.g., better management of aquaculture ponds (Forster, 1771) or to improve drinking water for cattle (West et al., 1786). While smaller ponds have long been popular with the general public and naturalists, they were mostly overlooked for their role as wildlife habitats by freshwater biologists and policy makers. It was not until the late-1980s that Pond Action (now known as the Freshwater Habitats Trust) was launched; the first NGO in the UK to focus on the ecology and conservation of ponds (Biggs et al., 2005). Over the past three decades in the UK an increase in the studies of pond ecology and their processes has led to an understanding of their importance within the landscape that is now widely recognised by researchers, land managers, and policy advocates alike (Jeffries, 2015). Recent greening requirements set out by EU regulations have stimulated the need to establish Ecological Focus Areas on 5 % of arable land (DEFRA, 2013). Currently focus lies on improving water quality of existing watercourses through the integration of surrounding buffer strips. The potential for the creation of new ponds and their considerable environmental benefits (e.g., nutrient retention, water storage, and improved biodiversity) is currently overlooked.

Aquatic rates and processes are more intense in small lakes and ponds leading to enhanced productivity and habitat composition. They are well known for their complexity and species richness, with small ponds being disproportionately rich in both macrophyte and invertebrate species compared to larger water bodies such as large ponds, lakes and rivers (Duarte, 1986; Jeffries, 2015; Williams et al., 2004). Whilst alpha (*a*) diversity, that is to say the local diversity observed at individual sites, often reveals a greater number of invertebrate species in rivers and streams

Table I.4: Pond and river invertebrate species richness and rarity comparison. Taken from Biggs et al. (2005).

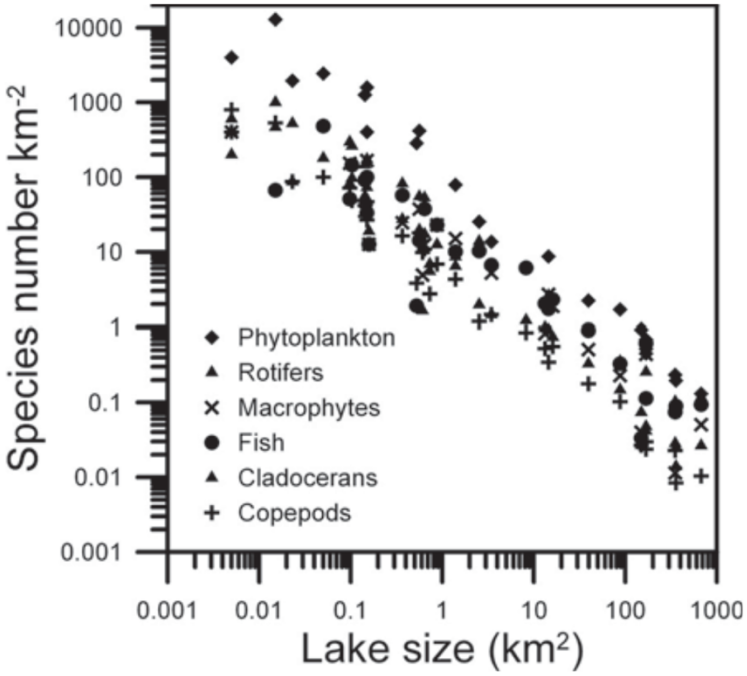
	Ponds (200 sites)	**Rivers (614 sites)**
Number of species	431	377
Nationally scarce species (occurring in 15-100 10 km squares)	78	41
Red Data Book species	26	13

Table I.5: Pond and lake aquatic plant species richness and rarity comparison. Taken from Biggs et al. (2005).

	Ponds (200 sites)	**Lakes (1100 sites)**
Number of species	72	89
Nationally scarce species (occurring in 15-100 10 km squares)	7	8
Red Data Book species	5	5

compared to individual ponds, the cumulative regional gamma (γ) diversity, observed among ponds collectively across the landscape, is often far greater. For example, in a comparison survey between the RIVPACS database (River Invertebrate Prediction and Classification System; a tool used to predict the ecological health of freshwater river systems) and ecological data from ponds, approximately 10 % more macro-invertebrate species and roughly double the number of uncommon species were found in ponds, despite there being roughly three times as many river sites (Tables I.4 and I.5; Biggs et al., 2005). While individually these systems are shown to be ecologically valuable, it is now known that the interconnected network of aquatic habitats that occur over the landscape enhances regional biodiversity of aquatic birds, plants, amphibians and invertebrates (Biggs et al., 2005; Elmberg et al., 1994; Scheffer et al., 2006). Collectively they are important for the maintenance and stability of regional γ biodiversity on the landscape scale. Lake biodiversity data has shown that small lakes contain more species of virtually all taxa, per unit area, than large lakes (Figure I.3), suggesting that a higher regional biodiversity can be maintained from multiple smaller systems equal in cumulative area to one singular large water body. This ecological importance for small aquatic systems is equally true for temporary ponds. They support a specialist flora and fauna unlike any found in permanent systems, that adds a significant contribution to γ biodiversity on the landscape scale (Biggs et al., 1994). Many of the species that colonise temporary systems are specially adapted to withstand drought through resistant propagules or by rapid re-colonisation, making these systems extremely resilient to environmental change compared to larger systems (Collinson et al., 1995; Jeffries, 1998, 2010). Given the high ecological diversity observed among ponds across the

Figure I.3: Species richness per unit area of various aquatic taxa in water bodies of different sizes. Taken from Downing (2010); original data from Dodson et al. (2000).



landscape, it is postulated that their geochemical processes and rates will vary among ponds of relatively close proximity.

4.C. Small Scale Studies, Big Implications.

A contributing factor that instigated much of the work carried out in recent years, not only on the distribution of water bodies, but also pond ecology, was prompted by a noted loss of ponds from agricultural lowlands of Europe and North America (Fairchild et al., 2013; Jeffries, 2015). Small ponds in these landscapes are susceptible to loss of habitat due to intensification of agricultural practices. Many modern agricultural practices have led to the indirect removal of ponds from the landscape, such as increasing land drainage and lowering the water table, which can indirectly remove several wetland environments across a large area, especially temporary features. Equally, direct removal was common practice in the early 20th C. as the usefulness of ponds had declined; e.g., farm ponds were used to wash down horses after ploughing yet the introduction of farm machinery removed this use. Ultimately, once ponds no longer provided a useful function, they were lost (Jeffries, 2015). Though with a deeper understanding of the ecological value of ponds within the agricultural landscape, these perceptions and practices are gradually changing.

Such temporal changes within lowland environments are often difficult to detect during 'snap shot' pond surveys; that is to say, surveys conducted over one season, be it from aerial images or ground surveying. Studies that use sequences of maps from several years often show complex patterns exhibiting periods of both net loss and gain of pond numbers (Fairchild et al., 2013; Jeffries, 2012, 2015). Use of maps for determining pond numbers, however, is always likely to be underestimates. The minimum size threshold for maps, below which features are not recorded, is often greater than many of the smallest aquatic features, such as the 4 m size threshold for U.K. Ordnance Survey maps (Jeffries, 2012). Temporary ponds are almost always missed in such surveys.

While the usefulness of pond surveys from maps should not be underestimated, it is evident that there is a need for detailed ground surveys on the numbers, and permanence, of small ponds within our landscapes. Such studies show significant variability in the number and areas of ponds between years and seasons, being especially influenced by extremes in weather (Jeffries, 2015). While it is unfeasible to conduct ground surveys on the large scale required for global modelling, such data is invaluable in helping us understand how the numbers and areas of small ponds change throughout time in response to climate. Furthermore, with rapid recent development with aerial drones, very high resolution imaging can be obtained quickly and cheaply.

4.D. Pond Succession

A compounding factor in assessing the temporal changes in pond numbers is that there is often a marked turnover of individual ponds. Use of historical maps to track pond numbers in south-east Northumberland, UK, revealed a slight increase in overall pond numbers since the mid-nineteenth century, from 222 to 257, yet only 24 of the original 222 ponds were still depicted on present maps (Jeffries, 2012). Whether such turnover of ponds is due to natural or anthropogenic causes is unclear. Regardless of the aforementioned anthropogenic removal of ponds from agricultural landscapes, highly productive small ponds can have very short lifespans; for example, 1 m^2 ponds of 30 cm depth in Northumberland, UK, have nearly completely filled with sediment since their creation in 1994 (Gilbert et al., 2014; Jeffries, 2008). Equally some ponds can persist for very long periods of time; East Anglian pingo ponds in south-east UK have remained relatively unchanged since their creation at the end of the last ice age approximately 11,000 years ago (Clay, 2015; Williams et al., 2001). This poses a problem for quantifying the long term numbers of ponds in the landscape; how long does a pond last?

Calculations of lake or pond lifespan have been estimated using the equation: $12.1\sqrt{L'}$; with L' being the average effective length and breadth (km) (Dean and Gorham, 1998; Downing, 2010; Straskraba, 1980): Figure I.4 displays the estimated life span of different sized water bodies calculated from this ratio (Downing, 2010). It is suggested from this relationship between size and lifespan that small lakes and ponds in nutrient enriched environments with high sediment burial rates may last only a few decades (Gilbert et al., 2014), whilst those systems with low burial rates may persist for > 1000 years. However, while the depth vs area ratio assumed by this equation may be true for lakes, it does not hold for many small systems that form in depressions within the landscape. Predicting the life span of a pond system based on its current size and burial rates is difficult as they are extremely susceptible to change. Equally, attempting to backdate ponds, using geochemical techniques such as radio isotope dating, is difficult due to the recent age of many sediments and the high levels of sediment disturbance experienced in small systems. Furthermore, how does one quantify the age of a temporary pond that may take many years of periodic inundation before pond species colonise? While predicting the life span of small ponds is difficult, ultimately all systems that are not anthropogenically removed go through a series of relatively predictable successions. While this can be broken down into many different stages, each populated by different groups of flora and fauna ecological communities, the life cycle can be characterised into three broad stages:

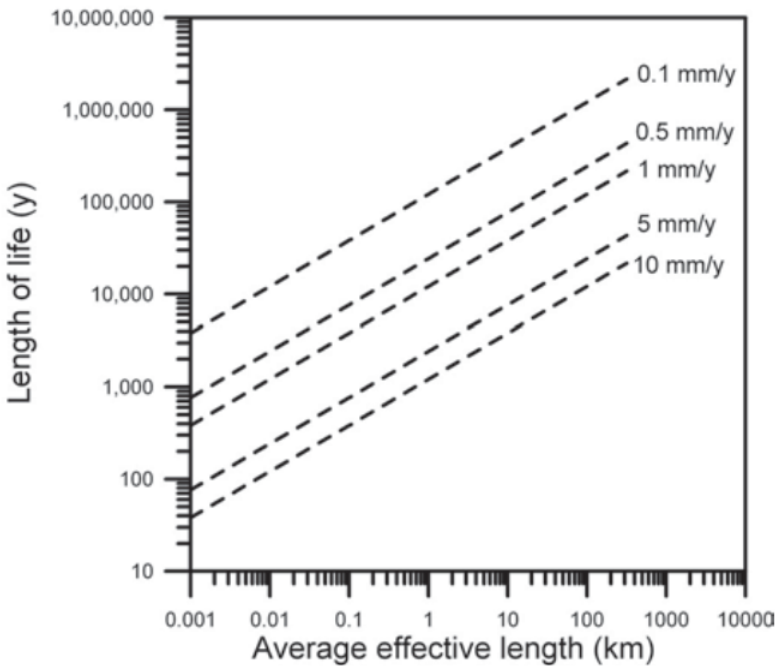
1) **Early successional ponds:** A depression in the landscape occurs, either by natural or anthropogenic causes (Table I.3), in which water collects and is held either permanently or for at least several months of the year. The first aquatic species arrive, known as pond

pioneer species. Those plant species that colonise first typically thrive on the open water and limiting nutrient conditions.

2) **Mid successional ponds:** Eventually rooted, emergent macrophyte communities begin to colonise, and in shallow ponds may cover the whole pond. These species often exhibit annual periods of rapid spring growth and winter decay, resulting in high sediment accumulation rates that begin to infill the pond.
3) **Late successional ponds:** emergent macrophyte communities now dominate the pond system, with sediment levels being so high that the pond is more 'marsh' like in character. In time sediment infill will exceed the water table, leading to drying out and colonisation by terrestrial vegetation. Ultimately the pond may be indistinguishable from the surrounding terrestrial landscape.

This description is undoubtedly broad, and in reality pond succession is characterised by many shorter ecological phases. However, the general principle remains; a pond is created, throughout its life it accumulates sediment, eventually it will fill, dry, and become terrestrialised. The length of time it takes for this process of pond succession to occur will vary greatly among systems.

Figure I.4: Potential lifetime of aquatic ecosystems of a range of sizes.



Taken from Downing (2010). The calculations were based on assumed rates of sedimentation spanning the range of those observed in oligotrophic to eutrophic lakes and the assumption that the mean depth (m) of a lake is around 12.1 $\sqrt{L'}$, where L' is the average effective length and breadth (km) and length is approximately double the breadth.

Management techniques, such as sediment dredging, are occasionally employed in an attempt to retain ponds at mid successional stages as this is typically when pond α diversity is at its greatest. While this is true, late successional ponds have their own unique assemblages and it is important to maintain a balance of young, mid, and old successional phased ponds across the landscape in order to maintain high γ diversity (Biggs et al., 1994).

While there are many estimations on the numbers and aerial coverage of ponds, no literature exists documenting the number of ponds in different successional stages in conjunction with spatial distribution. Given the difficulties in quantifying the actual number of ponds across the landscape, it is unlikely that documenting their successional stage will occur any time soon. Yet there is one crucial factor that needs to be recognised when trying to quantify the role of small ponds in the global carbon cycle; the variations in geochemical processing rates of carbon that occur at different stages of a pond's life cycle. Nutrient dynamics, rates of primary productivity, macrophyte composition, sediment burial rates, and processes of carbon remobilisation all differ depending on the successional stage of the pond.

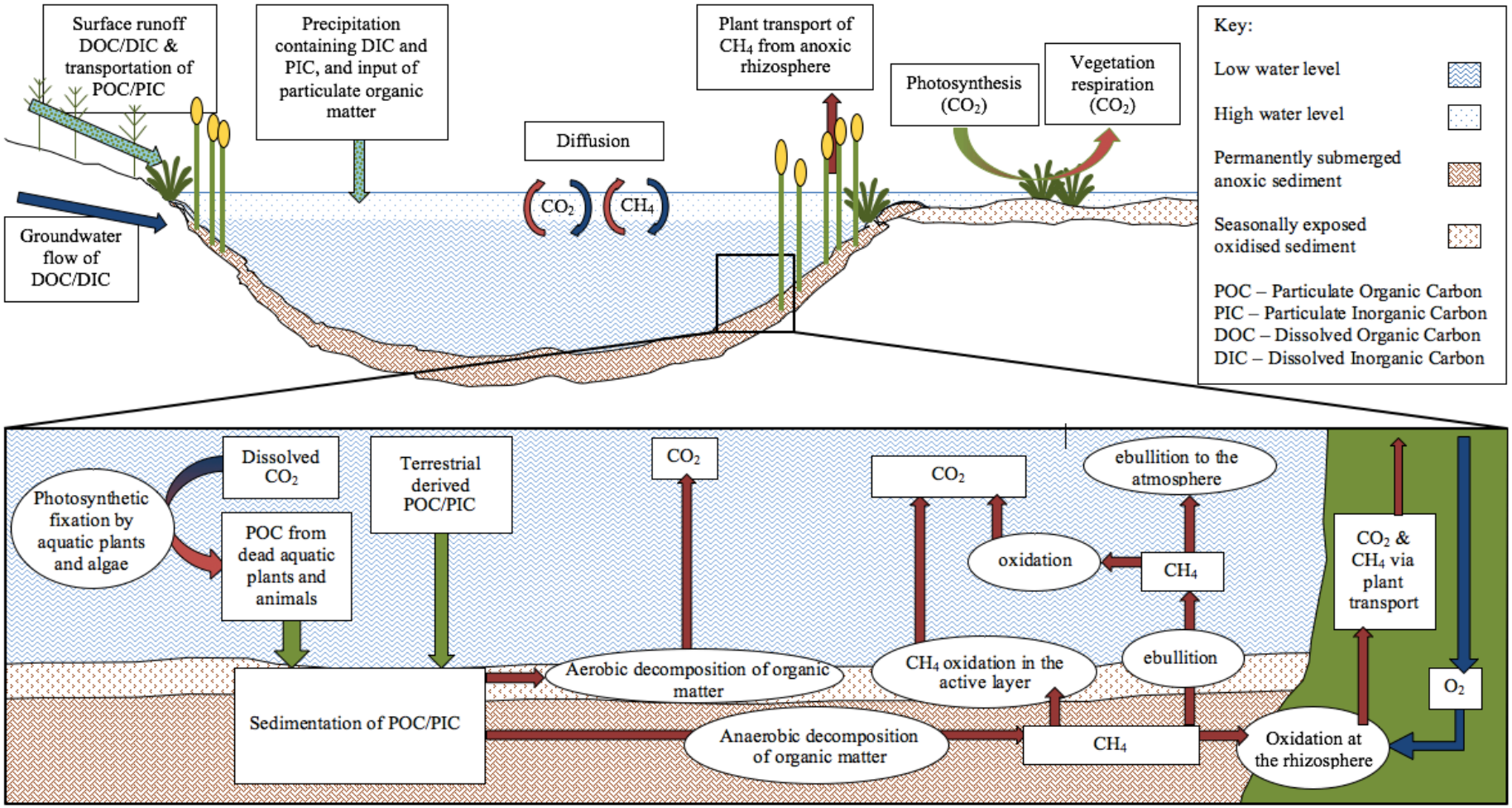
5. Pinning Down the Role of Aquatic Systems in the Global Carbon Cycle

Broadly speaking, for the majority of terrestrial environments, carbon enters the biological part of an ecosystem through photosynthetic assimilation of atmospheric carbon, known as gross primary production (GPP). This carbon supports the respiration of living organisms until death and decay when typically, *remineralisation* of organic matter via microbial degradation and photo-oxidation releases C back to the atmosphere as carbon dioxide (CO_2) or methane (CH_4) fluxes (*F*). The difference between GPP and the community respiration, or *F*, of a system is known as net ecosystem production (NEP). In the majority of systems the NEP is left with two fates: (1) *storage* within a system as living or dead biomass; (2) *export* from the system via erosion and transportation processes, where it may either be stored or remineralised in another system. It is the balance between rates of *GPP* and *remineralisation,* and the quantities of *storage* and *export* of *NEP*, that define how effective a system is as a C store. Typically NEP is quantified as units of C per area per unit time (e.g., '*x'* g C m^{-2} yr^{-1}) with a positive value for net autotrophic systems and negative value for net heterotrophic systems (Aufdenkampe et al., 2011).

One factor limiting greater understanding of terrestrial carbon stores is that NEP measured at local scales often does not extrapolate well to larger scales (Aufdenkampe et al., 2011; Ometto et al., 2005; Stephens et al., 2007). The assumption that all NEP is converted entirely to storage, without consideration of lateral export is a combining factor in these errors (Aufdenkampe et al., 2011). Early models assumed that inland waters played a minor role in this cycling of carbon, simply acting as fluvial conduits that transport eroded terrestrial organic matter to the oceans where remobilisation to the atmosphere occurred (Cole et al., 2007; Downing, 2010). Yet, recent studies have demonstrated that inland waters play a significant role in the cycling of terrestrial carbon prior to delivery to the oceans (Aufdenkampe et al., 2011; Cole et al., 2007).

The role of ponds within the terrestrial carbon cycle is relatively understudied in comparison to the extensive research documenting carbon storage, transportation, and remobilisation within lakes, reservoirs and river systems (Mulholland & Elwood, 1982; Dean & Gorham, 1998; Cole et al., 2007; Tranvik et al., 2009; Aufdenkampe et al., 2011; Pacheco et al., 2013). While every scientist claims their subject system is extremely complex, ponds certainly are complex. Figure I.5 shows a conceptual model and typical C mass balance for a pond system. Many environmental factors combine to dictate the sequestration, remobilisation and storage of C, and determining whether a pond is a net sink or source of C to the atmosphere is far from easy to determine.

Figure I.5: Conceptual model of the carbon cycle in a typical pond environment.



5.A. The Accumulation of Organic Matter

The role of an aquatic system in the carbon cycle depends largely on the volume of organic matter entering the system, either from GPP (autochthonous) or transported from the surrounding terrestrial catchment (allochthonous). The greater the abundance of organic material, either of autochthonous or allochthonous origin, the greater the potential not only for high levels of C storage, but also higher rates of respiration and release to the atmosphere.

There are many ways in which allochthonous organic matter may enter an aquatic system from the surrounding terrestrial landscape. Abrasive soil erosion at the water/bank interface plays a key role as a source of allochthonous sediment in rivers or large lakes, however in small ponds with no turbulent surface affects erosional processes are minimal. Instead erosion and transportation from the surrounding landscape via surface runoff plays a greater role on the import of allochthonous sediment in ponds, especially for systems with stream/riverine inputs (Cole et al., 2007; Huttunen et al., 2002). Equally, ponds in forested areas, or simply overlooked by a single tree, can become quickly filled with leaf matter that will likely be the dominant source of organic matter, far exceeding GPP (Biggs et al., 1994). Ultimately the volume of allochthonous inputs within a system depends largely on the topography and habitat type of the surrounding landscape and catchment. Contrastingly, autochthonous organic matter, originates from photosynthetic fixation of carbon within the system itself, either from dissolved organic carbon (DOC) in the water column by algae and submerged macrophytes, or through sequestration of atmospheric CO_2 by emergent macrophytes (Catalán et al., 2014). While the surrounding landscape type plays a key role in determining the availability and delivery of nutrients to aquatic system (Schiller et al., 2014), the rates of GPP in aquatic environments is predominantly dependant on the environmental variables of the system itself.

The volumes, and ratio of, allochthonous and autochthonous sediments in aquatic systems varies considerably, depending not only on the system itself, but also the catchment. The majority of large lakes and reservoirs are heterotrophic, receiving large amounts of sediment inputs from their catchments, transported by surface runoff and fluvial erosion via streams and rivers (Cole et al., 2007). Contrastingly most small lakes and ponds with no permanent stream/riverine inputs lack this supply of allochthonous organic matter, yet their small size and volume makes them ideally suited to high rates of autochthonous sediment production. This results in organic carbon (OC) burial rates that vary largely among inland waters, yet broadly speaking, they are repeatedly shown to have higher OC burial rates than terrestrial environments (Table I.6). Agricultural impoundments have the highest OC burial rates > 100 g m^{-2} yr^{-1} (max = 2122 g m^{-2} yr^{-1}), significantly greater than any other ecosystem (Downing et al., 2008).

Table I.6: Organic carbon burial rates in a variety of Earth's ecosystems.

Environment [a]	**Mean OC Burial rate ($g\ m^{-2}\ yr^{-1}$)**	**Range ($g\ m^{-2}\ yr^{-1}$)**
Agricultural impoundments	2122	148-17,392
Impoundments (Asia)	980	20-3300
Impoundments (Central Europe)	465	14-1700
Impoundments (United States)	350	52-2000
Impoundments (Africa)	260	-
Marine vegetated habitats	139	83-151
Small mesotrophic lakes	94	11-198
Abandoned agricultural land, returning to grassland	56	1.6-110
Mine spoils returning to forest and grassland	42	28-55
Wetlands and peat lands	31	8-105
Marine depositional areas	31	17-45
Abandoned agricultural land returning to forest	30	21-55
Small oligotrophic lakes	27	3-128
Large mesotrophic lakes	18	10-30
Large oligotrophic lakes	6	2-9
Boreal forest	4.9	0.8-11.7
Temperate forests	4.2	0.7-12
Tropical forests	2.4	2.3-2.4
Temperate grassland	2.2	-
Tundra	1.2	0.2-2.4
Temperate desert	0.8	-

Taken from Downing et al. (2008). [a] Data for lakes and impoundments are from Mulholland and Elwood (1982), data for terrestrial ecosystems including peatlands and wetlands are from Schlesinger (1997), data for marine ecosystems are from Duarte et al. (2005).

5.B. Ponds Lend Themselves to High Rates of Primary Productivity

Thermal stratification and limited light penetration in large lakes often leads to unproductive areas of open water. Rooted macrophyte communities typically only colonise the shoreline of many lakes, with the centre often being relatively inactive in terms of primary production due to limited light penetration (Allende et al., 2009; Middelboe and Markager, 1997; Weisner, 1991). Contrastingly, the whole volume of water within a pond is usually available for photosynthetic activity, with shallow ponds permitting growth of rooted macrophyte communities across the whole pond area (Della Bella et al., 2007). The small volume of ponds also means that they rapidly respond to temperature change, subsequently impacted by short term localised climatic variations, and can reach warm temperatures in direct sunlight on the coolest winter day. The result is that greater macrophyte coverage and growth periods in ponds results in higher rates of primary productivity per unit area than lakes.

The low water volume of small aquatic systems plays another key factor in enhancing the rates of primary productivity; determining the dilution factor of nutrient inputs. Poor land management and

intensification of agricultural landscapes has led to higher levels of nutrients in surface runoff entering many aquatic systems (Schiller et al., 2014). While large lakes with riverine inputs receive greater total nutrient loadings than ponds, they are subject to high dilution factors, rendering their impact on the system relatively low. Contrastingly the low volume of water in ponds results in a far lower dilution factor, with higher nutrient loadings per unit volume having a greater impact on the productivity of the systems. The small catchments and quick response to nutrient inputs also means that two pond systems that are located relatively close can receive largely different nutrient inputs. This may be a contributing factor to the ecological diversity of ponds across the landscape (Biggs et al., 1994; Jeffries, 1998, 2008). Whilst this quick response to nutrients may be a danger to ponds, in that eutrophication could happen from relatively minor nutrient spikes, it also serves as a form of protection for ponds across the landscape as a collective, as their small catchments protect them from pollution incidences that may be geographically close, but not directly within their catchment (Biggs et al., 2005).

5.C. Determining the Origin of Organic Material

Determining the origin of sediments is beneficial for understanding the role of pond systems in the carbon cycle. Rivers and lakes are known sites for remineralisation of terrestrial organic matter (Cole et al., 2007), yet the extent of this processing of terrestrial carbon in ponds remains undetermined. One distinguishing feature of allochthonous and autochthonous organic matter is that they have distinct biochemical compositions, specifically the ratio of carbon and nitrogen (C:N ratio) of the organic matter (Meyers and Ishiwatari, 1993).

Allochthonous organic matter of terrestrial origin is typically composed of compounds with highly complex structures, such as lignin which lends rigidity to vascular plants. These compounds are rich in carbon for its structural properties, and subsequently comparably low in nitrogen (Dean and Gorham, 1998) resulting in a high C:N ratio, typically 20-30:1 (Meyers and Ishiwatari, 1993). Contrastingly, autochthonous organic matter, typically comprised of algae and submerged macrophyte assemblages, is enriched in low-complexity compounds, such as proteins, that are low in C and subsequently comparably high in N, resulting in low C:N ratios of < 10:1 (Meyers and Ishiwatari, 1993).

As to whether sediment is allochthonous or autochthonous relates to the origin of the sediment, observing changes in the stratification of C:N ratios down sediment cores allows observation of changes in sediment sources throughout the past. The use of C:N ratios in deep ocean sediments aids in the determination of past vegetation changes and is a commonly used tool in climate reconstruction. Pond sediments tend not to be as clearly laminated as older lakes or oceanic sediments, however, it may be that it is possible to identify periods of differing vegetation growth in

a ponds life cycle; i.e., the different successional phases of a pond. There is potential for diagenesis of organic matter to affect C:N ratios over time throughout the depths of sediment as components of autochthonous organic matter are, in general, more sensitive to degradation processes as their structures are less complex and easier to degrade (Meyers and Ishiwatari, 1993).

5.D. Degradation, Remobilisation, and Emission to the Atmosphere

Aquatic systems can act as sinks and stores for carbon, both from fixation of atmospheric CO_2 and import of terrestrial carbon, yet a substantial portion is mineralised and released to the atmosphere. In aquatic systems the rates of carbon remobilisation and the form it takes when released to the atmosphere is based on two key processes: (1) degradation and mineralisation of organic matter; (2) movement of gas through the water column and exchange between the water and atmosphere interface.

5.D.i. Degradation of organic matter

Organic material undergoes many stages of degradation before it reaches its mineralised state. The rates and transformations depend on the environmental conditions of the pond and its sediment, or rather, the microbial communities that are best suited to those environmental conditions. Ultimately the final state of carbon in the degradation of organic matter is either CO_2 or CH_4 depending on the availability of oxygen.

In oxic environments, where oxygen is freely available, CO_2 is always preferentially produced. For example, in the surface layers of sediment and the water column itself, oxygen is abundant, or at least in 'healthy' non hyper-eutrophic systems. These oxic surface layers are often referred to as the *active layer* as this is where the highest rates of respiration occur. Below the active layer the availability of oxygen is limited and CO_2 is rarely produced. Within these anoxic, highly reductive environments CH_4 is the final state of carbon in decomposition. Methane production (methanogenesis) is carried out by anaerobic bacteria (methanogens) that utilise carbon compounds such as acetic acid (CH_3COOH), and methanol (CH_3OH) for energy production. However the metabolic rates of methanogens are much slower than those of bacteria that inhabit oxic conditions, meaning rates of CH_4 production in aquatic environments are typically much lower than those of CO_2 (Cole et al., 2007), even in extremely reductive systems such as wetlands and peatlands.

Generally, dissolved oxygen concentrations tend to be lower in ponds and small lakes than large ones (Crisman et al., 1998; Downing, 2010) enhancing CO_2 and CH_4 emissions. This low availability of oxygen means that methane concentrations and therefore potential evasion rates are greater in small lakes than large ones (Bastviken et al., 2004; Downing, 2010; Michmerhuizen et al., 1996). That said, certain environmental factors of small, shallow temporary ponds lend themselves to more

oxic conditions. Shallow ponds are especially susceptible to mixing of surface sediments, such as bioturbation from grazing cattle or wading birds, resulting in greater depth of the active layer and prolonged exposure of organic matter to oxidative conditions (Meyers and Ishiwatari, 1993). The depth of the active layer varies between systems, ranging from a few mm to tens of cm. The sediments of temporary systems are regularly exposed, to the atmosphere, drying completely under prolonged dry periods, resulting in oxidation of sediment layers. Furthermore, rewetting of desiccated sediments results in a sudden burst of microbial activity resulting in a spike in CO_2 emissions, or *hot spot*, from temporary systems (Catalán et al., 2014; Schiller et al., 2014). While such conditions limit anaerobic production of CH_4, they can lead to rapid oxidation with high rates of CO_2 production and emission. Even when anoxic conditions lend themselves to methanogenic activity several other factors can potentially limit CH_4 production. Many methanogenic bacteria preferentially degrade alternative electron acceptors over carbon when available, such as nitrate (NO_3^-) or sulphate (SO_4^-), reducing the rate of CH_4 production in certain environments; e.g., anoxic saline conditions (Saarnio et al., 2009). Equally metabolic rates of methanogenic bacteria slow considerably at low temperatures, meaning methane production often exhibits strong seasonal trends.

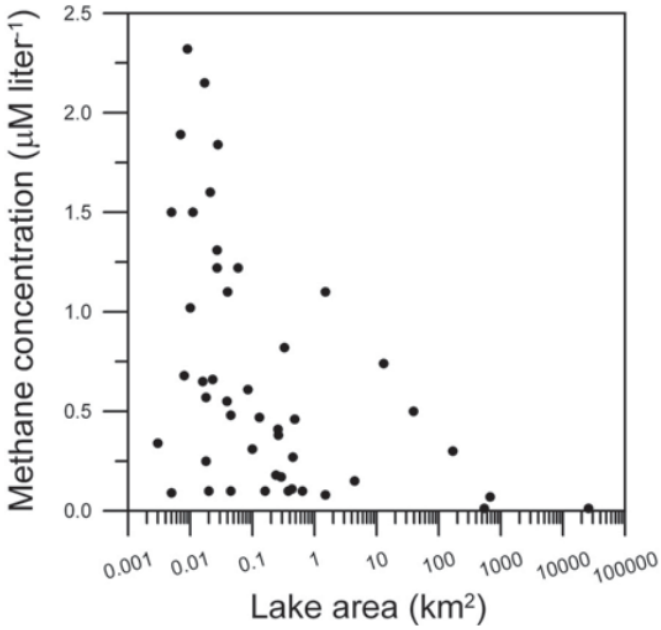
5.D.ii. Gas exchange with the atmosphere

Once the organic matter has been mineralised to CO_2 or CH_4, it then undergoes release to the atmosphere. For temporary ponds with exposed sediments CO_2 produced is released straight to the atmosphere without interruption (Catalán et al., 2014). However, for submerged sediments the water column acts as a barrier to the atmosphere through which any gas produced must first transfer. Typically, CO_2 produced in the active layer is diffused and dissolved into the water column, contributing to the dissolved inorganic carbon (DIC) fraction of a system. DIC may interconvert between species, namely dissolved CO_2, carbonic acid (H_2CO_3), bicarbonate (HCO_3^-), or carbonate (CO_3^{2-}), depending on the thermodynamic equilibriums of the system; i.e., their relative concentrations, concentrations of other buffering species, pH, and temperature (Aufdenkampe et al., 2011). Once in the water column, some dissolved CO_2 will be consumed by submerged macrophytes during photosynthesis. The remaining CO_2 will either be stored in the water column as DIC or degassed to the atmosphere. Gas exchange at the water and atmospheric boundary is dependent on the partial pressure of the system as defined by Henry's law of diffusion, which states the given volume of a gas that can be dissolved in a liquid, dependent on the temperature and pressure of the liquid and the atmosphere (Aufdenkampe et al., 2011). Essentially, if a body of water is under-saturated in HCO_3^-, then CO_2 will be drawn down, or taken in from the atmosphere acting as a carbon sink. Alternatively if a water body is supersaturated in HCO_3^-, then CO_2 will be degassed to the atmosphere, acting as a source. The rates at which CO_2 is exchanged between the water column and atmosphere is predominantly dependent on the partial pressure of CO_2, temperature, and wind speed across the water surface. Whilst large lakes have been shown to be consistently supersaturated

in dissolved CO_2, being considerable sources of carbon to the atmosphere (Aufdenkampe et al., 2011; Pacheco et al., 2013), small ponds have much more complex cycles, highly dependent on the respiration rates of macrophytes. Strong diurnal cycles of CO_2 flux rates have been observed in small ponds (Natchinmuthu et al., 2014), acting as sinks for atmospheric CO_2 in the day during peak macrophyte photosynthesis, and sources during the night, making quantification of their net gas fluxes challenging.

The processes by which CH_4 is degassed to the atmosphere are slightly different. Low concentrations of CH_4 that permeate upwards from anoxic to oxic sediments may be oxidised by methanotrophic bacteria, and converted to CO_2 within the sediment itself. Any CH_4 that manages to permeate through the oxic, active layer of sediment to the water column without being oxidised is then typically dissolved into the water column. However, dissolved CH_4 is quickly oxidised and converted to CO_2 and so very little CH_4 is actually released to the atmosphere through diffuse gaseous diffusion from the water column. The majority of CH_4 emissions from aquatic environments to the atmosphere occur through the process of *ebullition*. Pockets of gas build up in the subsurface anoxic sediments and are suddenly released as bubbles which rise to the waters surface, omitting prolonged residence in the oxidative environment of the water column and resulting in a quick release to the atmosphere. This process is a major contributor of CH_4 emissions in eutrophic lakes (Bridgham et al., 2013; Gonzalez-Valencia et al., 2013) though understanding of the extent of this process in small ponds is limited. That said, dissolved CH_4 concentrations, and therefore potential release rates, are higher in small aquatic systems compared to large lakes (Figure I.6).

Figure I.6: Methane concentrations in lakes around the world related to the size of lakes. Taken from Downing (2010); original data from Bastviken et al. (2004).



5.D.iii. Influence of vegetation

Beyond the organic matter inputs from decaying aquatic vegetation, the potential influence of living macrophyte communities in the degradation of organic material and subsequent gas emission is often overlooked. Many rooted vascular plants actively transfer oxygen from the atmosphere to their rhysosphere creating oxic conditions around their root systems (Couwenberg, 2009). In large reed bed systems it is likely that this has a significant contribution to creating oxic conditions in the sediments that prevent CH_4 production. Equally, this same mechanism can transfer CO_2 from the sediments directly to the atmosphere, acting as a *'shunt pathway'* that avoids retention in the water column (Couwenberg, 2009). However, quantifying releases of CO_2 through shunt pathways is difficult, requiring whole-system approaches to quantifying the net balance of carbon in heavily vegetated systems. Some studies even exclude wetlands from estimates of mass balance of aquatic ecosystems as their large vegetated areas behave functionally like terrestrial systems (Cole et al., 2007), though to do so discounts an extremely large portion of semi-terrestrial aquatic systems.

It may also be that some macrophyte communities play a significant role in determining CO_2 fluxes from temporary ponds. While temporary ponds in Mediterranean climates may remain dry for several months during summer, in temperate climates, where rainfall patterns are more varied, they often undergo several drying and rewetting phases during the summer season. Such events can lead to oxidation of sediments and large releases of CO_2, yet macrophyte communities that form thick blankets of vegetation have been observed to keep sediments hydrated and protected from desiccation well after standing water has evaporated, potentially minimising degradation and CO_2 emissions in between wet periods.

Given the infancy of research into carbon cycling in small ponds it is no surprise that the specific influence of macrophyte communities on the biogeochemical conditions of pond sediments and subsequent gas release is virtually untouched. Yet given their extensive coverage in small systems when compared to large lakes, it is likely that they play a significant role.

5.E. Gas Release Rates from Inland Waters

Despite ponds still being underrepresented in C budgets, increasing effort has been conducted in recent years to document the role of inland waters in the carbon cycle, proving that they are not just neutral conduits for organic matter transportation, but are actively cycling large volumes of C of both allochthonous and autochthonous origins. As is often the case with limnological studies, the lakes of the Earth have received the most interest, with whole system mass carbon budgets for large lakes revealing their true role in the carbon cycle (Dean and Gorham, 1998; Downing et al., 2006; Mulholland and Elwood, 1982; Pacheco et al., 2013). Many lakes have been shown to have gas emissions far exceeding their own net GPP, that can only be explained by a large input of terrestrial

carbon to the systems that is making up the excess emissions to the atmosphere (Cole et al., 2007). By looking at the net carbon balance of whole catchments it has been shown that large amounts of land-derived carbon is released to the atmosphere from freshwater ecosystems. Kling et al. (1991) estimated that 20 % of Arctic tundra NEP is remobilised to the atmosphere as gas fluxes from lakes and rivers. Similar values were reported for forested areas in Finland where lakes remobilised and released to the atmosphere 20 % of C accumulated by forests and soils (Cole et al., 2007; Kortelainen et al., 2006). At present the source of carbon evading freshwater systems can not be fully partitioned, with differentiating between gas release of terrestrial inputs or from aquatic inputs being problematic (Cole et al., 2007). However, whole system carbon budgets are highlighting that inland aquatic systems play an intricate and potentially important role in inland carbon cycling. Globally, inland waters are estimated to outgas 3.28 Pg C yr^{-1} (Aufdenkampe et al., 2011). This value is over 4 fold higher than the 0.75 Pg C yr^{-1} estimated by Cole et al. (2007), which omitted headwater streams due to sparse documentation of their coverage, and wetland environments because their emergent vegetation was said to function similar to semi-terrestrial environments; this has proven to be an incorrect assumption. The inclusion of wetland systems, inclusive of seasonally flooded riparian zones (e.g., flood plain of the Amazon river basin), adds an additional 2.08 Pg C yr^{-1} (Table I.7) to global estimates, mainly due to their extensive global coverage. Generally, emissions are higher from tropical environments than temperate and boreal environments (Aufdenkampe et al., 2011; Gonzalez-Valencia et al., 2013), with tropical wetlands frequently being reported as the having higher emission rates than other inland aquatic system (Bridgham et al., 2013; Morris et al., 2013). Annual CH_4 efflux to the atmosphere from lakes have been estimated at between 6-36 Tg C yr^{-1} (Bastviken et al., 2004). For context the riverine basin of the Amazon River and its floodplains alone are estimated to contribute an additional 22 Tg C yr^{-1} (Cole et al., 2007; Melack et al., 2004), predominantly originating from wetland carbon export (Abril et al., 2014). A conservative estimate of the relative importance of methane emissions suggests that the C gas efflux as CH_4 is 4 % of the C gas efflux of CO_2 (Cole et al., 2007).

One limitation to most global estimates of CO_2 and CH_4 emissions is an absence of estimates from small lakes and ponds, despite their increasing recognition as potentially significant contributors (Aufdenkampe et al., 2011; Cole et al., 2007). While the accurate quantification of the aerial extent and rates of processes within small lakes and ponds are difficult to obtain, it is likely that they make a considerable contribution to global C budgets that is currently unaccounted for. Elevated emissions have been observed from ponds and small wetlands in all climatic zones, including boreal and Arctic zones (Abnizova et al., 2012; Huttunen et al., 2002), temperate and Mediterranean climates (Catalán et al., 2014; Schiller et al., 2014; Torgersen and Branco, 2008), and tropical climates (Downing et al., 2008). A compounding factor is the diversity of pond ecologies that occur across the landscape, of both differing successional phases as well as broad pond types or land-use (Gilbert et al., 2014;

Table I.7: Estimated CO_2 outgassing from inland waters, for zones based on atmospheric circulation. Taken from Aufdenkampe et al., (2011).

Zone-Class	Area of inland waters (1000s km^2)	pCO_2 (ppm)	Gas exchange velocity (k_{600}^{-9} cm hr^{-1})	Areal outgassing (g C m^{-2} yr^{-1})	Zonal outgassing (Pg C yr^{-1})
	min-max	**median**	**median**	**median**	**median**
Tropical (0°-25°)					
Lakes and reservoirs	1840-1840	1900	4.0	240	0.45
Rivers (> 60-100 m wide)	146-146	3600	12.3	1600	0.23
Streams (< 60-100 m wide)	60-60	4300	17.2	2720	0.16
Wetlands	3080-6170	2900	2.4	240	1.12
Temperate (25°-50°)					
Lakes and reservoirs	880-1050	900	4.0	80	0.08
Rivers (> 60-100 m wide)	70-84	3200	6.0	720	0.05
Streams (< 60-100 m wide)	29-34	3500	13.1	2630	0.08
Wetlands	880-3530	2500	2.4	210	0.47
Boreal and Arctic (50°-90°)					
Lakes and reservoirs	80-1650	110	4.0	130	0.11
Rivers (> 60-100 m wide)	7-131	1300	6.0	260	0.02
Streams (< 60-100 m wide)	3-54	1300	13.1	560	0.02
Wetlands	280-5520	200	2.4	170	0.49
Global	**Area of inland waters (1000s km^2)**	**Percent of global land area**		**Zonal outgassing (Pg C yr^{-1})**	
Lakes and reservoirs	2800-4540	2.1-3.4		0.64	
Rivers (> 60-100 m wide)	220-360	0.2-0.3		0.30	
Streams (< 60-100 m wide)	90-150	0.1-0.1		0.26	
Wetlands	4240-15,220	3.2-11.4		2.08	
All inland waters	**7350 - 20,260**	**5.5 - 15.2**		**3.28**	

Huttunen et al., 2002), resulting in ponds as a collective being diverse in their geochemical processes and rates and subsequent C release. Regardless these are issues that need to be overcome should ponds be included in larger global carbon budgets. It has long been argued that much of the missing C sink may be stored in small aquatic habitats, including those of anthropogenic construction such as rice paddies or small farm ponds (Cole et al., 2007; Downing, 2010; Downing et al., 2008; Gilbert et al., 2014; Stallard, 1998). Only recently is the number of studies focusing on C release from ponds and small wetlands increasing, including focus on the frequently overlooked temporary systems (Catalán et al., 2014; Schiller et al., 2014).

6. Conclusion

Ponds and small wetlands form an intrinsic part of our landscapes and culture, found across all terrestrial biomes of the Earth, from frozen arctic tundra to dessert oasis. While there is no universally agreed definition of what physically constitutes a pond, they are well ingrained in our landscapes that come in many shapes and sizes, from ancient post glacial relics to recent temporary ponds. Despite being relatively understudied for most of the 20th C. from a geochemical perspective, ponds are now receiving increasing interest. The ecological importance of ponds is well recognised, contributing more to regional γ diversity than any other inland aquatic system. Considerable work has been conducted to determine the global coverage of small aquatic systems, with most recent estimates placing the global coverage of ponds and lakes of ≥ 0.002 km^2 as 5×10^6 km^2, equating to 3.7 % of the Earth's non-glaciated land surface, with this being dominated by large and intermediate sized lakes. Yet the exact extent of the smallest of these features (< 0.0001 km^2) remains undetermined as they are rarely detected in aerial surveys, compounding estimates of their biogeochemical processing rates.

Regardless of extrapolations, surveys suggest that small ponds are extremely active sites for C sequestration and storage, as well as remobilisation to the atmosphere, and that small ponds and wetlands have the potential to be considerable cyclers of C, and may be processing a significant amount of the missing carbon budget. Yet quantification of pond sediment C stocks, and C processing and flux rates are few, severely restricting our understanding of their role in the C cycle and their inclusion in regional or national C budgets. In order for ponds to be fully integrated into C budgets it is crucial that their C stocks, as well as biogeochemical processing rates are quantified.

Chapter II. A Regional Survey of Pond Sediments and Carbon Stocks in Druridge Bay, Northumberland

1. Introduction

Our understanding of the global carbon budget is incomplete, with a significant gap between top down global atmospheric models and quantified surface measurements resulting in a 'missing carbon sink' equivalent to roughly one-third of global fossil-fuel emissions (Aufdenkampe et al., 2011). It is believed the majority of this error lies within the terrestrial carbon sink, creating a substantial need to fully quantify the processes and intricate interactions within and between sub-compartments of the global carbon cycle (Cole et al., 2007).

One of the most poorly constrained sub-compartments of the terrestrial carbon budget is that of inland water bodies, predominantly small water bodies where their interaction with the surrounding terrestrial environment is often seamless. These small aquatic systems often go unquantified because: (1) their processing of C is considered to be closer to terrestrial environments and so are omitted from budgets of inland waters (Cole et al., 2007); (2) regional monitoring of C budgets often masks these small features, such as eddy covariance systems which typically cover a large footprint. However, the processes and rates of degradation and remobilisation of carbon are very different in small aquatic environments when compared to the surrounding terrestrial systems. While substantial research has recently been conducted into the role of inland seas, large lakes, reservoirs, and aquaculture impoundments as sinks for carbon sequestration (Boyd et al., 2010; Dean and Gorham, 1998; Downing, 2010; Lehner and Döll, 2004), smaller water bodies have been almost wholly neglected in audits of the global carbon cycle (Alonso et al., 2012; Downing, 2010; ICCP, 2001, 2007, 2014; USCCSP, 2003).

Developments over recent years have highlighted the potential significance of ponds in global cycles. A negative correlation exists between the size and frequency of larger water bodies across the landscape, however constraints in satellite imagery and limited regional data hinder the ability to quantify ponds smaller than 1000 m^2 (Downing et al., 2012). Regardless it is likely they have significant global coverage. With revised estimations of the global coverage of small wetland environments, and better understanding of the rates of carbon sequestration in small lakes and ponds, inland waters are now believed to process about 1 Pg yr^{-1} more C than previously thought (Downing, 2010), potentially dominating terrestrial carbon processing (Boyd et al., 2010; Cole et al., 2007; Downing, 2010; Meyers and Ishiwatari, 1993).

However, most studies focus specifically on aquaculture ponds (Boyd et al., 2010; Ntengwe and Edema, 2008; Xinglong and Boyd, 2006), and of those studies that do focus on small natural water bodies, most overlook the diversity of pond types and the heterogeneity of pond wildlife that can occur across landscapes (Williams et al., 2004). Only a few notable studies focus specifically on the C dynamics within ponds of varying vegetation classification (Gilbert et al., 2014) and among ponds with differing hydrological regimes (Gilbert et al., 2014; Macrae et al., 2004), significantly restricting regional extrapolations.

2. Aims & Objectives

Given that at the landscape scale pond biodiversity is characteristically heterogeneous (Biggs et al., 2005; Jeffries, 2010; Williams et al., 2004) it is hypothesised that the carbon stocks within sediments of different pond types will also be varying; e.g., with pond permanence or adjacent land-use. By grouping all ponds within the landscape together key features regarding the individual characteristics of a pond that dictate a higher C storage capability may be overlooked. The aim of this chapter is to accurately quantify the C stocks stored within sediments of ponds across Druridge Bay, Northumberland, UK. This was designed to include both the high variability of pond types, variations in vegetation coverage, ponds of differing hydrological patterns, and varying sizes.

Specific objectives are to:

1) Quantify the amounts of C stored within the sediments of a range of ponds across Druridge Bay.
2) Explore the spatial distribution of C within the sediment of individual ponds, elucidating the potential errors in relation to the number of sediment core samples.
3) Identify the spatial heterogeneity of C stocks among different *'ecological types'* across the landscape; e.g., ponds of differing type, vegetation classification, and permanence.
4) Identify variations in C stocks among superficially similar ponds; i.e., ponds of the same type, vegetation classification, and permanence.

3. Site Description

The research conducted within this chapter focuses on the region of Druridge Bay, Northumberland, UK. Northumberland itself is largely upland dominated by the Cheviot Hills and surrounding moorland, however, running parallel to the coast is a lowland strip dominated by agricultural landscape. The Northumberland coastal plain has a relatively cool, dry, temperate climate, with summer mean maximum temperatures seldom > 20 °C, and a rain shadow from the hills to the West resulting in annual rainfall of usually < 800 mm yr^{-1} (Gilbert et al., 2014; Lunn, 2004) though this is largely dependant on the influence of the North Atlantic Oscillation (Fowler and Kilsby, 2002; George et al., 2004).

3.A. Druridge Bay

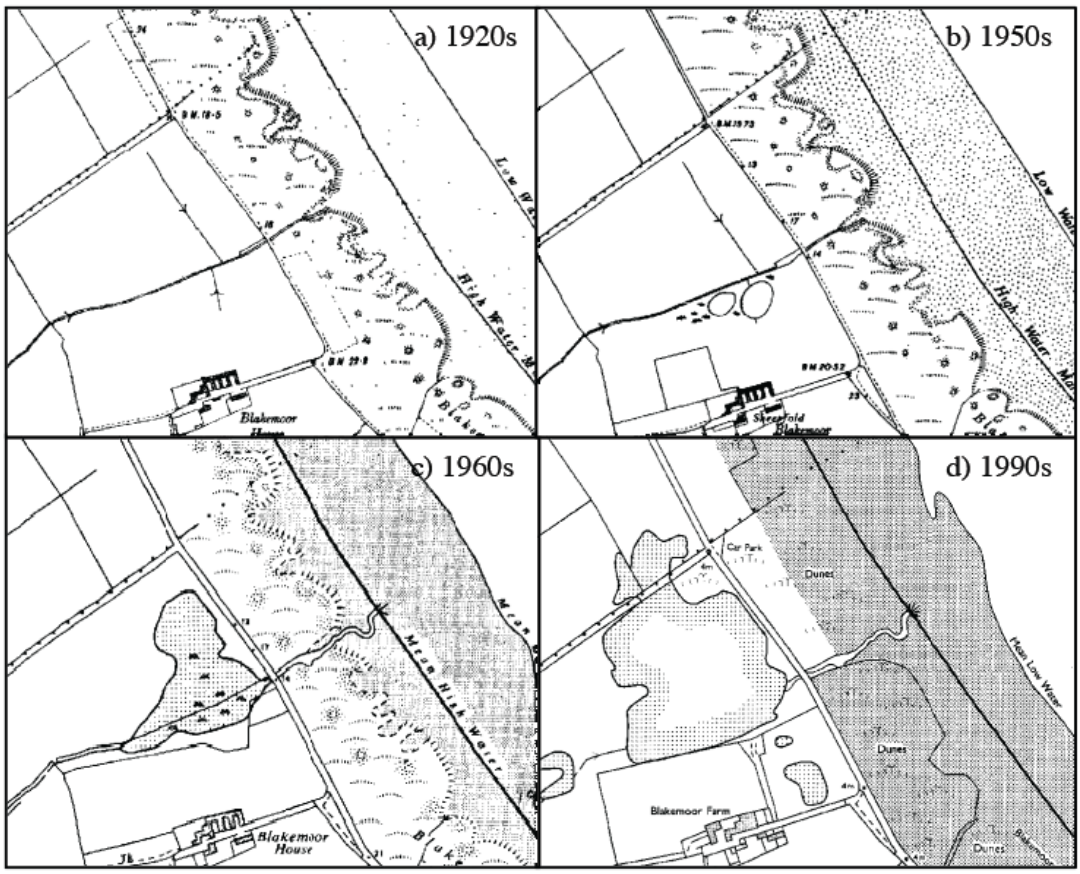
Within this Northumberland coastal plain lies Druridge Bay (Figure II.1), an 11 km stretch of sand dune coastline stretching from Amble in the north (NU265042; Lat: 55.336468, Long: -1.5790701) to Cresswell in the south (NZ293935; Lat: 55.235092, long: -1.5395880). Behind the beach and sand

---

Figure II.1: Map of the UK and Druridge Bay, showing the locations of sampling sites at: (a) Hauxley Nature Reserve; (b) Druridge Bay Country Park sand dunes; (c) Blakemoor Farm. See Figure II.5 for detailed images of sampling sites.

dunes, Druridge Bay is an area of rich lowland agricultural farmland, with both arable and pasture fields dominating the landscape. However, the region has a strong mining heritage, and since the majority of activity ceased, several large ex-opencast coal mines have been converted to wetland habitats for nature conservation now covering ~ 10 % of the area (Jeffries, 2012). Coupled with this reclamation of opencast sites, large extents of land subsidence from abandoned underlying coal mine-shafts have occurred, resulting in new depressions forming across the landscape (Jeffries, 2012). Since the 1950s many pools have naturally formed within these depressions, such as Druridge Pools near Cresswell (Figure II.2), and subsequently smaller ponds and dune slacks now occupy ~ 2 % of the landscape, the majority of which are < 400 m^2. Due to the strong seasonal extremes observed in the region many of the smallest aquatic systems are temporary pools and flashes of < 10 m^2, occurring during periods of prolonged rainfall and dissipating during warmer summer months. The temporal nature of many of the aquatic systems means that there is often large inter annual variations in the number and extent of wetlands in the region that can have significant impact on the invertebrate and macrophyte communities that occupy them (Jeffries, 2010, 2015). The large constructed wetlands combined with the smaller temporary ponds form an intricate network of wetland environments that are ubiquitous across Druridge Bay.

Figure II.2: Historical maps showing the development of Druridge Pools over 70 years due to land subsidence around Blakemoor Farm. Images from Edina (2015).



The importance of these aquatic systems for conservation is widely recognised. This is equally true for Druridge Bay. Ponds and small wetland environments are repeatedly highlighted in the Northumberland Biodiversity Action Plan (Jaggs, 2008) as being important sites, not only crucial for amphibians but also coastal and farmland birds and upland waders. Yet conflicts can arise. The majority of Druridge Bay is agricultural land, with the sporadic appearance of these ponds in the middle of fields hindering agricultural activity. They pose a significant financial implication to farmers as seasonal flooding of arable fields can significantly reduce crop yield. Increasing pressure on farmers to enhance the productivity of agricultural land can lead to infilling of depressions or land drainage, both factors highlighted as major causes of loss of wetland environments in the Northumberland Biodiversity Action Plan (Jaggs, 2008).

4. Methodology

4.A. Pond Selection

4.A.i. Identification of broad Pond Types

To test the hypothesis that C stocks among ponds would be as varied as their biodiversity, pond selection was identified as an important process prior to sampling. The purpose was not to explore the detailed variations of plant communities themselves, but to incorporate the gross variations in plants and adjacent land uses that occur across the landscape which may affect C sequestration. This approach has been adopted in previous work (Gilbert et al., 2014; Jeffries, 2015), identifying 4 distinct Pond Types: Arable Field ponds; Pasture Field ponds; Dune Slack ponds; and Classically Vegetated ponds. These four Pond Types represent the broad variations across Druridge Bay; details of the four categories can be seen in Table II.1 along with visual images in Figures II.3 and II.4. This study used these four Pond Types as a guide to selecting individual ponds and an initial method for grouping ponds in analysis. Further methods of grouping ponds for comparative analysis are described in Section 4.B.

Table II.1: Broad characterisation of four Pond Types across Druridge Bay. Taken from Gilbert et al. (2014). Data for conductivity and vegetation coverage acquired during extensive regional surveys by Jeffries (2010, 2015).

Pond type	Characteristics. Conductivity (µS cm^{-1}), as mean (min, max)
Arable field ponds	Shallow (< 30 cm), usually temporary ponds in arable cereal and oil seed rape fields. Bare ploughed soil substrate, with no or limited (< 5%) vegetation cover, primarily ubiquitous weeds of disturbed ground; e.g., common knotgrass [*Polygonum aviculare*] and wild chamomile [*Chamomilla suaveolens*], OV18 community (Rodwell, 2000). Mean Cond. = 383 µS cm^{-1} (89–1200).
Pasture field ponds	Shallow (< 30 cm) permanent and temporary ponds in sheep or cattle pasture fields. Soil substrate, extensive vegetation (> 90%), mostly seeded fodder (e.g., perennial ryegrass [*Lolium perenne*]) or amphibious grasses (e.g., flote grass [*Glyceria fluitans*]). Mean Cond. = 581 µS cm^{-1} (234–1151).
Dune Slack ponds	Shallow (< 30 cm), temporary, on landward side of dunes. Organic rich mud substrate over sandy soil. Occasional brackish water inundation. Extensive natural vegetation (>90%) characteristic of such sites (e.g., common silverweed [*Potentilla anserine*]). Mean Cond. = 728 µS cm^{-1} (244–1392).
Classically Vegetated ponds	Permanent with extensive deep zones (> 0.5 m) as well as shallow swamp zones. Typically with organic rich, anoxic, uncompacted substrate. Extensive but patchily vegetated (20–60%) with characteristic pond emergents (e.g., branched bur-reed, [*Sparganium erectum*]) and submerged species (e.g., water-starworts [*Callitriche* spp.]). Mean Cond. = 817 µS cm^{-1} (555–1436).

Figure II.3: Images of arable and pasture field ponds. (A) pasture field pond in and amongst ridge and furrow land; (B) cattle wallowing in a temporary field pond; (C) arable field pond situated in and amongst oil seed rape; (D) arable field pond during summer drying with crop spraying right up to the pond edge.

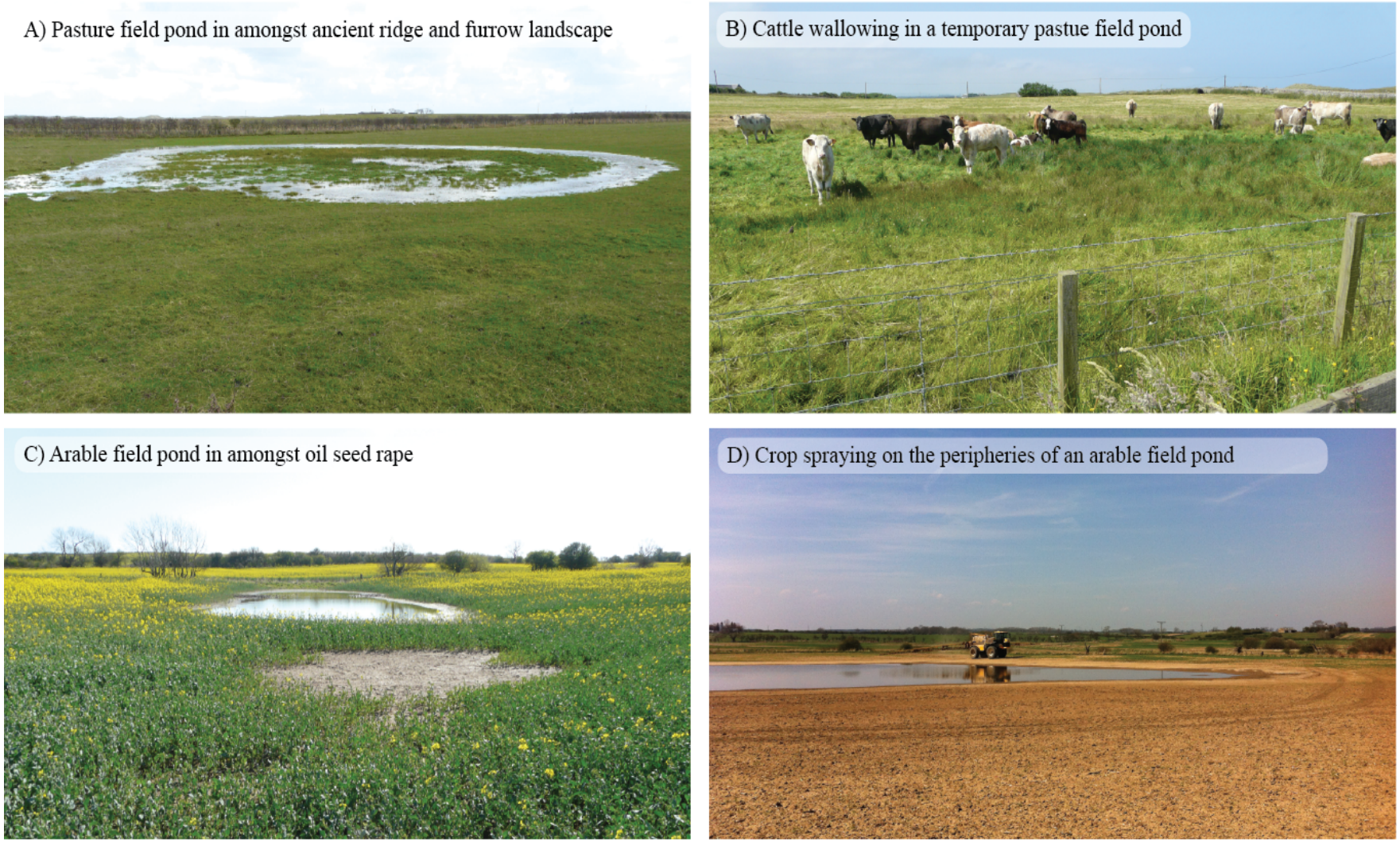


Figure II.4: Images of dunes slack and naturally vegetated ponds: (A) dune slack pond in and amongst sand dunes with Druridge Bay Country Park in the back ground; (B) classically vegetated pond with heavily vegetated peripheries; (C) deep anoxic sediments on the peripheries of a classically vegetated pond during a summer dry period.

4.A.ii. Individual pond selection

Ten ponds were selected from each of the four Pond Types (i.e., Classically Vegetated, Arable, Pasture, and Dune Slack ponds), totalling 40 ponds across Druridge Bay from three regions; Hauxley Nature Reserve (NU282027; Lat: 55.317742, Long: -1.5569258), Druridge Bay Country Park sand dunes (NU274001; Lat: 55.294439, Long: -1.5693069), Blakemoor Farm (NZ283940; Lat: 55.239644, Long: -1.5559387). Individual ponds within the four Pond Types were selected to provide as broad coverage of environmental variables as possible, encompassing ponds of varying size, depth, and permanence, within four broad land uses found across Druridge Bay: arable land, pasture land, dune slacks and natural wetland mosaics. However, permission for access and ease of access were also factors in determining pond choice. Figure II.5 shows the location of each individual pond, with Table II.2 detailing pond area, permanence, and broad Vegetation Type (discussed further in Section 4.B.i.). It should be noted that the numbering system used for the ponds in this study is based on the numbers allocated to ponds in previous studies (Gilbert et al., 2014; Zealand and Jeffries, 2009) and as such has been kept the same for ease of referencing between studies. Due to ease of access most ponds were located in the southern region of Druridge Bay on Blakemoor Farm where open access was granted (previously owned by Alcan Farms, recently sold to the Crown Estate and leased to Velcourt Farms).

4.B. Additional Methods of Grouping Ponds

Whilst the four broad Pond Types taken from Gilbert et al. (2014) were used to select individual ponds based on gross variations among ponds across the landscape, they form just one method of separating ponds into groups for analysis. Several other variables were documented that also serve as methods for grouping ponds during statistical analysis. Along with the Pond Types identified in Section 4.A.i. and in Table II.1, additional grouping criteria are Vegetation Type, Pond Permanence, Surrounding Land Use Type, and Pond Surface Area.

4.B.i. Vegetation Type

While the four Pond Types used to select ponds represent the broad variations among ponds across the landscape, grouping ponds solely by Vegetation Type allows analysis of specific plant communities that may be better suited for enhanced C storage, regardless of surrounding lands or location. As part of a study conducted by Jeffries (2015) pond macrophyte communities were surveyed across Druridge Bay to explore differences in vegetation and to see if plant communities supported the separation of ponds into the four broad Pond Types detailed in Table II.1. Macrophyte surveying was conducted using standard methods for UK National Vegetation Survey, quantifying the abundance of each taxon using quadrat identification and the DOMIN scale (for further detail see Jeffries, 2015).

Figure II.5: Location of the forty ponds across Druridge Bay: (A) Hauxley Nature Reserve; (B) Druridge Bay Country Park; (C) Blakemoor Farm. For descriptions of pond types see Table II.2. * = ponds from which ten replicate sediment cores were collected

Table II.2: Details of individual ponds, grouped by Pond Type, and detailing: Pond Area, Permanence; and Vegetation Type.

Pond ID	Pond maximum area (m^2)	Pond Permanence (Does it dry out?)	Vegetation Type (Group Nº)	Pond ID	Pond maximum area (m^2)	Pond Permanence (Does it dry out?)	Vegetation Type (Group Nº)
Classically Vegetated Ponds				**Pasture Field ponds**			
Hx 1	1077	Sometimes	1.1	18	618	Yes	2.1
Hx 2	487	Sometimes	1.1	24	3111	Yes	2.1
4	1517	Never	1.2	25	2124	Never	2.1
15	1612	Sometimes	1.2	26	2350	Yes	2.1
16	996	Sometimes	2.1	27	1008	Sometimes	1.2
29c	5513	Never	1.2	28	49	Yes	1.2
32a	4417	Never	1.2	29	151	Yes	1.2
40a	1401	Yes	1.2	30	3603	Yes	2.1
46	1835	Never	1.2	31	2446	Never	2.1
54	1472	Sometimes	2.1	31a	344	Yes	2.1
Arable Field ponds				**Dune Slack ponds**			
34	403	Yes	2.2	3	1721	Sometimes	1.2
35	2766	Yes	2.2	5	1110	Yes	1.2
38	6675	Sometimes	2.2	6	466	Yes	1.2
40	795	Sometimes	2.2	7	1055	Yes	1.2
43	407	Yes	2.2	8	463	Yes	1.2
44	987	Yes	2.2	9	401	Yes	1.2
47a	845	Yes	2.2	9a	984	Yes	1.2
48	3902	Sometimes	2.2	CP 1	366	Sometimes	1.1
49	1161	Sometimes	2.2	CP 2	141	Yes	1.1
55	435	Yes	2.2	CP 3	142	Sometimes	1.1

Pond area was measured using ArcMap 10 and digitised from base-map imagery; see Section 4.B.vi. Pond Permanence was established from a 3 year ground survey by Jeffries (2015); see Section 4.B.iii; Yes = dries out every year, Sometimes = dries some years but not others, Never = never recorded to dry out. Vegetation Type was established from 2014 macrophyte identification, conducted by Jeffries (2015), and is grouped according to the results of a two-tier TWINSPAN; see Section 4.B.i and Table II.3.

In this study, the vegetation data from 2014 was subject to a two-tier TWINSPAN (two-way indicator species analysis) whereby the ponds were separated into four groups based on the presence or absence of certain key indicator species; a technique previously shown to separate ponds into distinct broad ecological types (Jeffries 2012). Table II.3 shows the breakdown of the TWINSPAN and the key indicator species for each group, as well as a broad description of the groups of ponds. Whilst most ponds fall into convergent categories when grouped by either Pond Type or Vegetation Type, (i.e., Arable Field ponds, Pasture Field ponds, and Dune Slack ponds are all in roughly similar groups when grouped by either Pond Type or Vegetation Type) there is some overlap in key indicator species among ponds.

Table II.3: Results of the two tier TWINSPAN as used to group ponds by Vegetation Type.

Tier 1		Tier 2		Characteristics
Division 1	**Group 1** **Ponds:** Hx.1, Hx.2, CP.1, CP.2, CP.3, 3, 4, 5, 6, 7, 8, 9, 9a, 15, 27, 28, 29, 29c, 32a, 40a, 46 **Indicator species:** • *Agrostis stolonifera*	Division 2	**Group 1.1** **Ponds:** Hx.1, Hx.2, CP.1, CP.2, CP.3	Ponds solely from Hauxley Nature Reserve and Dune Slack ponds from Country Park. All ponds are heavily vegetated with *Phragmites australis,* and other large reeded macrophyte communities. All ponds are well established systems and dry out only during extended periods of low rainfall. **Indicator species:** • *Phragmites australis* • *Iris pseudacorus*
			Group 1.2 **Ponds:** 3, 4, 5, 6, 7, 8, 9, 9a, 15, 27, 28, 29, 29c, 32a, 40a, 46	Mostly sand dune slack ponds from the dunes neighbouring Blakemoor Farm (ponds 3, 5, 6, 7, 8, 9, & 9a) with the rest comprised of ponds that fall under the 'Classically Vegetated' category when grouped by Pond Type. All systems are well established and densely populated by rooted macrophyte communities with little to no bare ground. **Indicator species:** • *Agrostis stolonifera*
	Group 2 **Ponds:** 16, 18, 24, 25, 26, 30, 31, 31a, 34, 35, 38, 40, 43, 44, 47a, 48, 49, 54, 55 **Indicator species:** • *Matricaria matricarioides* • *Alopecurus geniculatus* • BARE GROUND	Division 3	**Group 2.1** **Ponds:** 16, 18, 24, 25, 26, 30, 31, 54, 31a	With the exception of pond 54, all ponds are located in pasture fields. All ponds are temporary, drying during summer and so are dominated by aquatic grasses such as *Alopecurus geniculatus*, which thrive on wet/dry cycle conditions. Most ponds are usually completely covered with low lying vegetation with little to no bare ground. **Indicator species:** • *Alopecurus geniculatus*
			Group 2.2 **Ponds:** 34, 35, 38, 40, 43, 44, 48, 49, 55, 47a	Ponds located in arable fields, all of which dry during summer months and are often ploughed over. **Indicator species:** • *Polygonum aviculare* • *Orache spp. A prostrate and .C rubrum* • *Tipleurospermum inodorum* • BARE GROUND

4.B.ii. Surrounding Land Use

Ponds may also be grouped by their surrounding Land Use. This is similar to grouping ponds by Pond Type in that all Arable Field Ponds are surrounded by arable fields, Pasture Field Ponds surrounded by pasture fields, and Dune Slack Ponds surrounded by sand dunes. The difference however lies within those Classically Vegetated ponds identified in Pond Types, that lie in and amongst all the aforementioned land use types. Grouping ponds by Land Use type alone restricts analysis to the identifying land uses and catchment that may influence C storage; i.e., nutrient input or agricultural influences.

4.B.iii. Pond permanence

Many of the ponds included in this survey are temporary features, fluctuating in size and permanence depending on precipitation patterns. Many ponds dry out entirely during periods of low rainfall, with the frequency and duration of these drying periods varying between years depending upon annual variations in rainfall. Data of Pond Permanence was obtained from a study by Jeffries (2015) in which each site was examined every few months: late November, January, March, late May and August. The survey period ran from November 2010 until November 2013 inclusive, recording whether the ponds either dry out during summer, never fully dry out, or sometimes dry or not depending on inter-annual rainfall variations. Of the ponds selected for this survey (n = 40), ~ half (n = 21) were observed to dry out on an annual basis, 13 occasionally dried or did not depending on inter-annual rainfall variations, and only 6 ponds never dried out during summer months. Whilst having only 6 ponds that never dry (15 % of the total number of ponds) may be perceived as unbalanced, it highlights a uniqueness to this study; that in selecting ponds from ground walking the site considerably more temporal ponds are observed than if ponds had been selected from aerial images or maps. Details of individual Pond Permanence can be found in Table II.2.

4.B.iv. Pond area

Pond area was mapped in ArcMap 10 using basemap aerial imagery. As many of the ponds in this study are temporary, and certainly all vary in size depending on rainfall, inter-annual pond sizes can vary significantly depending on annual rainfall fluctuations. Furthermore, due to the lack of direct inflow, summer minimum inundation varies depending on rainfall events with the majority of ponds drying out. As such only winter maximum extent was mapped, which was clearly identifiable from differences in vegetation type from surrounding land use. As the mapping of ponds in this project was conducted from one satellite image (rather than repeated over several years to gain an average), pond areas should be treated with caution, and viewed as a 'snap shot'. However, pond area in this study is simply used to extrapolate to whole pond C stocks estimates, not for regional extrapolations. Should regional extrapolations be required further study documenting the true seasonal fluctuations

of pond coverage should be conducted. Pond size at maximum inundation averaged 1534 m^2 (± 1495 SD, min = 49, max = 6675); individual pond maximum areas can be found in Table II.2.

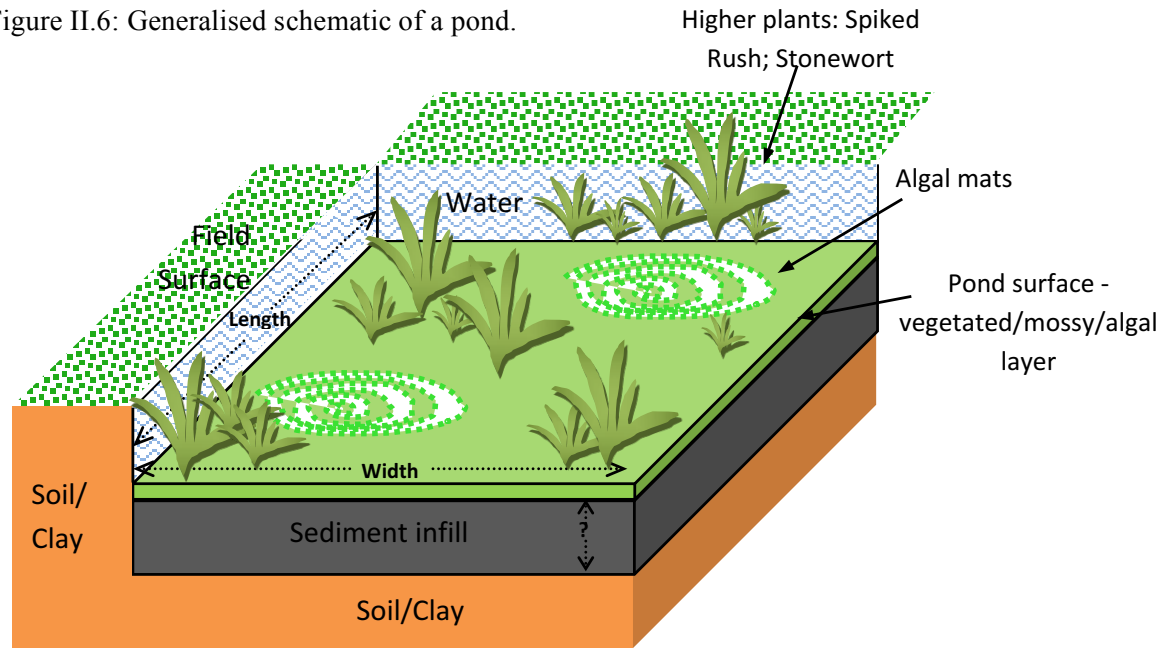
4.C. Sampling Strategy

4.C.i. Problems encountered and trialing of different coring techniques

There were numerous problems in the early stages of this research associated with collecting core samples, as no traditional methods were suitable for the hugely varied range of environments and ecosystems encountered. Figure II.6 is a generalised schematic of a pond highlighting some of the key features, such as water depth and vegetation. The primary difficulties encountered were:

1) **Water depth** of ponds sampled in this survey ranged from zero to 70 cm. How does one remove an intact core from beneath 70 cm of water?
2) **Sediment density and composition** varied markedly among ponds due to differences in hydrological patterns (e.g., temporary ponds with a dried-out desiccated surface can be quite difficult to penetrate) and land-use type (e.g., clay based substrate of arable fields were considerably denser than sandy substrates found in dune-slack ponds).
3) **Vegetation type**, and the amount of flora covering the surface of many ponds varied considerably, from algal mats to densely matted higher plants, and not just from site to site but also throughout the year for the same site, as seen in Figure II.7. The primary difficulty with vegetation was penetrating the dense rooted layer of sediments in heavily vegetated ponds, without disturbing the underlying sediments, and again often conducted under > 10 cm of water.

Figure II.6: Generalised schematic of a pond.



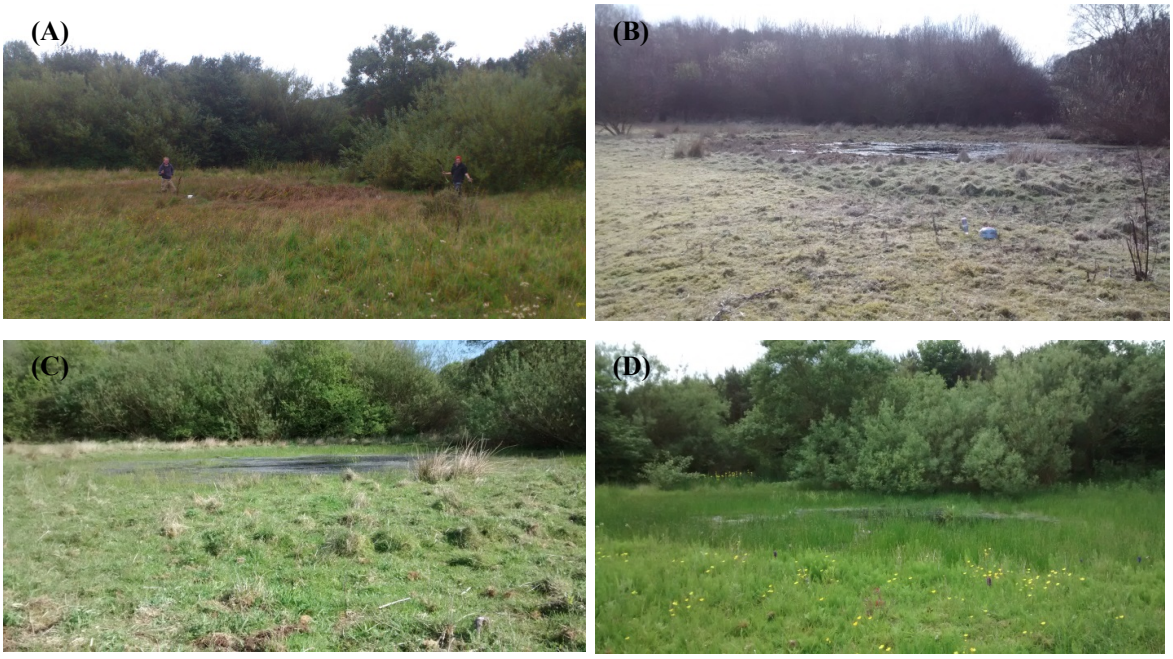


Figure II.7: Large natural pond at Hauxley Nature Reserve shown throughout 2014/15: (A) November; (B) February; (C) May; (D) June.

---

4) **Friction** between corer and sediment resulted in varied levels of compaction, an important issue that is not addressed in academic literature.

A significant amount of effort was directed at developing a robust, universally applicable methodology that could be used on all pond and sediment types. An overview of coring techniques trialled is described as follows.

4.C.i.a Plastic pole corer

Initially samples were extracted using 6.35 cm diameter plastic tubing. The tubing was forced into pond sediment, extracting approximately a 15-20 cm core. Initially the samples were collected from the centre of the pond, as the centre remained submerged for the longest period annually (Gilbert, 2011). A pilot study was previously carried out in which 3.18 cm diameter tubing was used to collect cores, however sample weight was insufficient for analytical testing. The method was therefore improved by increasing tube diameter to collect sufficient material. The samples were extruded by physical pushing of an internal plunger. Each core length was measured (with core lengths up to 20 cm), and photographs taken of each core. The cores were dissected into 1-2 cm sections, depending on the physical characteristics of the sediment; typically for very soft water logged sediments it was very difficult to section every 1 cm. Figure II.8 shows a conceptual model of the core alongside a core photograph representing transitions of different core sections. In an early pilot study sediments were dried within the plastic corer before dissecting, however this proved difficult to cut into accurate sections and loss of sediment occurred from dust produced when cutting. The

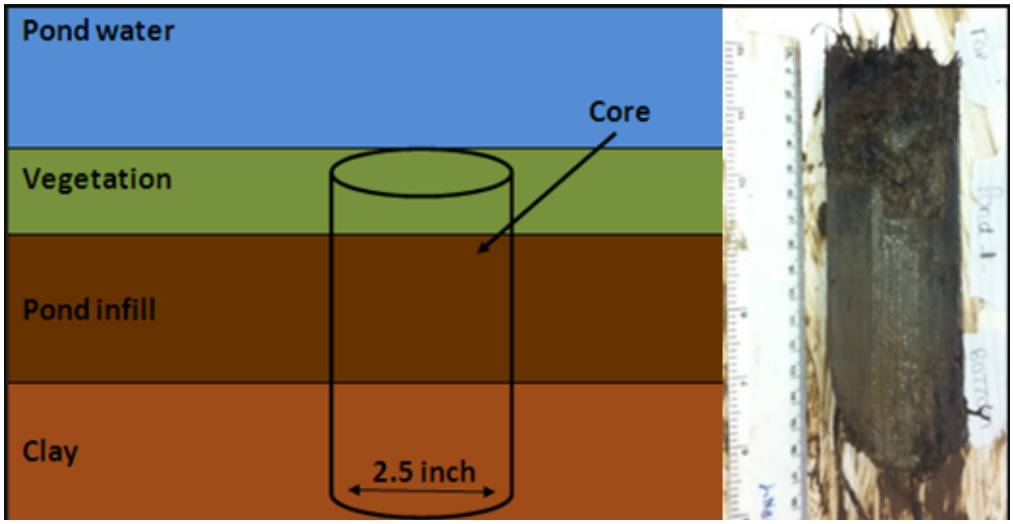


Figure II.8: Conceptual model of an extruded core.

---

use of this plastic coring technique worked well on 'medium' density sediments, that is to say, those not too hard or soft.

4.C.i.b Dry ice corer

While sediments of firmer consistency (i.e., medium/high density) could be extracted using a simple plastic pole corer, this technique was not suitable for the extremely low density sediments that were permanently saturated. Such sampling was attempted using freeze core extraction, as used in (Gilbert et al., 2014). This comprised a hollow, wedge shaped metal corer, which was driven into the low density sediments and filled with dry ice (solid phase CO_2 at < -78.5 °C), which rapidly cools the corer freezing the surrounding sediment. When extracted, a thin layer of sediment is frozen to the outside of the corer, keeping intact any laminations (Munsiri et al., 1995). Samples can then be transported, stored, and processed in the same manner as those collected using a pole corer. While this method does not allow such easy dissection and separation, as large amounts of root organic matter are also frozen within the sample, it does provide a useful approach to extracting unconsolidated sediments whilst keeping depth horizons intact.

4.C.i.c Open face metal corer

High density sediments, typically those of temporary ponds in arable fields with clay based substrate, proved especially difficult to sample. The relatively blunt base of most pole corers (Section 4.C.i.a.) could not penetrate to any significant depth, and extraction of sediment cores from the tubing by way of the plunger being extremely difficult due to high levels of compaction and friction. To overcome this an open faced metal corer (typically used for coring soils) was trialled. With a sharpened end penetrating to greater depth, and open face allowing for ease of access to the extracted sediment core for dissection, this technique had relative success within high density sediments, but was inappropriate for lower density sediments which would simply fall out of the open face.

After trialling the above coring techniques it became apparent that no currently available coring technique was applicable to all pond and sediment types. In a previous study (Gilbert et al., 2014) two techniques (the above described pole core and dry ice core techniques) were adopted for sampling differing sediment types, however a key outcome was that the use of two techniques caused uncertainty in the results between the sampling methods and reproducibility. Given the variety and number of ponds to be sampled in this study, it was apparent that a more effective and reproducible methodology needed to be developed, and consequently focus turned to developing a new custom designed corer that would allow core collection across all environments and precise dissection in the field. It must be clearly stated that this development was wholly collaborative, including the author, Scott Taylor and David Cooke.

The main implication identified from these early attempts was accurately calculating sediment dry bulk density and any subsequent compaction of the sediment in the core extrusion process. Bulk density is arguably one of the most important values required to calculate carbon storage in sediments and, as density results from sediment cores are often extrapolated to cover an entire pond system, it is crucial that volume and mass values are obtained as accurately and reproducibly as possible.

4.C.ii. Development of a universal sediment corer

4.C.ii.a Attempt 1

As can be seen in Figure II.9 a custom made metal corer was constructed, using chromium-vanadium steel (High Polish 2P). Mechanically polished stainless steel is widely used, including both building internal and external applications. The surface appearance, corrosion resistance and dirt retention of mechanically finished stainless steel surfaces can vary widely, depending, in part, upon the nature of the abrasive medium used and the polishing practice. The 1P/2P finish (technical grade for polish finishing) is of the highest standard, giving a very fine, clean cut with minimal micro-crevices. This helps optimise the corrosion resistance and minimising friction between corer and sediments. The bottom of the corer, i.e., the first point of contact with material, was filed to produce sharp cutting edge, facilitating easy penetration of both dense vegetation layers or desiccated hardened sediment. A plastic tube was inserted inside the metal corer for collection of sediment. At this stage attempts were made to dissect cores into 1 cm sections in the field, but this proved unsuccessful. Also the width (40 mm) and shortness of the corer (250 mm) proved inadequate in many environments, particularly with the wetter sediments, with significant loses during removal from pond (i.e., the sediment just dropped out of plastic inner tube).

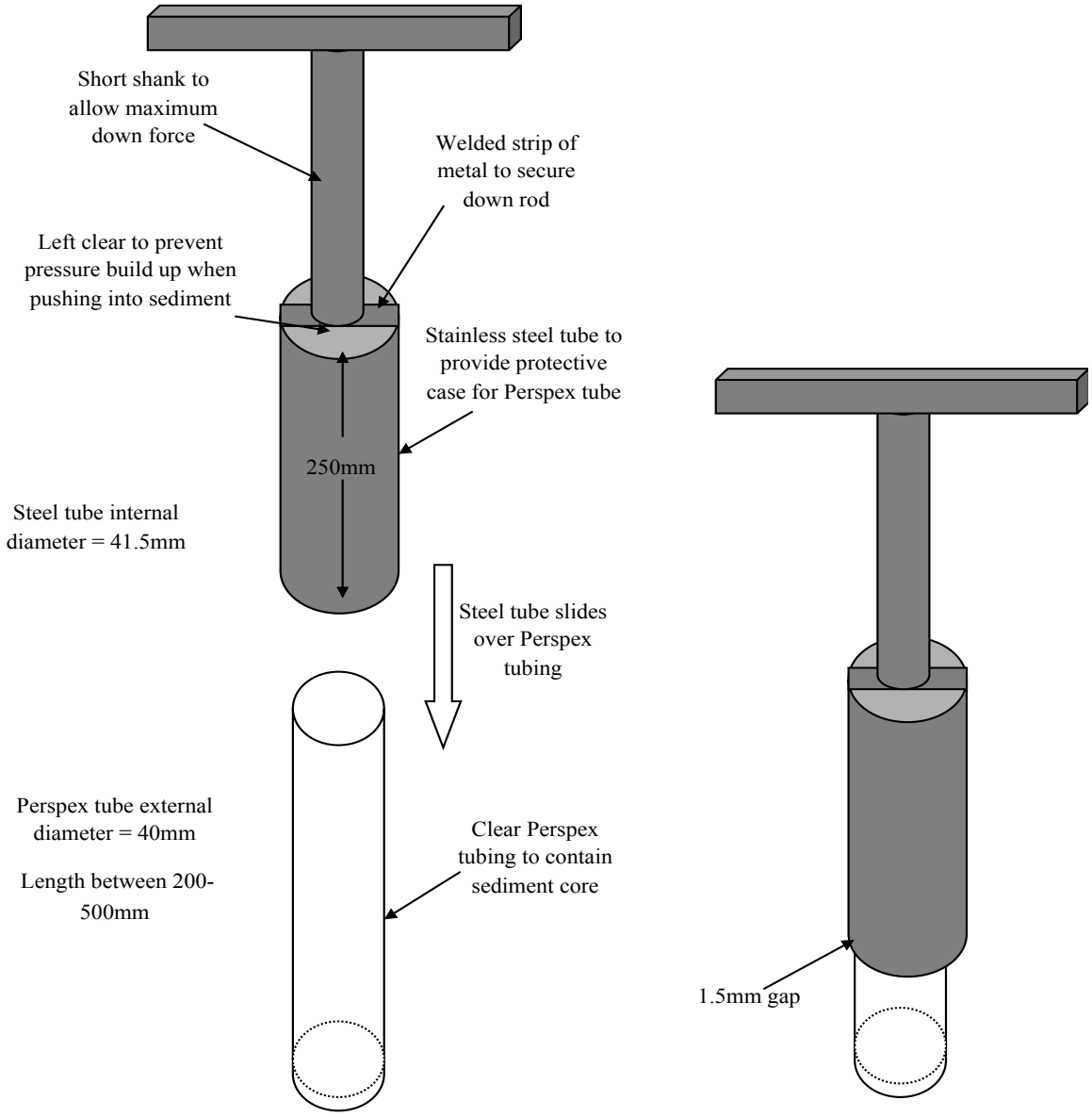


Figure II.9: Schematic of the universal corer - attempt 1.

4.C.ii.b Attempt 2; working version

The corer in Figure II.9 below has since been developed which has overcome all problems previously encountered, allowing the sampling of sediments regardless of their structural integrity. The new corer also provided an accurate gauge on compaction, by use of internal graduated plunger, and allowed the efficient extrusion and dissection of cores at consistent 1 cm intervals in the field, critical for accurate density measurements to be made. This final design of sediment corer was used for the collection of all sediment cores and subsequent data reported within this thesis.

4.C.iii. Sediment core collection

Sediment cores were collected using the universal sediment corer, designed and constructed specifically for this project (as described above in Section 4.C.ii). The corer was driven manually into the sediment as far as possible, typically reaching the more compacted soil base, that acts as a plug to seal in the softer sediment layers above (Figure II.10.B. and II.10.C.). Upon removal of the corer excess water was drained via a small hole at the top, and the length of the core was measured via the internal plunger, allowing for calculation of compaction during the removal of the sediment core. The sediment core was extruded and dissected 1 cm at a time, measured by 1 cm markings along the length of the internal plunger, recording the exact length of each core section for calculation of the volume (Figure II.10.D and II.10.E). Dissecting the core in this manner was found to be more accurate than extruding the core intact and dissecting in the lab. Upon dissection each section was wrapped in foil and placed in a paper sample bag and transported back to Northumbria University, Newcastle upon Tyne, and stored in refrigeration prior to analysis.

It should be noted that as the sampling technique relied on manual force to core the sediment, depth is largely a factor of the sampler's ability to penetrate the substrate. Quite simply put this is a factor of the sampler's strength, or downward force, yet all sediment cores were collected by the same sampler (i.e., myself) reducing the level of human error in determining core depth. That said, moisture content and density of the sediment both play a key role in the ability to penetrate to greater depths and so temporal differences in sampling times may have influenced the sampler's ability to core to greater depths; i.e., temporary ponds cored in summer may have recently been subject to drying periods with a decrease in sediment moisture content, making coring more difficult compared to the softer high moisture content sediments of ponds that remain hydrated or temporary ponds sampled in winter.

All sediment cores were collected between April-December 2014, and while many ponds dry during summer months, all ponds had standing water at the time of sampling. Sediment cores were collected from the centre of each pond, or as close to the centre as possible where water level was above the height of waders. In some cases this was in amongst the vegetation whilst open water in others. One sediment core was collected from each of the forty ponds, prioritising a higher pond count with just one core per pond (rather than fewer ponds with more cores per pond) in order to reduce the risk of pseudoreplication and gain a broader understanding of the variations in sediment C levels among individual ponds across the landscape.

4.C.iv. Analysis of sediment C distribution within individual ponds

Collecting only one sediment core from each pond may raise questions as to the representativeness of C distributions within individual ponds. To combat this a second approach was adopted.

Figure II.10: Images of sediment coring: (A) a deconstructed corer and intact sediment core; (B) coring of an arable field pond; (C) cross sectional view of the sediment corer; (D) extruding a sediment core for dissection in the field by myself (left) and Scott Taylor (right); (E) dissecting a sediment core into 1 cm sections.

Four ponds were selected, one from each of the four Pond Types (i.e., Arable Field, Pasture Field, Dune Slack, and Classically Vegetated ponds), and, using the same coring method as described in Section 4.C.iii, ten sediment cores were collected from across each pond. This allowed assessment of the heterogeneity of C levels within individual ponds, analysis of the accuracy of extrapolating results from just one sediment core, and an assessment of the increase in precision gained by increasing sediment core numbers when sampling small water bodies.

4.C.v. Soil sampling

Alongside the sediment samples, soil cores were also collected to provide comparison between the C stocks within pond sediments and that of the background surrounding soil. Nine sediment cores were collected, comprising three cores from each of the three Land Use Types (pasture, arable and sand dunes), and processed in the same manner as the sediment cores. It must be clearly stated these were not collected as controls, but purely for comparison purposes.

4.D. Sample Preparation

4.D.i. Measurement of sediment properties

4.D.i.a Moisture content

Within 24 hours of coring, individual samples were weighed to acquire the wet weight of each section. Samples were then placed in a drying cabinet at ~ 40 °C for ~ 7 days until a constant weight was achieved and the dry weight recorded. The moisture content (% moisture) of each individual sediment section was calculated following Equation II.1.

Equation II.1:

$$\%\ moisture\ content = \frac{wet\ weight - dry\ weight}{wet\ weight} * 100$$

It should be noted that the sample bags and foil used during sediment collection were pre-marked with a sample ID and pre-weighed prior to core collection. This allowed for the wet and dry weights to be recorded by simply re-weighing the bags with the samples inside rather than removing samples from the bags, avoiding inaccuracies in the weighing process such as sample loss and cross contamination.

4.D.i.b Dry bulk density

Along with moisture content, dry bulk density (DBD) was also calculated. Quantified as the mass of sediment within a given volume (g cm^{-3}), DBD was calculated from the recorded dry weight and volume of each individual sediment section (Equation II.2), and reflects the actual mass of sediment left within an individual section once the water content has been removed. This is crucial to know as

it allows for the calculation of C density within individual sediment sections and absolute C stocks (discussed further in Section 4.E.iii.b-c).

Equation II.2:

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

4.D.ii. Grinding and sieving

Dried samples were ground using a pestle and mortar, removing large vegetated material (i.e., twigs or roots) as undecomposed organic matter would considerably increase the C values upon analysis. Ground samples were then sieved to 0.5 µm, cleaning all equipment with acetone between samples. Samples were then sealed in 10 ml vials and frozen (< 4 °C) prior to analysis.

4.E. Analysis

4.E.i. Carbon & Nitrogen analysis

Total carbon and nitrogen analysis was performed on all sediment and soil samples. Prior to analysis samples were removed from the freezer and left at room temperature for two hours to return to their atmospheric moisture content. Analysis was performed by dry combustion using Total Elemental Analysis (TEA), specifically a Thermo Scientific FLASH 2000 Series Organic Elemental Analyser. The system operates through flash combustion, with resulting gases carried by a helium flow, passing through both a copper sulphate and a magnesium perchlorate filter, separated out by a GC column, and detected by a thermal conductivity detector (TCD). An Aspartic Acid certified reference material was used to calibrate the TEA prior to the running of each batch of samples. Approximately 5 mg of sample (weight recorded to 0.001 mg) was analysed, with oven temperature set at 980 °C and a run time of 360 seconds, ensuring full combustion and detection of both N and C peaks.

4.E.ii. Data validation

During C and N analysis by TEA every 10th sample was run in triplicate, followed by a blank sample (n = 121) processed and run identically to the sediment samples as described in Section 4.E.i. From the blanks the LoD (Limit of Detection) and LoQ (Limit of Quantification) were calculated using Equations II.3 and II.4 respectively.

Equation II.3:

$$\mathrm{LoD} = \mathrm{mean}_{\mathrm{blanks}} + (3 \times \mathrm{standard\ deviation}_{\mathrm{blanks}})$$

Equation II.4:

$$\mathrm{LoQ} = \mathrm{mean}_{\mathrm{blanks}} + (10 \times \mathrm{standard\ deviation}_{\mathrm{blanks}})$$

Triplicates of samples were used to calculate the precision of analysis (% RSD; % Relative Standard Deviation) as in Equation II.5.

Equation II.5:

$$\% \, RSD = \frac{standard\ deviation_{\ triplicates}}{mean_{triplicates}} \times 100$$

The LoD was calculated to be 0.46 % C with the LoQ being 1.43 % C. Triplicates (n = 98) comprised a full range of depths and averaged 7.81 % RSD with the majority of samples being < 10 % RSD. Only 11 triplicates were > 15 % RSD, of which 9 were triplicates of samples from Dune Slack Ponds.

4.E.iii. Carbon quantifications

4.E.iii.a C concentration – % C

The direct output from the TEA analysis is a % value of C and N, calculated from the specific weight of the sample analysed. This can be described as the C concentration, or % C, within the particulate matter of an individual sediment section.

4.E.iii.b C density – mg C cm^{-3}

Whilst the % C within an individual sample provides information on the organic matter content of the sediment, it is also important to know the absolute mass, or *C density*, within each sediment section relative to the overall dry mass of particulate matter. By factoring in sediment DBD, C density (mg C cm^{-3}) within individual sediment layers can be calculated (Equation II.6) allowing for variations in DBD over depth and among ponds.

Equation II.6:

$$\text{C density} = \frac{\%\ \text{C}}{100} \times \text{DBD}_{\text{sediment section}} \times 1000$$

$$\text{mg C cm}^{-3} = \frac{\%\ \text{C}}{100} \times \text{g cm}^{-3} \times 1000$$

4.E.iii.c Carbon stock – kg C m$^{-2}_{< 10\ cm}$

For further extrapolations to whole pond estimates and comparisons among systems it is easier to work in absolute values; i.e., to quantify the absolute mass of C stored within a given depth and limit complications arising from factoring changes over depth. In this study Carbon Stock refers to the absolute mass of C stored in the upper 10 cm of sediment, expressed as kg C m$^{-2}_{< 10\ cm}$ (Equation II.7).

Equation II.7:

$$\text{Carbon stock m}^{-2} = \frac{\sum \text{C density cm}^{-3}{}_{\text{upper 10 cm sediment}} \times 10{,}000}{1{,}000{,}000}$$

$$\text{Kg C m}^{-2}{}_{<10\text{ cm}} = \frac{\sum \text{mg C cm}^{-3}{}_{<10\text{ cm}} \times 10{,}000}{1{,}000{,}000}$$

4.E.iii.d Carbon:Nitrogen ratio

Along with C, concentrations, the TEA also provides analysis of nitrogen concentrations (% N). Alone this data has little implication for this thesis, and is therefore not directly included in the results or discussion. However, when the concentration of C and N are compared as a ratio (Equation II.8) they provide insight into the composition of the organic matter within the sediments (Meyers and Ishiwatari, 1993).

Equation II.8:

$$\text{C: N ratio} = \frac{\%\text{ C}}{\%\text{ N}}$$

4.E.iv. Statistical calculations

Within this regional study of sediment C stocks there was a considerable amount of data collected, and as such it was important to choose the correct statistical model for analysis. Within the data there are numerous variables (e.g., sediment C stock, % C, or moisture content), and several factors (e.g., Pond Type, Vegetation Type, or Pond Permanence) which are potentially significant in determining sediment C stocks. As such, a mixed model Repeat Measures, Analysis of Variance (ANOVA) was performed using IBM SPSS Statistics 22. The ANOVA is also used as the main statistical tool within both Chapters III and IV, and therefore is covered in detail here to act as a reference point for later chapters.

4.E.iv.a Background to ANOVA

ANOVA is a statistical model specifically designed for testing the statistical significance among the means of two or more groups at once. As such it is particularly practical for this study as it allows for direct comparison of the means of a dependant variable (e.g., % C), among several groups within one of the factors (e.g., Pond Type), whilst still maintaining individual subjects (individual ponds/cores); e.g., the % C measurements (dependant variable) from individual ponds (subjects) can be grouped by Vegetation Type (factor).

A particularly useful element of ANOVA is the inclusion of *Repeat Measures* into the analysis. Several measurements from individual subjects (e.g., the carbon in individual 1 cm slices down a core) are repeat measures rather than wholly independent measurements, meaning they are more likely to be similar to one another (in the case of individual 1 cm slices, likely to be more similar to the slices immediately above or below) than those measurements from other subjects (other

ponds/cores) within the same group (e.g., Classically Vegetated Pond Type). ANOVA allows for the placement of a covariance structure on these repeat measurements. In the case of repeat measurements from the sediment cores (i.e., individual sediment sections over depth), an autoregressive[1] (AR[1]) covariance structure was applied, which assumes that adjacent measurements are more closely correlated than those further apart; i.e., sediment layers at depths of 1 cm and 2 cm are more closely correlated than those at 1 cm and 10 cm depths.

From this model, factors can be run as fixed (i.e., the model assumes all potential groupings were sampled) or random factors (i.e., the model assumes individual ponds sampled are a random sample of all the ponds across the landscape) in order to create the most appropriate model. Whilst several methods exist for creating/selecting the best model (e.g., the specific set up of random factors and co-variables), all models in this thesis were selected to be the most parsimonious; i.e., the model which provides the greatest level of information for the lowest complexity (number of parameters).

Lastly, whilst ANOVA tests if there is any significant difference among groups of means, it does not directly state where those differences are. As such a post-hoc Bonferroni test was applied to all ANOVA models within this thesis, which conducts pairwise comparisons among groups within the fixed factor (e.g., among different Vegetation Types), stating the statistical significant difference between them. Bonferroni test was selected over others as it assumes statistical test are non-independent making it a more conservative analysis, reducing the risk of making a type 1 error; i.e., a false rejection of the null-hypothesis, or a false positive significant difference. All statistical significance is reported to 95 % confidence interval for both overall ANOVA models and post-hoc comparisons.

In summary, ANOVA is a complex yet extremely powerful tool for handling the large dataset obtained throughout this research. Whilst the datasets may be different in each chapter, the general method of the above described model is the same for each. For reference, a brief description of the statistical models used is provided within each methods section.

4.E.iv.b The use of ANOVA within this chapter – regional sediment survey

Within this sediment survey of ponds from Druridge Bay, individual ponds/cores were considered to be subjects, and run as random factors of the total population of ponds/sediments in the landscape. Individual sediment sections over depth were run as repeat measures with an AR[1] covariance structure. Sediment characteristics (i.e., moisture content and DBD) and geochemical properties (i.e., % C, C:N ratio, C density, and C stocks) were the dependent variables, with the fixed factors being: Pond Type (i.e., Classically Vegetated, Arable, Pasture, and Dune Slacks); Vegetation Type

categorised by TWINSPAN (Groups 1.1, 1.2, 2.1, and 2.2); Pond Permanence (dries up, never dries, and sometimes dries); Land Use Type (Arable, Pasture, and Sand Dune); and Sediment Layers (< 5 cm, 5-10 cm, and > 10 cm). While the results of each statistical permutation are discussed in detail within separate sections, Table II.5 shows the primary statistical analysis and results for ease of reference.

Data was tested for normality using tests and graphical plots (e.g. Shapiro-Wilk test, stem and leaf plots. Tests and plots run in SPSS). Data were normally distributed for some measures, and for some pond types (e.g. for some of the four pond types defined by Pond Type at Druridge Bay), but not in all cases. Square root or log transformations normalised most data. Whilst typically results would be log-transformed to reduce the skew of the data, this was found to have little impact on the statistical analysis produced by ANOVA, and did not change the statistical outcome of the results. As such all statistical analysis was conducted on the un-transformed data for transparency. Equally, whilst non-parametric percentage data is typically Arcsine transformed, this should only be conducted on count-derived data; the % C values in this study are not count-derived, but direct measurements, and as such were not transformed prior to statistical analysis.

4.F. Method Development

As with much of this research, a large element consisted of developing a robust methodology. Whilst the laboratory analysis of sediment samples is relatively well established, sampling techniques vary widely. The following section documents some of the method development and trials that were faced during the early stages of this research.

4.F.i. Field weighing trial

In order to record moisture content more accurately, weighing of sediment sections in the field immediately after dissection was trialled on 5 sediment cores (*n* individual dissected slices = 102). Portable weighing scales, enclosed in a plastic container for protection from the wind, were used to weigh each sediment section immediately after dissection. The samples were weighed again the following day within Northumbria University laboratory, and the two weights compared to determine the accuracy of wet weights after 24 hours. No significant difference was found between field weights and those recorded in the lab (two-sample t-test, $p = 0.939$). Subsequently sediment sections were weighed in the lab as weighing in the field was subject to uneven surfaces and disturbance from wind, despite the use of shielding.

4.F.ii. Drying samples

Calculation of the % C by TEA is based on the input weight of sediment analysed. It is therefore crucial that analysis by TEA is conducted on dried samples, as a high sediment moisture content

would result in a false calculation. Whilst sediments were dried prior to grinding and sieving they were then frozen prior to analysis, a process that can alter their moisture content. To determine if repeated drying of samples in a drying cabinet after freezing was required a comparison study was conducted. Sediment samples ($n = 10$) were weighed in duplicates with one set analysed with repeated drying and the other set non-repeated drying, and compared using a paired t-test. The mean difference in weight ($M \pm SD = -0.29 \pm 0.44$ g) was not significantly different ($t = 0.45$, $df = 18$, two-tail $p = 0.66$) providing evidence that the further drying of samples was not necessary.

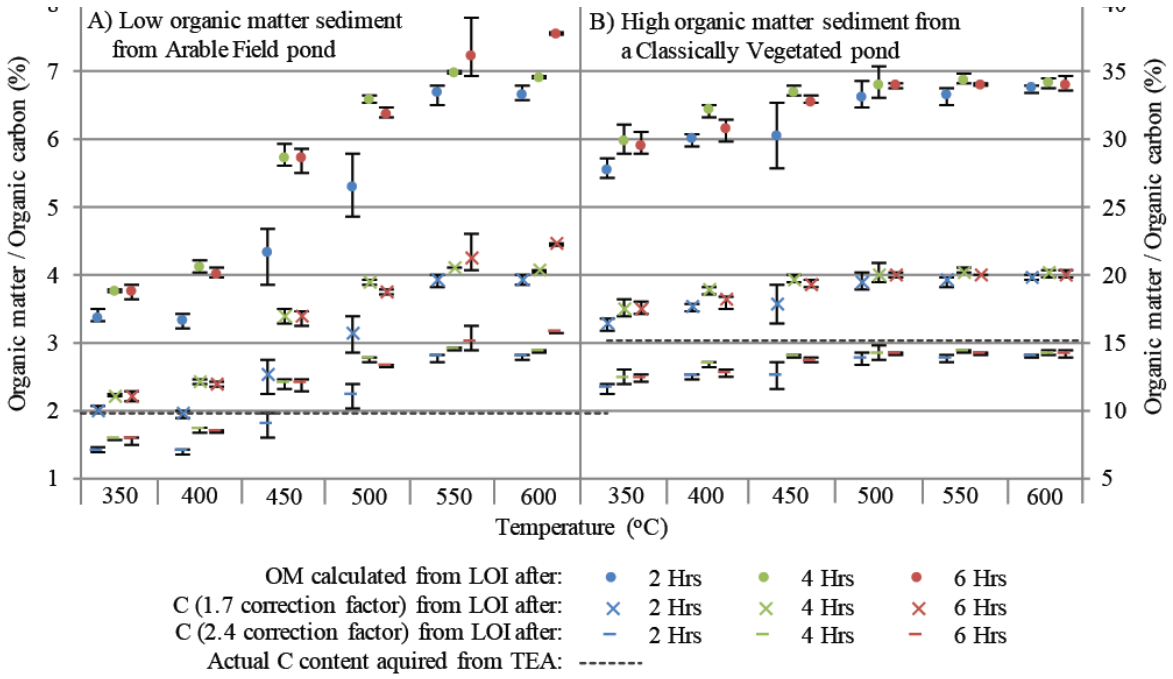
4.F.iii. LOI

At the time of project inception the TEA (as described in Section 4.E.i) used to analyse sediment C and N concentrations was not yet available, and as such loss on ignition (LOI) was originally proposed to analyse sediment C concentrations. LOI operates by incinerating the sample at a given temperature and time and taking the loss in weight as the organic matter (OM) content of the sample. The C content is then calculated by applying a correction factor that assumes a given % of OM is C. However, throughout literature there is a lack of consensus over the correct running time and temperature at which to run the sample. Too short a period, or low temperature, may result in incomplete combustion and underestimation of the OM content, whilst too long combustion at too high temperature may result in a loss of moisture bound within clay particles and an overestimation of the OM content. Furthermore, correction factors differ depending on the composition of the OM, often ranging from 1.7 to 2.4. To combat this two homogenised sediment samples (one with low C value and one with high C) were analysed on LOI at a series of temperature and time permutations, ranging from 350-600 °C increasing at 50 °C increments, and running at each temperature for 2, 4 and 6 hours, with each sample run in triplicate. Alongside this each sample was sent for analysis by TEA at Newcastle University to provide an accurate measure of C content; TEA is the current standard and recognised as being far more accurate than LOI. The purpose of this exercise was to use the % OC value from the TEA analysis to establish the most accurate temperature and time duration for running sediments on LOI, along with an accurate correction factor, which would then be used as the protocol for C analysis for this project.

Considerable differences were observed across the range of temperatures and time permutations used (Figure II.11), with calculated organic matter content increasing with increase in temperature. While this suggests incomplete combustion at lower temperatures this may also be due to the loss of inorganic C or particle bound moisture at higher temperatures. Equally, different running times resulted in variable OM estimations, and while it might be expected that a longer combustion period would result in higher OM concentration, this was not always the case. Combined, these factors resulted in large variation in OM concentration, which is carried over to estimated % C concentration when the correction factors are applied. Few of the % C results from the run time/temperature

permutations of LOI matched the results given by the current standard method of TEA. For this reason, the proposed method of LOI was dismissed, and with the arrival of a TEA at Northumbria University, all analysis of sediment C concentrations (% C) reported in this thesis was conducted by TEA.

Figure II.11: Experimental analysis of loss on ignition (LOI), used to determine the correct temperature and running time for comparison to TEA.



5. Results

5.A. Within Pond Variations - a Study of Replicate Cores within Individual Ponds

To assess the accuracy of extrapolating from single sediment cores to whole pond C stocks, the spatial distribution of C within individual ponds was first explored. Four ponds were selected from the four broad Pond Types initially identified across Druridge Bay, picking one pond of each (i.e., Classically Vegetated, Arable, Pasture and Dune Slack ponds), with 10 replicate sediment cores collected from each individual pond. Respective of pond area, the sampling densities (number of samples per given area) of the four ponds were 18, 15, 28, 273 samples per hectare for the Classically Vegetated, Arable, Pasture, and Dune Slack ponds respectively (Table II.4).

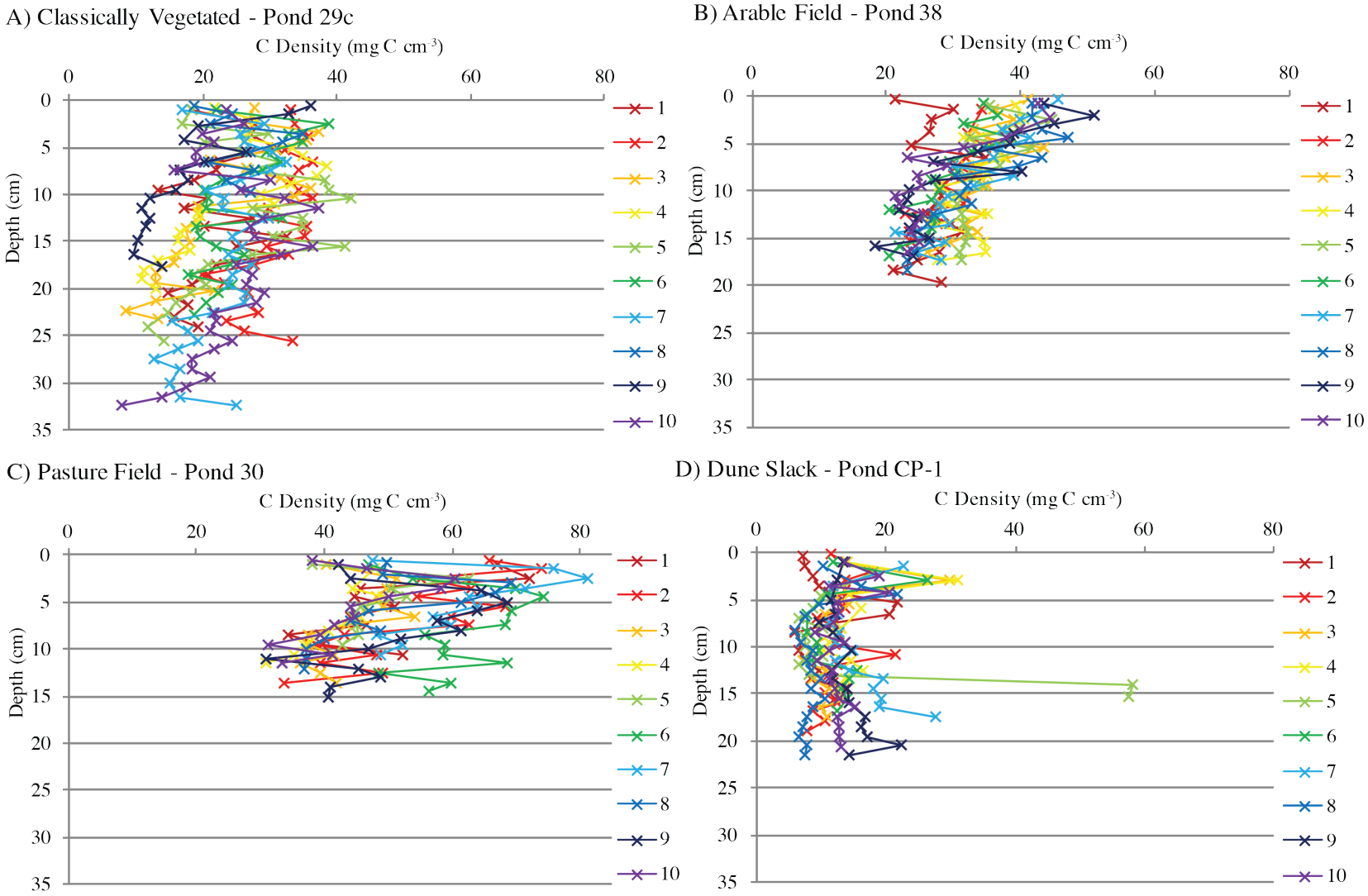
Both the concentration of C (% C) and C density (mg C cm^{-3}; Figure II.12) typically decreased over depth within ponds, with the greatest variations observed in the upper most sediment layers. The C density at similar depths among replicate cores from the same pond varied considerably, often by > 20 mg C cm^{-3} at similar depths (i.e., > 100 %). Of the four ponds the C density within sediment sections were most variable among cores from the Classically Vegetated pond and the Pasture pond. Equally it was these two systems that had the greatest variation in sediment C concentrations (% C) in the upper most layers.

Despite the obvious graphical differences, little statistical difference was found at similar depths among replicate cores from the same ponds, either in % C or in C density in individual sediment sections. No significant difference in % C was observed among the replicate cores from the Classically Vegetated pond ($F = 0.644$, df = 9.0, 9.3, $p = 0.739$), Arable field pond ($F = 0.504$, df = 9.0, 9.1, $p = 0.504$), Pasture field pond ($F = 1.078$, df = 9.0, 118.1, $p = 0.384$), or the Dune Slack pond ($F = 0.742$, df = 9.0, 9.6, $p = 0.668$). Equally, no significant difference in C density was observed among replicate cores from the Classically Vegetated pond ($F = 1.080$, df = 9.0, 25.6, $p = > 0.5$), the Arable Field pond ($F = 0.619$, df = 9.0, 17.7, $p = 0.766$), or the Dune Slack pond ($F = 1.106$, df = 9, 23.0, $p = 0.397$). A significant difference was observed among the replicate cores

Table II.4: Details of sampling densities and precision of analysis for assessment of the heterogeneity of C stocks within individual ponds.

	Pond ID	Area (m^2)	Sampling density (N° samples per hectare)	Precision of sampling (% RSD)
Classically Vegetated pond	29c	5513	18	14.39
Arable Field pond	38	6675	15	9.31
Pasture Field pond	30	3603	27	12.47
Dune Slack pond	CP1	366	273	15.43

Figure II.12: C density in replicate sediment cores from four ponds across Druridge Bay.



of the Pasture Field pond (F = 5.616, df = 9.0, 117.0, p = < 0.000), yet only with sediment sections in cores 6 and 7 having significantly higher C densities than cores 3, 4, and 10 (p = < 0.05).

While C density over depth varied among replicate cores, though not significantly, when quantifying total C stocks in the upper 10 cm of sediment (kg C $m^{-2}_{< 10\ cm}$), the margins of error are relatively low (Figure II.13). Overall the mean estimated masses of C stored were 2.68 (95 % CI = 2.43, 2.93), 3.55 (3.33, 3.77), 5.27 (4.84, 5.69), and 1.34 (1.21, 1.48) kg C $m^{-2}_{< 10\ cm}$ for the Classically Vegetated, Arable, Pasture and Dune Slack ponds respectively. The relatively low variation in calculated C stocks among replicate cores from individual ponds equates to a relatively high level of precision of sampling, with % RSDs of 15.2, 9.8, 13.2, and 16.3 % for the Classically Vegetated, Arable, Pasture, and Dune Slack ponds respectively.

5.B. Regional Survey – Differences Among Ponds throughout the Landscape

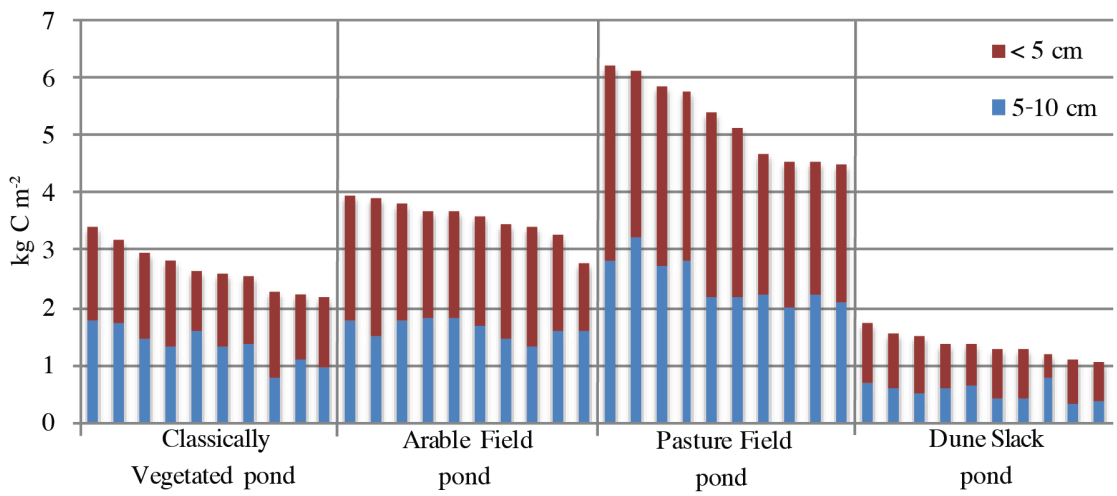
Whilst Section 5.A. focused on the distribution of C within individual ponds and the accuracy among replicate cores, this section focuses on the singular sediment cores collected from 40 ponds across Druridge Bay, Northumberland.

5.B.i. Sediment characteristics

5.B.i.a Core length

Of the singular sediment cores collected (n = 40) from the 40 ponds across Druridge Bay, sediment core length averaged 16.9 ± 5.9 cm (± SD, range = 9.2-33.0 cm) typically being longer from Dune Slack ponds and shortest from Arable Field ponds (mean ± SD = 19.0 ± 5.7 and 15.5 ± 4.4 cm respectively). This variation is largely a factor of sediment moisture content and sediment density

Figure II.13: C stock in the upper 10 cm of replicate cores from four ponds across Druridge Bay.



controlling the researcher's ability to penetrate to a greater depth, and while there was some variation among Pond Types, no significant difference in length was observed (p = > 0.05). Again, it should be noted that core length plays little role in determining C Stock estimates (kg C m^{-2}) within this study as all calculations are based solely on the upper 10 cm of sediment to allow for direct comparison among ponds, as well as with other studies; no cores collected were shorter than 10 cm.

5.B.i.b Dry bulk density

Mean DBD across all sediment samples was 1.04 ± 0.43 g cm^{-3} (± SD, n = 199, range = 0.09-2.15 g cm^{-3}). An increase in DBD over depth was observed (F = 60.15, df = 2, 79, p = < 0.000) being significantly lower in the top 5 cm (0.67 ± 0.34 g cm^{-3}) than the 5-10 cm section (1.09 ± 0.36 g cm^{-3}, p = < 0.001) and > 10 cm section (1.28 ± 0.26 g cm^{-3}, p = < 0.001), and was significantly higher in the > 10 cm section than the 5-10 cm section (p = < 0.001; Figure II.15). Of the top 5 cm layers the lowest mean DBD was in sediments of Dune Slack ponds (mean = 0.52 ± 0.40 g cm^{-3}) and highest in the compacted sediments of Arable Field ponds (mean = 0.89 ± 0.24 g cm^{-3}).

Overall no statistical significant difference in DBD was observed among Pond Types, Vegetation Type defined by TWINSPAN, Pond Permanence (Table II.5), or Land Use Type (p = > 0.05). Equally, when comparing the DBD of sediments to that of the surrounding soil, only the sediments of ponds in arable fields were significantly lower (F = 5.81, df = 1, 24, p = < 0.05), with no significant difference observed between the DBD of sediments from Dune Slack ponds, Pasture ponds, and Classically Vegetated ponds, and their respective surrounding soils (All p values = > 0.05; Figure II.14).

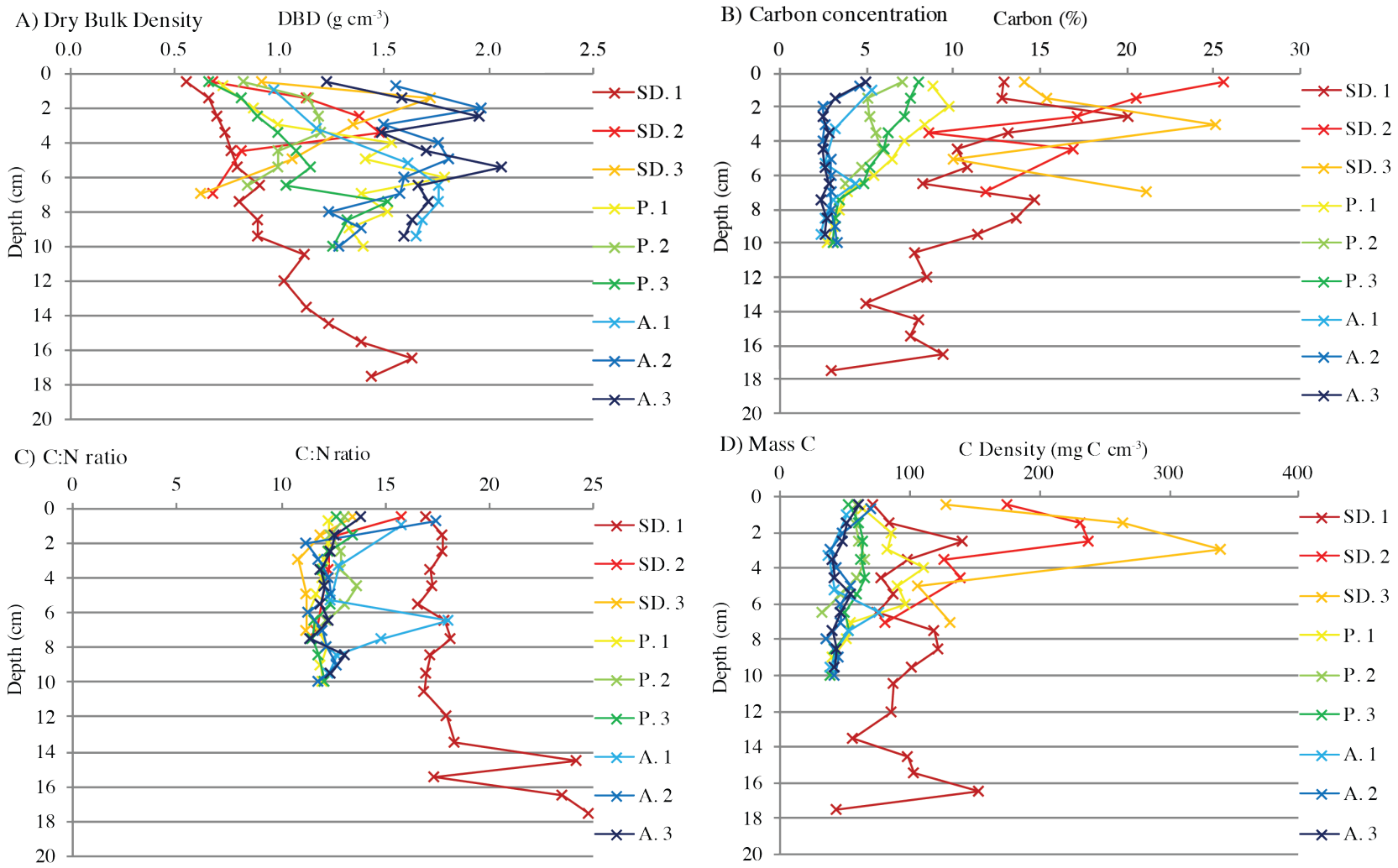
5.B.i.c Moisture content

In contrast to DBD, sediment moisture content (% moisture; Figure II.16) decreased with depth (F = 59.1, df = 2, 77, p = < 0.05), being significantly higher in the upper 5 cm (mean ± SD = 53 ± 16 %) than the 5-10 cm section (38 ± 13 % p = < 0.05), and > 10 cm section (30 ± 6 %, p = < 0.05). However, as with DBD, no statistical difference was observed in moisture content among ponds when grouped by Pond Type, Vegetation Type, Pond Permanence (Table II.5), or Land Use Type (p = > 0.05). However, sediment moisture content was significantly higher in ponds located in pasture fields (F = 7.76, df = 1, 21, p = < 0.05), and in arable fields when compared to the adjacent soils (F = 7.87, df = 1, 17, p = < 0.05), but no difference was observed between Dune Slack ponds, and their adjacent soil (p = > 0.05).

Table II.5: Primary statistical results from SPSS ANOVA conducted on measured variables as run by differing grouping methods. Pairwise comparisons show the difference among groups with those of statistical significance highlighted in yellow.

		Measured variable				
		DBD ($g\ cm^{-3}$)	**Moisture content (%)**	**C concentration (% C)**	**C:N ratio**	**C density ($mg\ C\ cm^{-3}$)**
Grouping methods	**Pond Type:** 1 = Classically Vegetated ponds 2 = Arable Field ponds 3 = Pasture Field ponds 4 = Dune Slack ponds	F = 0.518 df = 3, 47.927 p = 0.672 Pairwise = *p value* 1-2 = 1.000 1-3 = 1.000 1-4 = 1.000 2-3 = 1.000 2-4 = 1.000 3-4 = 1.000	F = 0.405 df = 3, 39.493 p = 0.750 Pairwise = *p value* 1-2 = 1.000 1-3 = 1.000 1-4 = 1.000 2-3 = 1.000 2-4 = 1.000 3-4 = 1.000	F = 2.828 df = 3, 38.170 p = 0.051 Pairwise = *p value* 1-2 = 1.000 1-3 = 1.000 1-4 = 0.639 2-3 = 0.950 **2-4 = 0.037** 3-4 = 0.955	F = 4.018 df = 3, 38.853 p = 0.014 Pairwise = *p value* **1-2 = 0.040** 1-3 = 0.108 1-4 = 1.000 2-3 = 1.000 2-4 = 0.142 3-4 = 0.348	F = 6.446 df = 3, 40.089 p = 0.001 Pairwise = *p value* 1-2 = 1.000 1-3 = 0.151 **1-4 = 0.005** 2-3 = 0.144 **2-4 = 0.004** 3-4 = 1.000
	Vegetation Type: 1 = Group 1.1 2 = Group 1.2 3 = Group 2.1 4 = Group 2.2	F = 0.998 df = 3, 47.890 p = 0.402 Pairwise = *p value* 1-2 = 1.000 1-3 = 1.000 1-4 = 1.000 2-3 = 1.000 2-4 = 0.706 3-4 = 1.000	F = 0.683 df = 3, 39.276 p = 0.598 Pairwise = *p value* 1-2 = 1.000 1-3 = 1.000 1-4 = 1.000 2-3 = 1.000 2-4 = 1.000 3-4 = 1.000	F = 2.626 df = 3, 38.729 p = 0.064 Pairwise = *p value* 1-2 = 1.000 1-3 = 1.000 1-4 = 1.000 2-3 = 0.727 2-4 = 0.056 3-4 = 1.000	F = 25.064 df = 3, 34.546 p = < 0.000 Pairwise = *p value* **1-2 = < 0.000** **1-3 = < 0.000** **1-4 = < 0.000** 2-3 = 1.000 2-4 = 0.672 3-4 = 1.000	F = 4.115 df = 3, 39.888 p = 0.012 Pairwise = *p value* 1-2 = 0.086 1-3 = 1.000 1-4 = 1.000 2-3 = 1.000 **2-4 = 0.024** 3-4 = 0.967
	Pond Permanence: 1 = Never dries 2 = Sometimes dries 3 = Always dries	F = 0.394 df = 2, 45.254 p = 0.677 Pairwise = *p value* 1-2 = 1.000 1-3 = 1.000 2-3 = 1.000	F = 0.098 df = 2, 37.482 p = 0.906 Pairwise = *p value* 1-2 = 1.000 1-3 = 1.000 2-3 = 1.000	F = 1.637 df = 2, 35.787 p = 0.209 Pairwise = *p value* 1-2 = 1.000 1-3 = 0.505 2-3 = 0.403	F = 1.324 df = 2, 37.312 p = 0.278 Pairwise = *p value* 1-2 = 0.933 1-3 = 1.000 2-3 = 0.364	F = 6.486 df = 2, 36.835 p = 0.004 Pairwise = *p value* 1-2 = 1.000 **1-3 = 0.047** **2-3 = 0.008**

Figure II.14: Soil dry bulk density, C concentration, C:N ratio, and C density from the different land use types across Druridge Bay.



SD =Sand Dunes; P = Pasture fields; A = Arable fields

Figure II.15: Dry bulk density of sediment cores, grouped by Pond Type.

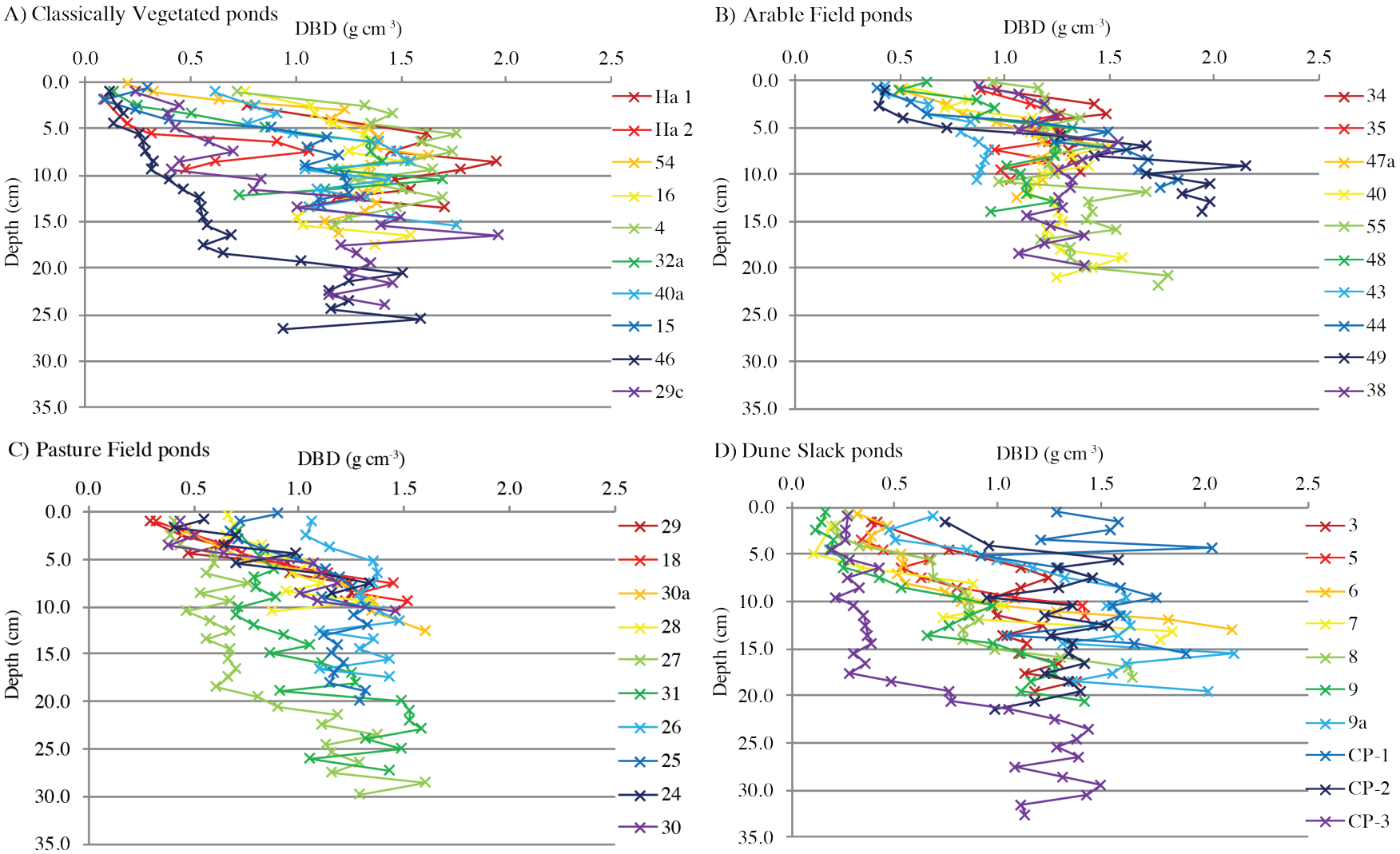
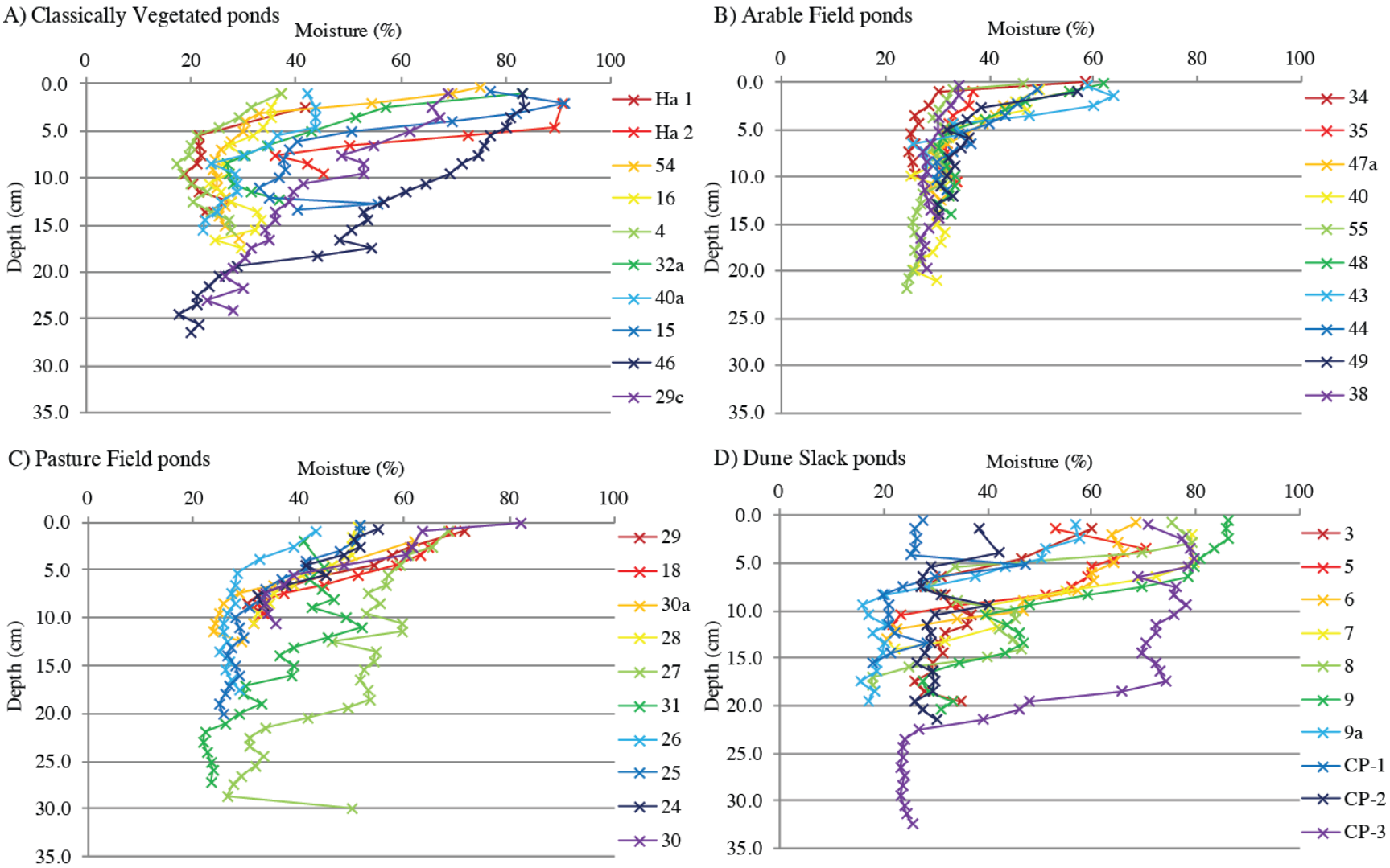


Figure II.16: Moisture content of sediment cores, grouped by Pond Type



5.B.ii. Sediment carbon and C:N ratios

5.B.ii.a Carbon concentration

The mean C concentration (% C) of all sediment samples was 5.6 % C (± SD = 5.9, range = 0.1-37.4), with considerable variation over depth, being responsible for 49 % of the overall sample variation observed, with the remaining 51 % attributed to variations among individual ponds.

Within cores (Figure II.17), % C was significantly higher (F = 6.261, df = 2, 80, p = < 0.05) in the upper most 5 cm layer (mean ± SD = 9.3 ± 7.8 %) than the 5-10 cm (5.6 ± 5.2 %, p = < 0.05), and > 10 cm sections (3.4 ± 1.9 %, p = < 0.05), yet the upper most layers also displayed the highest variation. Among Pond Types (F = 2.828, df = 3, 38, p = 0.051), Dune Slack ponds had the highest mean C concentration (9.1 ± 8.8 %) and were significantly higher than sediments from Arable Field ponds (2.9 ± 0.9 %, p = < 0.05), but not from Classically Vegetated ponds (4.4 ± 4.5 %, p = > 0.05) or Pasture Field ponds (5.6 ± 3.3 %, p = > 0.05).

No relationship was found between the % C within pond sediments when grouped by Pond Permanence (F = 1.637, df = 2, 35, p = > 0.05), nor when ponds were grouped by Vegetation Type (F = 2.626, df = 3, 38, p = > 0.05). Equally, no significant difference was found between % C within pond sediments and the % C values of their respective surrounding soils (F = 0.436, df = 1, 53, p = > 0.05; Figure II.14.B).

5.B.ii.b C:N ratio

The mean carbon to nitrogen (C:N) ratio for all sediments was 14.8 ± 6.3 (± SD, range = 4.0-55.1). When grouped by Pond Type (Table II.5, Figure II.18), a significant difference in C:N ratios was observed (F = 4.018, df = 3, 38, p = 0.014), yet only among sediments of Classically Vegetated ponds (16.2 ± 7.4) and Arable Field ponds (12.0 ± 1.0, p = 0.040). No significant difference was found among other Pond Type pairwise comparisons (p = > 0.05). However, when ponds were grouped by their Vegetation Type significant difference was observed (F = 25.064, df = 3, 35, p = < 0.000), with ponds in Group 1.1 (25.6 ± 11.4) having significantly higher C:N ratios than ponds in Group 1.2 (13.5 ± 1.9, p = < 0.000), Group 2.1 (13.7 ± 3.0, p = < 0.000), and ponds in Group 2.2 (12.0 ± 1.0, p = < 0.000). No significant difference in C:N ratio was observed between ponds when grouped by Pond Permanence (F = 1.324, df = 2, 37, p = > 0.05) nor when pond sediments were compared to the C:N ratio of the background soils (F = 0.380, df = 1, 52, p = > 0.05; Figure II.14.C). Equally, C:N ratios varied little over depth with no significant difference observed between sediment layers (F = 2.7, df = 2, 40, p = > 0.05).

Figure II.17: Carbon concentration of sediment cores, grouped by Pond Type.

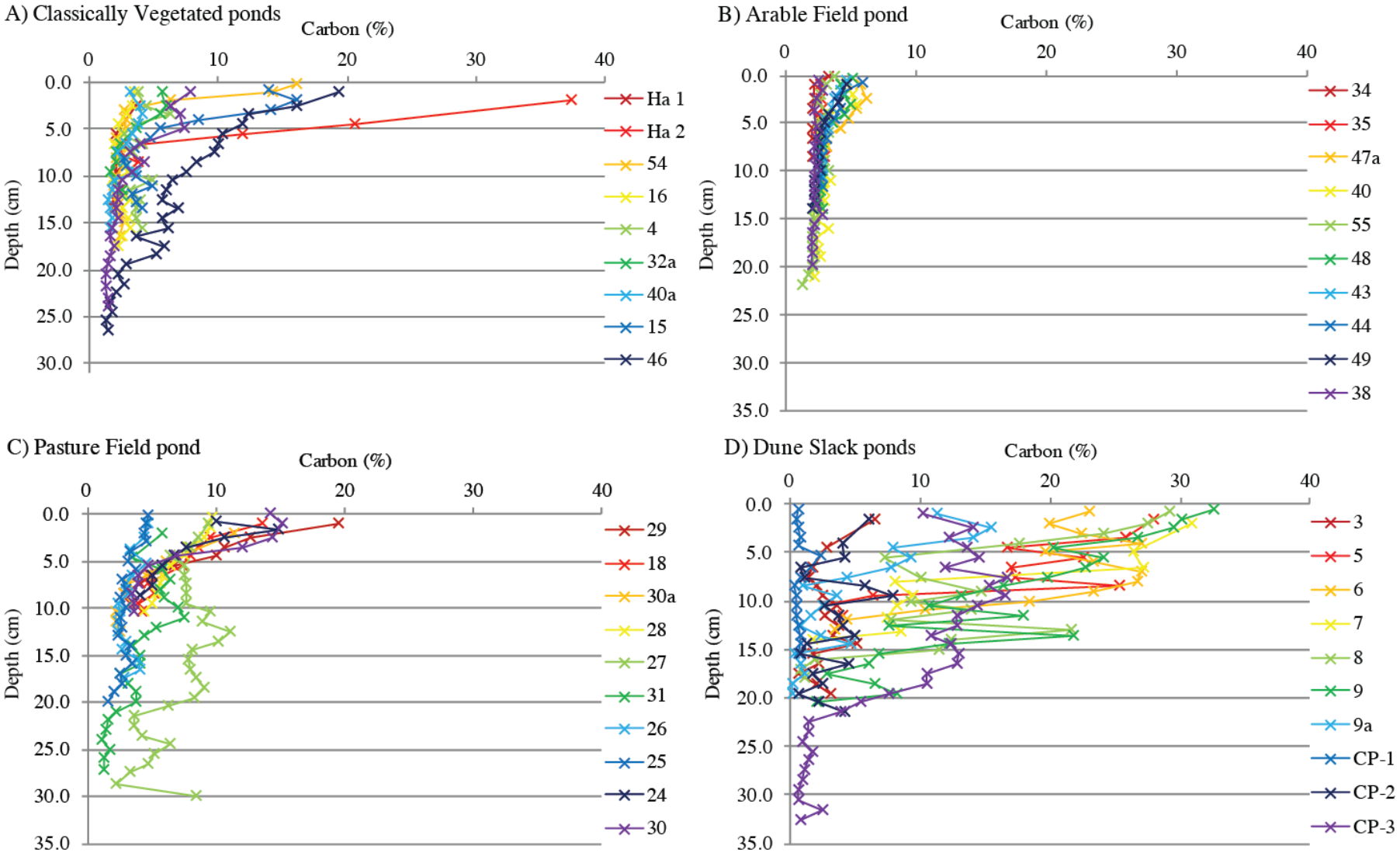
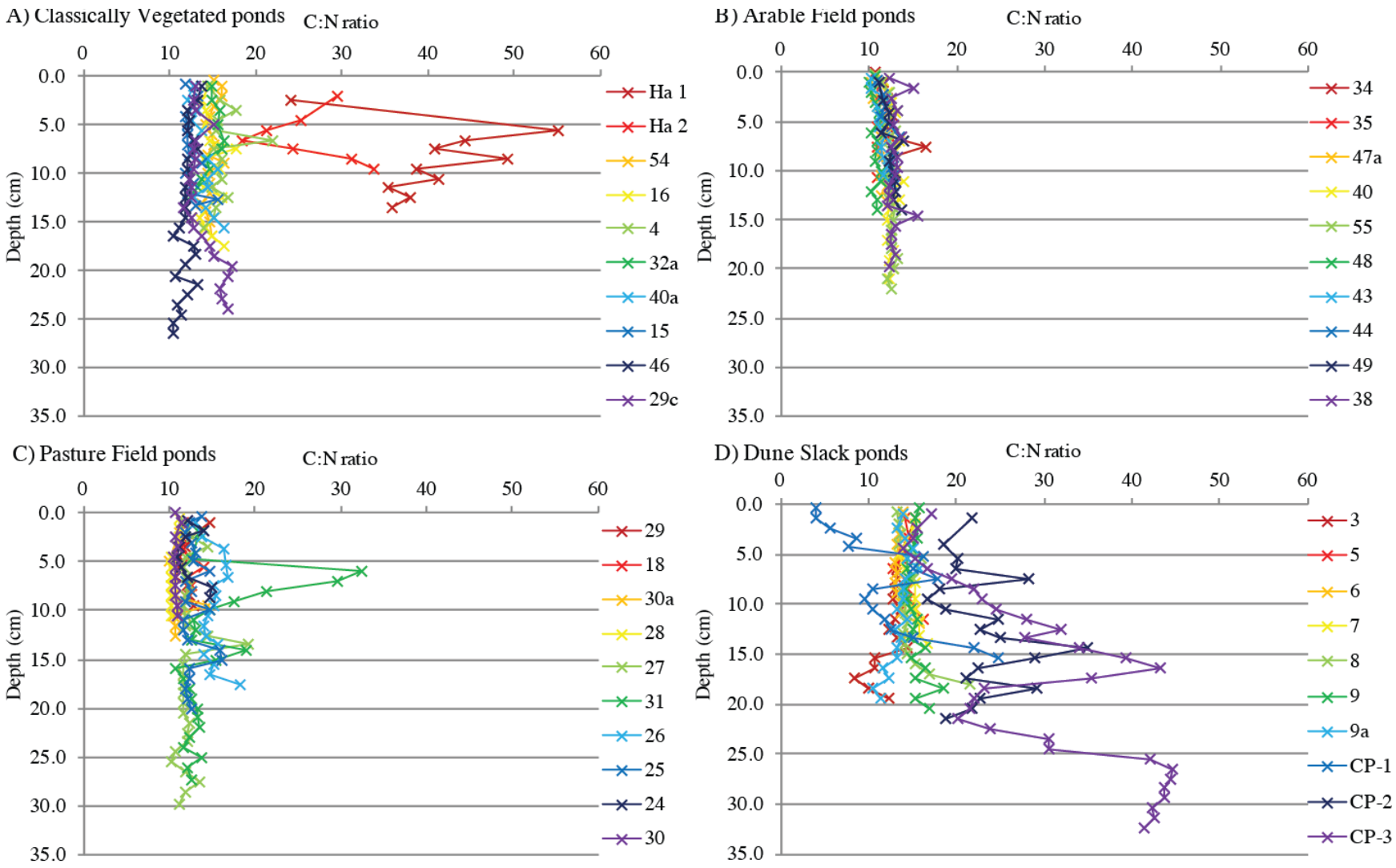


Figure II.18: C:N ratios of sediment cores, grouped by Pond Type.



5.B.ii.c Carbon density within individual sediment sections

The mean C density (mg C cm^{-3}) of all individual sediment sections was 41.2 ± 25.6 mg C cm^{-3} (± SD, range = 1.1-201.8 mg C cm^{-3}) with variations over depth (F = 6.26, df = 2, 80, p = < 0.05) being significantly higher in the 5-10 cm section (mean ± SD = 46.4 ± 25.9 mg C cm^{-3}) than the top 5 cm (40.4 ± 18.4 mg C cm^{-3}, p = < 0.05) and the > 10 cm section (39.8 ± 19.5 mg C cm^{-3}, p = < 0.05).

When grouped by Pond Type (Table II.5, Figure II.19) only the C densities in individual sediment sections from Dune Slack ponds (53.4 ± 40.9 mg C cm^{-3}) was found to be statistically different from other Pond Types (F = 6.446, df = 3, 40, p = 0.001), being significantly higher than those from Arable Field ponds (31.8 ± 7.6 mg C cm^{-3}, p = 0.004) and Classically Vegetated ponds (31.2 ± 11.7 mg C cm^{-3}, p = 0.005), with no difference observed among other Pond Type pairwise comparisons (All p values = > 0.05). Significant difference in C density was also observed among ponds when grouped by Vegetation Type (F = 4.115, df = 3, 40, p = 0.012), with Ponds in Group 1.2 being significantly higher than those in Group 2.2 (p = 0.024), though with no difference among other pairwise comparisons (All p = > 0.05). Note that although no significant difference was observed between Groups 1.1 and the elevated Group 1.2, the mean C density in Group 1.1 was the lowest of all groups; this lack of significant difference is a result of higher range of values.

A significant difference was observed between ponds when grouped by Pond Permanence (F = 6.486, df = 2, 37, p = 0.004) with C density being significantly higher in sediment sections from ponds that dry up on an annual basis (n = 20, mean ± SD = 51.8 ± 32.3 mg C cm^{-3}) compared to those that only occasionally dry (n = 13, 34.0 ± 14.9 mg C cm^{-3}, p = 0.008) and those that never completely dry out (n = 6, 32.0 ± 13.2 mg C cm^{-3}, p = 0.047). Equally, the C density of soil core samples (79.2 ± 53.6 mg C cm^{-3}; Figure II.14.D) were observed to be significantly higher than the C density within sections of sediment cores themselves (41.2 ± 25.6 mg C cm^{-3}; F = 16.39, df = 1, 47, p = < 0.05).

5.C. C Stocks

The overall mean C stock of sediments surveyed in this study was 4.36 kg C $m^{-2}_{< 10\ cm}$ (± SD = 2.21, range = 1.17-12.32 kg C $m^{-2}_{< 10\ cm}$). While graphically it appears that smaller ponds have higher sediment C stocks (Figure II.20.D), no statistical relationship was found between pond area and sediment C stock, though some variation was observed among different pond groupings.

Grouping results by Pond Type (Table II.5, Figure II.20.A) revealed a significant difference in sediment C stocks (F = 6.737, df = 3, 40, p = 0.001), with Dune slack ponds (mean ± SD = 6.18 ± 3.39 kg C $m^{-2}_{< 10\ cm}$, range = 1.17-12.32) being significantly higher than Classically Vegetated

Figure II.19: C density within sediment cores, grouped by Pond Type.

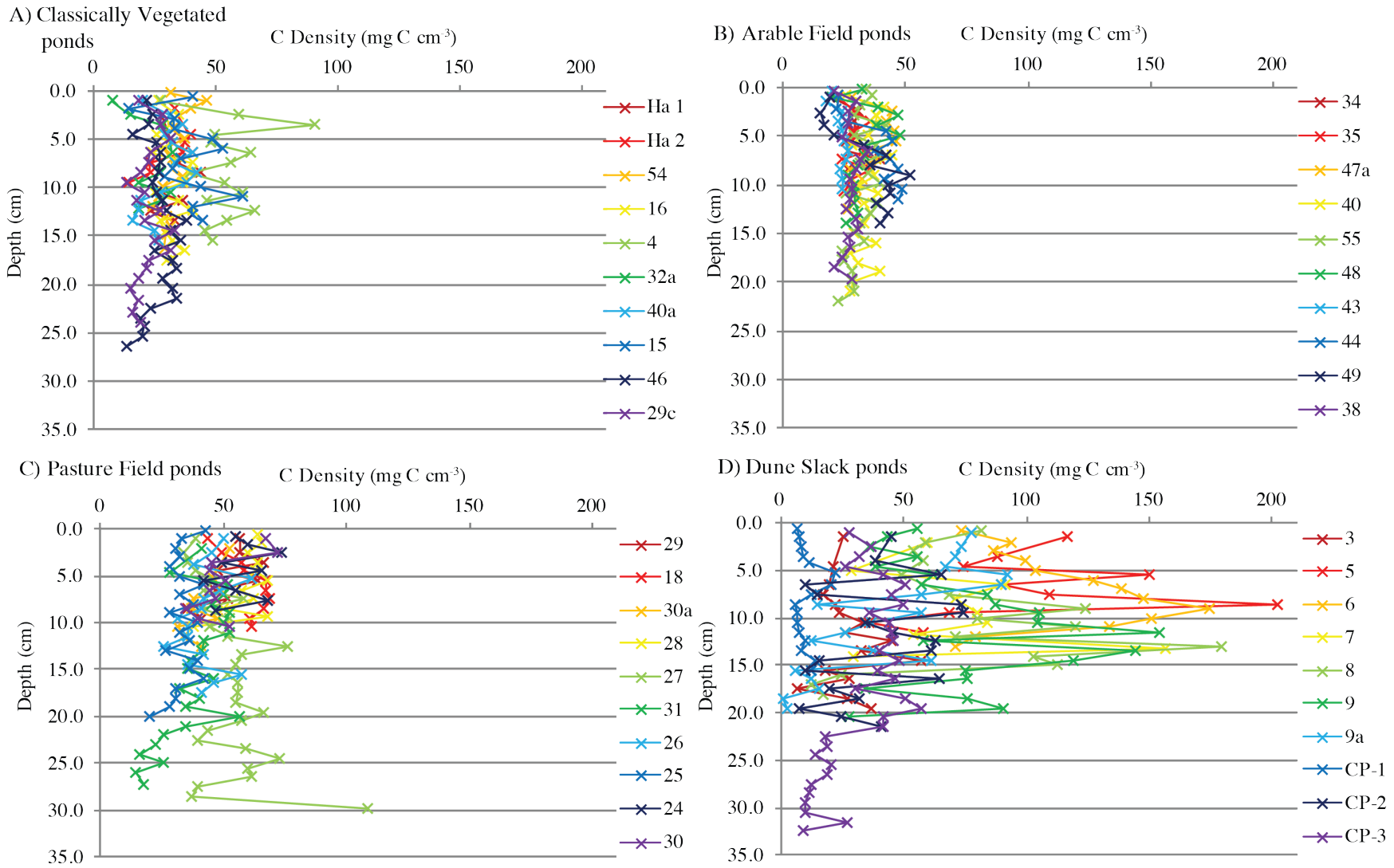
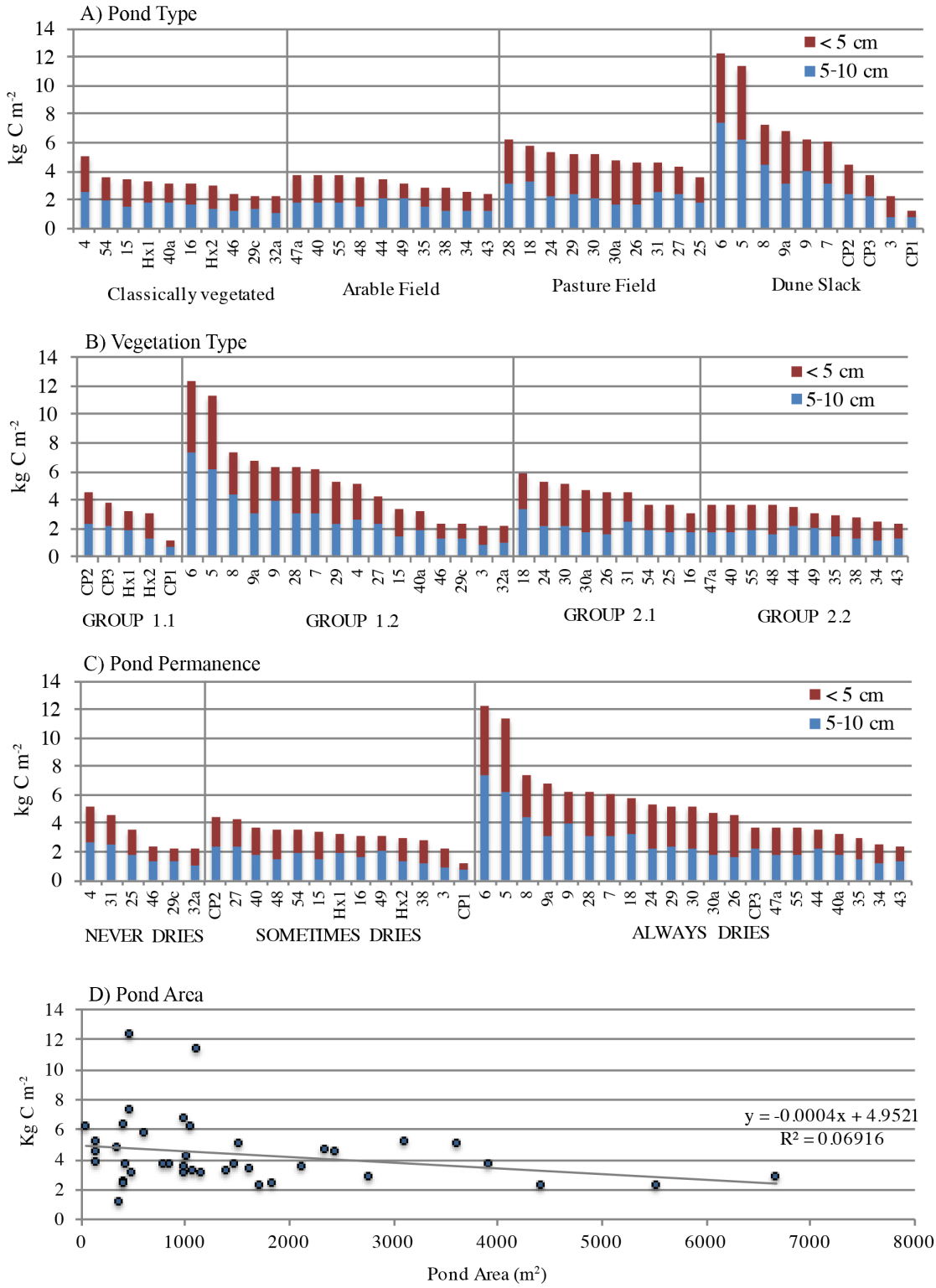


Figure II.20: Pond sediment C stocks [upper 10 cm] grouped by: (A) Pond Type; (B) Vegetation Type; (C) Pond Permanence; (D) Pond Area.



ponds (mean ± SD = 3.14 ± 0.80 kg C $m^{-2}_{< 10\ cm}$, range = 2.23-5.11, $p = 0.003$) and Arable Field ponds (3.17 ± 0.50 kg C $m^{-2}_{< 10\ cm}$, range = 2.35-3.69, $p = 0.003$), but not significantly different from Pasture Field Ponds (4.94 ± 0.73 kg C $m^{-2}_{< 10\ cm}$, range = 3.58-6.24, $p = 0.793$). No significant difference was observed in pairwise comparisons among Classically Vegetated, Arable, and Pasture field ponds (All $p = > 0.05$).

When grouped by Vegetation Type (Figure II.20.B) as defined by TWINSPAN (F = 3.397, df = 3, 40, $p = 0.027$), sediment C stocks of ponds in vegetation Group 1.2 (mean ± SD = 5.41 ± 3.06 kg C $m^{-2}_{< 10\ cm}$, range = 2.23-12.32) were found to be significantly higher than Group 2.2 (3.17 ± 0.52 kg C $m^{-2}_{< 10\ cm}$, range = 2.35-3.69, $p = 0.044$) but not significantly different from Group 1.1 (3.13 ± 1.24 kg C $m^{-2}_{< 10\ cm}$, range = 1.17-4.50) or Group 2.1 (4.49 ± 0.89 kg C $m^{-2}_{< 10\ cm}$, range = 3.12-5.82). No significant difference was observed in any other pairwise comparisons (All $p = > 0.05$).

When grouped by Pond Permanence (Figure II.20.C) significant difference was observed (F = 5.947, df = 3, 40, $p = 0.005$), with ponds that dry on an annual basis (mean ± SD = 5.36 ± 1.27 kg C $m^{-2}_{< 10\ cm}$, range = 2.35-12.32) being significantly higher than ponds that occasionally dry (3.20 ± 0.85 kg C $m^{-2}_{< 10\ cm}$, range = 1.17-4.50, $p = 0.009$), but not significantly different from those that never dry (3.35 ± 1.27 kg C $m^{-2}_{< 10\ cm}$, range = 2.23-5.11, $p = 0.091$).

6. Discussion

6.A. Within Pond C Distribution - a Study of Replicate Cores

While studies have documented variations in sediment C distribution within individual aquatic systems (Pittman et al., 2013), this is the first study to focus specifically on C stock distributions within small (< 10,000 m^2) natural ponds. Moreover, these systems are shallow, with three being temporary, and as such are more likely to be subject to uneven sediment deposition than deeper water bodies due to high levels of sediment disturbance, predominantly from agricultural activities (e.g., grazing cattle or farm machinery). This study provides a unique survey of the C distribution across individual ponds that is crucial for validating C stock estimates where numbers of sediment cores are limited.

6.A.i. Variations in C concentration and C density among replicate cores

Within individual ponds, both the C concentration (% C) and C density (mg C cm^{-3}), varied markedly at similar depths among replicate cores, often by > 100 %, especially in the upper sediment layers (Figure II.12). This suggests that C distribution does vary within an individual pond, however, no statistical difference was observed in % C among replicate cores from the same ponds. Significant difference was observed in C density among replicate cores, yet only among the sediment cores collected from the Pasture Field pond; no significant difference was observed among cores from the Arable, Classically Vegetated or Dune Slack ponds. It should be noted that whilst this lack of significant difference among replicate cores suggests that the levels of C across an individual pond are relatively evenly distributed, it may also be that the high level of variation over depth within individual cores is obscuring any differences in statistical analysis. This does not mean the statistical analysis chosen is inappropriate, simply the most appropriate model for such large datasets (i.e., the mixed model ANOVA with bonferroni post-hoc comparison) are more conservative, reducing the risk of making a Type I error: assuming a 'false positive'. The different concentrations of C among replicate cores, especially in the upper most layers of the Classically Vegetated and Pasture ponds, suggests that certain areas of a pond are better suited to higher levels of C preservation and storage.

Given the temporary nature of these ponds it is likely that sediment nearer the centre of a pond that stays submerged for longer periods may be subject to higher levels of sediment anoxia, favourable for C preservation, and subsequently higher C concentrations compared to pond peripheries where sediment is more frequently subject to oxic conditions during periods of low water level. Alternatively, certain locations within a pond may be localised focal points for sediment deposition, a trend often seen in impoundments with currents, where sediment C concentrations increase from inflow to outflow (Pittman et al., 2013; Shotbolt et al., 2005; Vanni et al., 2011). However, none of the ponds in this study have permanent inflow or outflow beyond surface runoff channels meaning

it is highly unlikely that currents are responsible for any variations observed. Localised areas of dense vegetation often form, with subsequent vegetation decay potentially leading to higher rates of organic matter deposition than areas of open water. However, in the absence of burial rates this theory remains speculative.

6.A.ii. Variations in calculated C stocks among replicate cores

Whilst C concentrations (% C) and C density (mg C cm^{-3}) were considerably variable in the upper layers, both within and among cores, there was comparably little variation in the calculated C stock (kg C $m^{-2}_{< 10\ cm}$) among replicate cores from individual ponds (Figure II.13). At the 95 % CI, the margins of error were all < 11 % of the mean, being 9.4, 6.1, 8.2 and 10.8 % (mean = 8.4 %) for the Classically Vegetated, Arable, Pasture, and Dune Slack ponds respectively. Furthermore, the high levels of precision (i.e., the low % RSDs) 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, sediment C distribution appears to be less variable.

While the mean % RSDs of sediment core replication were < 16 % for each of the four ponds it should be noted that the precision of sampling was best in the Arable Field pond and worst in the Dune Slack pond. This difference in precision of replicate cores among the four Ponds Types is similar to the broader trend in C stocks seen among ponds at the landscape level as noted in the regional survey (discussed further in Section 6.B), suggesting that overall C stocks are least variable in Arable Field ponds and highest in Dune Slack ponds. Interestingly, 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 relatively even irrespective of pond size. These factors combined give strong evidence to the accuracy of the C stock calculations and the ability to predict C stocks from singular core sampling strategy used in the regional survey.

6.A.iii. Outcomes

While this study on shallow temporary systems indicates that the calculated C stock (kg C $m^{-2}_{< 10\ cm}$) is relatively even regardless of the sediment core location within a pond, it is important to recognise its limitations. Given the large variability of C stocks among ponds in the regional study (Figure II.13) it would be inappropriate to assume that the four ponds in this survey are representative of their broad Pond Types (i.e., Classically Vegetated, Arable, Pasture, and Dune Slack ponds). Rather, as the range of calculated C stocks from this study of replicates (1.05-6.20 kg C $m^{-2}_{< 10\ cm}$) are broadly similar to the overall range observed from single core calculations in the regional survey (1.2-12.3 kg C $m^{-2}_{< 10\ cm}$; only two ponds > 7.3 kg C $m^{-2}_{< 10\ cm}$) it can be said that the four ponds represent the natural diversity of ponds across the landscape. As such, rather than using individual

margins of error for the four Pond Types when calculating C stocks from singular cores in our regional survey, it is more appropriate to apply the mean margin of error; i.e., 8.4 % at the 95 % CI. That is to say, when calculating C stocks from individual sediment cores we can assume, with 95 % confidence, that the estimated C stock is representative of the entire pond within 8.4 %.

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) and density (mg C cm^{-3}) may vary when compared among replicate cores from individual ponds, when calculating the actual C stock (kg C $m^{-2}_{< 10\ cm}$) the margin of error in estimations is comparably low, with C stock estimations from individual sediment cores being relatively representative of sediments across the pond.

6.B. Regional Survey of Pond Sediments and their C Stocks

This regional survey of sediment C stocks from forty ponds across the lowland agricultural landscape of Druridge Bay, Northumberland, provides one of the most comprehensive surveys to date of small, shallow, temporary and naturally occurring ponds. The relatively small size range (range = 49-6674 m^2) and temporary nature of ponds explored in this survey represent a habitat type largely understudied in inland waters, with the majority of studies focusing on water bodies > 10,000 m^2 (Downing, 2012). Despite the widespread occurrence of these habitats across our landscape and their high processing rates, they are rarely explored for their role in cycling of C and all but wholly missed from C budgets.

6.B.i. Overview of sediment C concentration and C stocks

The mean C concentrations (5.6 %) observed for all sediment samples is lower than those reported for large mesotrophic lakes (20 %, Brunskill et al., 1971; 7 %, Gorham et al., 1974; 12 %, Dean et al., 1993) impoundments (38 %, Pittman et al., 2013), and mangroves (8.5 %, Duarte et al., 2004). Yet they are higher than those reported for coastal salt marshes (5.4 %, Duarte et al., 2004), marine sediments (range = 0.7-1.5 %, Hedges & Keil, 1995) and aquaculture ponds (range = 1-7 %, Boyd et al., 2010; Adhikari et al., 2012).

The large range observed in this study among all sediment samples (0.1-37.4 % C) highlights the natural variation, both among systems and over depth within cores, with nearly equal variation in C concentrations observed among individual ponds, as between sediment layers over depth of individual cores. This is largely due to the C concentrations being significantly higher in the upper 5 cm than the 5-10 and > 10 cm sections as is typical of recently deposited sediments (Munsiri et al., 2003) due to decomposition and remobilisation of organic material over time in sediment layers of greater depth and age (Munsiri et al., 2005, Pitmann et al., 2013).

The mean C:N ratio of 14.8:1 is slightly higher than observed for larger impoundments (10:1, Pittman et al., 2013), and large lakes (9:1, Dean, 1993; 7:1, Gorham et al., 1974) suggesting greater organic matter inputs from rooted macrophyte communities in smaller ponds compared to phytoplankton dominated larger water bodies. The majority of sediments exhibited no significant change in C:N ratios over depth, suggesting a relatively consistent source of organic material inputs throughout the build up of sediment over a pond's lifetime. It should be noted however, that this lack of change in C:N ratio over depth may be a result of preferential degradation of algae based compounds over time, which typically have low C:N ratios, resulting in a seemingly consistent C:N ratio throughout the depth. Alternatively, it may be a result of mixing among layers due to secondary disturbance.

The mean calculated C stock (± SD = 4.36 ± 2.21 kg C $m^{-2}_{< 10\ cm}$, range = 1.17-12.32) is in the mid-range 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 CS, 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 recent development of these pond systems (roughly over the past 20-100 years) this represents a rapid accrual of C within pond sediments.

However, considerable variation was observed in the calculated C stocks (kg C $m^{-2}_{< 10\ cm}$) among individual ponds, suggesting that certain ponds are better suited to C storage than others. In this study pond groupings (e.g., Vegetation Type or Pond Permanence) were assessed to identify certain characteristics that may be beneficial to higher levels of C storage within pond sediments.

6.B.ii. Which type of pond is best suited to C storage?

While the grouping methods used in this study (i.e., Pond Type, Vegetation Type, Pond Permanence and Land Use Type) are surficial characterisations, being dependent on a visual assessment of their surface characteristics, strong evidence exists that these are powerful defining characteristics for predicting ecological variations (Jeffries, 2010, 2015). It is therefore likely that these characteristics will influence the accumulation, preservation, and subsequent C stocks of pond sediments. Quantifying differences among ponds of differing characteristics is crucial for furthering our understanding of C budgets as well as their inclusion in both regional and national scale C budgets.

6.B.ii.a Pond Area

Within this study there was marked variation in the C stocks (kg C $m^{-2}_{< 10\ cm}$) of sediments among ponds. A negative slope was observed between pond area and C stocks, with a higher range observed for ponds < 1500 m^2. However, no statistical significant difference was observed between pond area and C stocks, suggesting that at this small scale, pond area has little impact in determining C capture

or storage capability. This may in part be due to large variations in the ecological (i.e., Pond Type and Vegetation Type) and physiological (i.e., Pond Permanence) characteristics seen among the ponds sampled in this study. It is these variations that provide the most insight into which ponds are best suited to C storage.

6.B.ii.b Pond Type

When ponds were grouped by Pond Type, Dune Slack ponds had the highest mean C stock of all Pond Types, being significantly higher than Classically Vegetated and Arable Field ponds. This initial finding suggests that it is these Dune Slack ponds that are best suited to C storage. However, it should be noted that whilst 6 of the 7 highest pond C stock values recorded in this study were Dune Slack ponds, the lowest C stock of all ponds was also from a Sand Dune pond, indicating that these ecosystems have a large variability in their C storage. Equally, it should also be noted that the relatively limited area nationally means they are of relatively low importance compared to other pond systems when extrapolating their C capture potential to a regional scale; i.e., dune slack ponds are restricted to coastal regions whilst arable field ponds, pasture field ponds, and classically vegetated ponds occur regularly across agricultural landscapes nationwide, comprising a greater cumulative surface area nationally.

6.B.ii.c Vegetation Type

Grouping ponds by Vegetation Type as defined by TWINSPAN (see Table II.3) provides some further indication as to which ponds are best suited to C storage. Ponds in vegetation Group 1.2 had the highest mean C stock, being significantly higher than vegetation Group 2.2, suggesting it is ponds with vegetation dominated by the grass, *Agrostis stolonifera*, that are best suited to C storage. Yet, this group also showed a large variation, having 4 of the 5 lowest C stock values of all ponds, meaning that Vegetation Type alone does not provide a clear indication of pond C stock. Equally it should also be noted that it was Group 1.1 that had the lowest mean C stock, despite the lack of statistical difference with Group 1.2; likely due to the higher range of values. This suggest that ponds dominated by common reed, *Phragmites australis*, or yellow iris, *Iris pseudacorus,* are likely to have lower C stocks.

Furthermore, ponds in vegetation Group 1.1 with the lowest mean C stock were comprised solely of ponds from the Hauxley Nature Reserve and Country Park sand dunes, and no ponds from the Blakemoor site (see Figure II.20.B). Equally 6 of the top 7 C stock values recorded were from a series of Dune Slack ponds at Blakemore Farm, all within close vicinity to one another, without which the range of values in Group 1.2 would be comparable to the three other vegetation groups. From this it appears that there is some localisation of C stocks among ponds, or rather, that localised environmental factors can result in neighbouring ponds being broadly similar in their physiological characteristics, their resulting ecological appearance, and, it appears, their subsequent C stock. All 6

of the ponds with the highest C stocks (ponds 5, 6, 7, 8, 9 and 9a) are located within the dune slacks at Druridge Bay and have been observed to have high conductivity during routine field monitoring conducted by Dr Jeffries, Northumbria University. It may be that this localised factor is resulting in elevated C stocks, either through the growth of plant communities best suited to these conditions (i.e., vegetation Group 1.2), or through slower degradation rates or organic matter as a result of the increased salinity.

6.B.ii.d Pond permanence

Pond Permanence plays a strong role in influencing the geochemical processes within sediments, as typically slower rates of organic matter degradation are observed in anoxic sediments. However, this is not reflected in the C stock values found within this study. Sediments that were annually exposed to drying were found to contain significantly higher C stocks than sediments that remained submerged or only sometimes dried. This is counter intuitive as it would be assumed that those ponds which remain permanently aquatic and anoxic would have the highest levels of C preservation and burial rates. This highlights one of the major difficulties in interpreting C storage capability within temporary ponds; the influence of % C and DBD in the calculation of C density and overall C stocks.

6.B.iii. Importance of DBD and % C

High sediment C concentrations (% C) typically represent systems with large inputs of organic matter and high levels of C preservation; i.e., net C GPP occurs at a higher rate than the ecosystem C remobilisation and respiration rates. As C remobilisation is slowest in anoxic sediments, % C is typically correlated with sediment moisture content, and decreases over depth with sediment age. Yet whilst high sediment C concentrations represent systems favourable for C preservation and storage, this does not always translate to a high C density (mg C cm^{-3}) within sediment layers, and cumulative C stocks (kg C $m^{-2}_{< 10\ cm}$).

Mass of C is calculated by multiplying dry bulk density (DBD) by % C of the individual sediment layers, and as such DBD is equally important in determining sediment C stocks. The low DBD of upper sediment layers, and its increase over depth typically contrasts C concentrations, which are higher in the upper layers and decrease over depth, resulting in the two opposing trends over depth roughly cancelling each other out. Subsequently little variation in C density is seen over depth (Figure II.19) which in turn is used to calculate C stock. This highlights the importance of calculating C density, rather than solely % C, which may lead to misinterpretation of the effectiveness of C storage capability among ponds.

DBD is also negatively correlated with sediment moisture content, the two of which are both highly influenced by pond permanence. Permanently saturated sediments typically have low DBD, and high moisture content and % C, whilst temporary ponds that dry typically have lower moisture contents

and % C, and higher DBD. This, however, was not the case within this study. When grouped by Pond Permanence, % C was highest, and DBD lowest, in ponds that dried on an annual basis. Yet similarly to when ponds were grouped by Vegetation Type, this is due to the presence of the 6 high C stock Dune Slack ponds (5, 6, 7, 8, 9 and 9a) within this group. Without these 6 ponds, little variation was observed between ponds of differing pond permanence.

These differences in % C and DBD highlight the importance of calculating the C density and C stocks for assessment of sediment C storage capability rather than relying solely on % C. However, complication arises when trying to quantify the absolute C stock of sediment alone.

6.B.iv. Establishing true sediment volume and differentiating from underlying soil.

The majority of naturally occurring ponds, certainly in this study, have formed in depressions in the landscape, as apposed to being constructed with a non-permeable base (e.g., concrete based ponds in aquaculture). As such, sediment deposition in naturally occurring ponds overlays the original base soil that predates the ponds arrival. This creates a problem when trying to calculate true sediment volume and C stocks: differentiating within a sediment core between accumulated sediment base and underlying soil.

Initially, the approach for coring of sediments adopted in this study allowed for clear visual delamination between base soil and substrate: trial coring of sediments from Classically Vegetated and Dune Slack ponds showed a clear visual difference between the dark, organic matter rich, anoxic accumulated sediment layer, and the dense, clay based underlying substrate. But as more pond sediments were cored it became apparent that this clear layering is not always the case, especially for ponds in arable and pasture fields. A further complication for this study is the relatively high levels of sediment disturbance that can occur in shallow aquatic systems. Shallow ponds are often subject to bioturbation from wildlife, where they make ideal fishing spots for wading birds or small aquatic mammals (e.g., otters), or as watering holes for larger mammals (e.g., cattle or deer). Furthermore, high levels of sediment mixing are exacerbated in wetlands situated in or amongst agricultural land. Where ponds are situated in pasture fields, livestock are often free to enter pond boundaries creating a high level of sediment disturbance; this was often the case with pasture ponds on Blakemoor Farm in this study. Temporary ponds in arable fields are subject to mechanical disturbance from farm vehicles during their summer dry phase, where they are often ploughed and seeded along with the rest of the field. The result is a lack of clear sediment lamination in many shallow aquatic systems and frequently no clear boundary between accumulated sediment base and underlying soil.

It may be perceived that sediment and soil properties would be considerably different. However, in this study comparison between the C density (mg C cm^{-3}) of pond sediments to that of the surrounding soil revealed that for each Land Use Type (i.e., Pasture, Arable, and Sand Dune) the C density within pond sediments was significantly lower than that of the surrounding soil. Equally, no significant difference was observed in % C, meaning that these two factors alone can not be used to differentiate between sediment and soils. In this study, attempts were made to use XRF to differentiate between the geochemical composition of sediment and soil: this was not run on all samples, simply on sediment layers from one core as an exploratory trial and as a proof of concept that this technique may be used in future studies. However, this proved unsuccessful with no clear difference observed throughout the core, likely due to high levels of sediment mixing. Consequently, it is not possible to differentiate between accumulated sediment base and underlying soil: a result of this is that absolute sediment volume, and subsequently absolute C stock, can not be accurately quantified. Therefore, in this study when C stocks were quantified a conservative depth of the upper 10 cm was used.

6.B.v. Sediment accumulation and C burial rates

Comparing C stocks between aquatic systems and terrestrial systems (e.g., between pond sediments and soils) is often inappropriate as their C processing systems and rates are very different. Consequently, C burial rates are often the preferred value for comparing the C capture potential of systems and their potential effectiveness in climate change mitigation. While C stocks provide amounts of C stored within a system, C burial rates quantify the amount of C accumulated by the system over a given period, typically on an annual basis, calculated from sediment accumulation rates. Just as identifying the boundary between background soil and accumulated sediment is crucial for accurately quantifying C stocks, establishing sediment accumulation rates is crucial for accurately quantifying C burial rates.

Sediment accumulation rates can be measured using several techniques however the majority are unsuitable for use in shallow pond systems. Sediment traps, which collect deposited sediment and is measured after a given period, are often unsuitable as the high levels of bioturbation in small ponds means re-suspended matter is also collected, resulting in an over estimate of sediment accumulation. Equally due to the young nature of the sediments in this study (< 20-100 years old; relative to lake sediments being > 100-10,000 years old) many geochemical proxy techniques, such as radioisotope dating, are inappropriate as they are often only traceable over extensive geological timespans. Many studies of larger water bodies use bathymetric measurements to measure the physical sediment volume accumulation: a process based on calculating loss of water volume from a known depth based on digital mapping of the sediment by sonar (Downing et al., 2008). However, due to the

shallow and temporary nature of the ponds in this study, and their extensive vegetation coverage, bathymetric measurements would be virtually impossible to obtain.

Given the unsuitability of these techniques, the most appropriate method for calculating sediment accumulation rates in small ponds is to establish a *net accumulation* since the ponds construction calculated by dividing total sediment accumulation by the pond's age. While this undoubtedly obscures variations in sediment accumulation rates during different successional stages of a pond's life cycle, it provides a relatively accurate estimate of overall sediment burial rates. However, in this study, due to the lack of definable accumulated sediment base layer and underlying soil boundary it is difficult to calculate the net accumulation of sediment since the ponds origin. Equally, an accurate date of origin is required for this assessment to be made. For constructed ponds this is usually known, however, for the natural ponds in this study a date of origin is difficult to determine. Maps and aerial images can provide some insight into this, however they frequently miss the smallest and temporary features of the landscape. Effort was made in this study to obtain historical aerial images from WWII monitoring flights (roughly the suspected age of many of the ponds) however these proved unobtainable within the time-constraints of this thesis.

7. Conclusions

This study comprises one of the most comprehensive surveys of pond sediments to date (> 600 individual sediment samples analysed from 40 ponds), unique in that it focuses specifically on the smallest, shallow, and temporary aquatic habitats across a lowland agricultural landscape.

In the study of the C distribution within the sediments of individual ponds C concentrations (% C) and the C density (mg C cm^{-3}) were considerably variable in the upper layers, both within and among cores. Yet there was comparably little variation in the calculated C stock (kg C $m^{-2}_{< 10\ cm}$) among replicate cores from individual ponds with no significant difference observed. This suggests an even distribution of C across pond sediments meaning that calculated C stocks from singular sediment cores are relatively representative of the whole pond. Equally no relationship was observed between sampling density and the precision of sampling. Combined these factors suggest that a high level of accuracy can be gained from estimating pond sediment C stocks from singular sediment cores for ponds < 10 000 m^2.

Calculated C stocks for the 40 ponds in the regional survey (mean ± SD = 4.36 ± 2.21 kg C $m^{-2}_{< 10\ cm}$, range = 1.17-12.32) were in the mid range of those reported for ecosystems across the UK (CS, 2007), yet considerable variation was observed among individual ponds suggesting that certain ponds are better suited to C accumulation and storage than others. When comparing different grouping methods, it was revealed that C stocks were highest in the saline Dune Slack ponds, specifically those densely vegetated and characterised by *Agrostis stolonifera*, and that dry on an annual basis, suggesting it is these habitats that are best suited for high levels of C storage.

However, there is complication in determining total sediment C stocks as difficulty lies in differentiating between accumulated sediment base, and the underlying soil, further compounded by the lack of difference observed between sediment cores and soil cores. Equally, in the absence of known pond age it is not possible to calculate sediment accumulation and subsequent C burial rates, meaning that comparisons with other systems based solely on C stocks, providing limited information as to the true C capture capability of the systems. Whilst the aim of this study was to quantify C stocks from ponds across the landscape, it is crucial that future efforts are focused upon quantifying absolute C stocks and sediment burial rates within small natural ponds, so that their C burial rates can be accurately quantified, and only then can any potential importance in the C cycle and climate change mitigation and policy be determined.

Chapter III. Comparative Survey of Pond Sediments from Three Broadly Different Ecological Systems

1. Introduction

Chapter II explored variations in sediment C distribution within individual ponds as well as amongst groups of ponds across the lowland agricultural landscape of Druridge Bay, Northumberland. Sediment C stocks were broadly similar among replicate cores within individual ponds simplifying extrapolations from individual sediment cores, yet significant differences were observed in the amounts of C stored among different groups of ponds across the landscape. Whilst the focus was among ponds of differing hydrological and ecological classification, the ponds as a whole were all broadly similar, in that they were all situated within a lowland agricultural landscape in a small area of North East England, and as such did not account for the broad biogeographical variations that occur across England as a whole.

Despite its relatively small size, the island nature of Great Britain results in considerable climatic variation among different regions, which in turn results in distinct ecological differences, with ponds having their own representative flora and fauna. As stated in a review of UK carbon storage by habitat, "differentiation…between England's distinct wetland types and their individual contribution to the UKs carbon balance" is a key knowledge gap underpinning their full inclusion in C budgets (Alonso et al., 2012). In an attempt to elucidate the biogeographical variations in C stocks among ponds across England, this chapter focuses on three sets of ponds from climatically and biogeographically distinct regions.

2. Aims & Objectives

Different regions of the UK have different climatic influences which subsequently impact the ecological variations in pond types. Given the variation in C stocks seen in Northumberland alone within Chapter II, it is hypothesised that there will be considerable variation among different biogeographical regions of England. The aim of this chapter is to quantify C stocks from a variety of biogeographical and biodiverse pond habitat types across England.

Specific objectives are to:

1) Quantify the amount of C stored within the sediments of ponds from a set of geographically, climatically, and biodiverse systems at a national level.
2) Elucidate any variations in C stocks observed among different ponds at a national scale.
3) Provide preliminary estimates of C stocks within pond sediments nationally.

3. Site Descriptions

This chapter focuses on ponds from three biogeographically distinct regions across England; the lowland heathland of the Lizard Peninsula, Cornwall; lowland peat bog ponds at Askham Bogs, Yorkshire; and postglacial pingo ponds at Thompson Common, on the Breakland of Norfolk (Figure III.1).

These three regions were chosen as they have relatively different climates (at least for the UK) due to their different exposure to influencing weather systems. Cornwall, being in the South West of England is heavily influenced by tropical maritime air from the south creating a climate that is not too dissimilar from that of the Mediterranean (Bilton et al., 2009). Norfolk, being in the South East receives a greater influence from air masses flowing directly off mainland continental Europe, originating from the Saharan sub-tropical high pressure area (Hallett et al., 2004). Yorkshire and Northumbria being further north receive less direct influence from the southerly weather systems that keep Cornwall and Norfolk warm. Instead they tend to receive greater influence from Arctic

---

Figure III.1: Map of England showing sampling locations at: (A) Lizard Peninsula, Cornwall; (B) Askham bog, Yorkshire; (C) Thompson Common, Norfolk; see Figures III.4, 6, and 8 for detailed site maps; (D) Druridge Bay, Northumberland, shows the sampling location of Chapter II.

maritime northerlies, or Polar continental easterly weather systems (Fowler and Kilsby, 2002; George et al., 2004).

The result is that these three regions have relatively different climates and weather patterns (Figures III.2-3). Mean summer temperatures in Cornwall (~ 14-16 °C) are typically lower than Norfolk (16-17 °C), yet milder winters on average sustain mean temperatures > 6 °C, compared to 3-5 °C in Norfolk. Yorkshire on average has cooler summers and winters than both Cornwall and Norfolk. Mean summer rainfall between the three locations is usually similar (~ 200-250 mm) however mean winter rainfall in Cornwall (~ 300-500 mm) is on average greater than that of Norfolk and Yorkshire (both on average ~ 150-250 mm). For comparison, Druridge Bay, Northumberland typically has: cooler summers (14-15 °C) than the other regions; similar winter temperatures to Yorkshire; summer and winter rainfall similar to Yorkshire and Norfolk.

Along with site specific geomorphological characteristics, these subtle differences in climates have resulted in three distinct pond-scapes. Warm temperatures and shallow soils on the Lizard Peninsula, Cornwall, have created an array of temporary ponds, with flora and fauna biological classifications similar to ponds from the Mediterranean (Bilton et al., 2009; Hopkins, 1978). Ponds on Thompson Common, Norfolk, are remnants of post-glacial retreat and permafrost melt and are long standing, mostly permanent features of the landscape (Clay, 2015). Askham Bogs, Yorkshire, is a site of ancient fenland that has been used by local communities for peat extractions since Roman times resulting in a multitude of peat-extraction ponds (Hogg et al., 1995).

3.A. Cornwall - Lizard Peninsula Temporary Mediterranean Ponds

The Lizard Peninsula (lat: 49.966922, long: -5.200686; Figure III.4-5) is the most southerly point of British mainland and is an Area of Outstanding Natural Beauty (AONB), designated Character Area by Natural England, and holds several SSSIs for both its flora and fauna, as well as its geology. The region is typically warm yet wet, receiving milder winters than the other study regions, yet higher annual rainfall. The peninsula comprises three main geological units (serpentinites, 'ocean complex', and metamorphic basement; BGS 1975) which form the best preserved exposed ophiolite in the UK: all ponds surveyed on the Lizard Peninsula in this study were located on a region with base geology of serpentine. The unique geology of the area results in relatively shallow soils and a landscape of slow-draining upland heath and lowland unimproved grassland. Combined with high annual precipitation rates and warm climate the Lizard Peninsula is densely populated with aquatic systems, the vast majority of which are temporary.

Many of the larger pond systems on the Lizard peninsula appear to be constructed, believed to be dug for watering cattle, whilst some sites are the result of small-scale quarrying for serpentine,

Figure III.2: Mean summer and winter temperatures of the UK. Taken from Met Office (2016). See Figure III.1 for regional sampling sites.

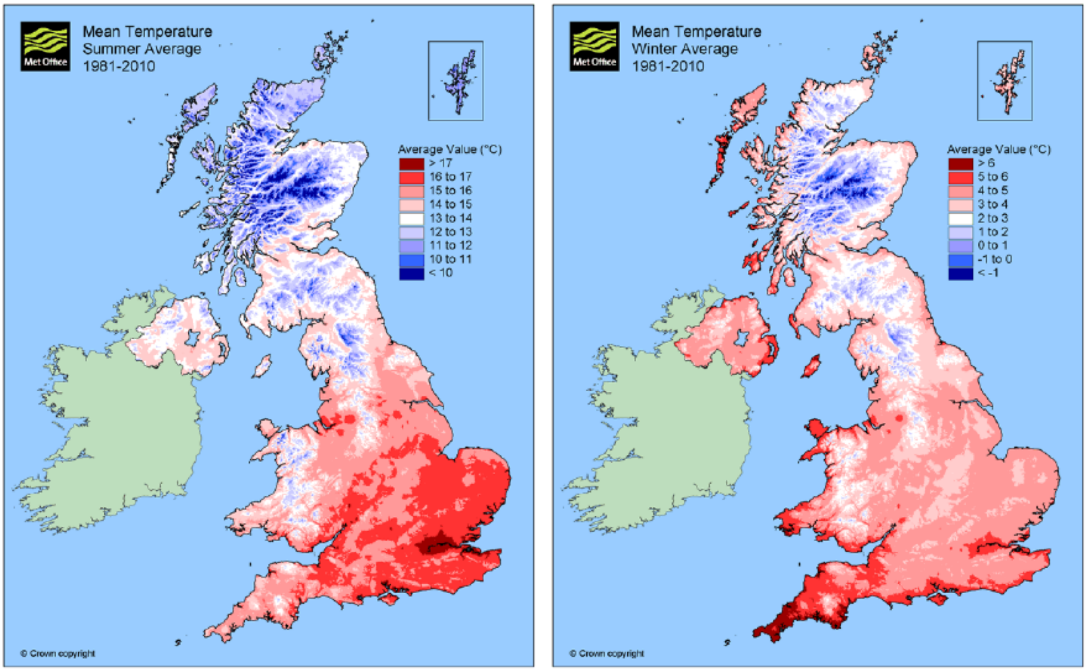


Figure III.3. Mean summer and winter rainfall across the UK. Taken from Met Office (2016). See Figure III.1 for regional sampling sites.

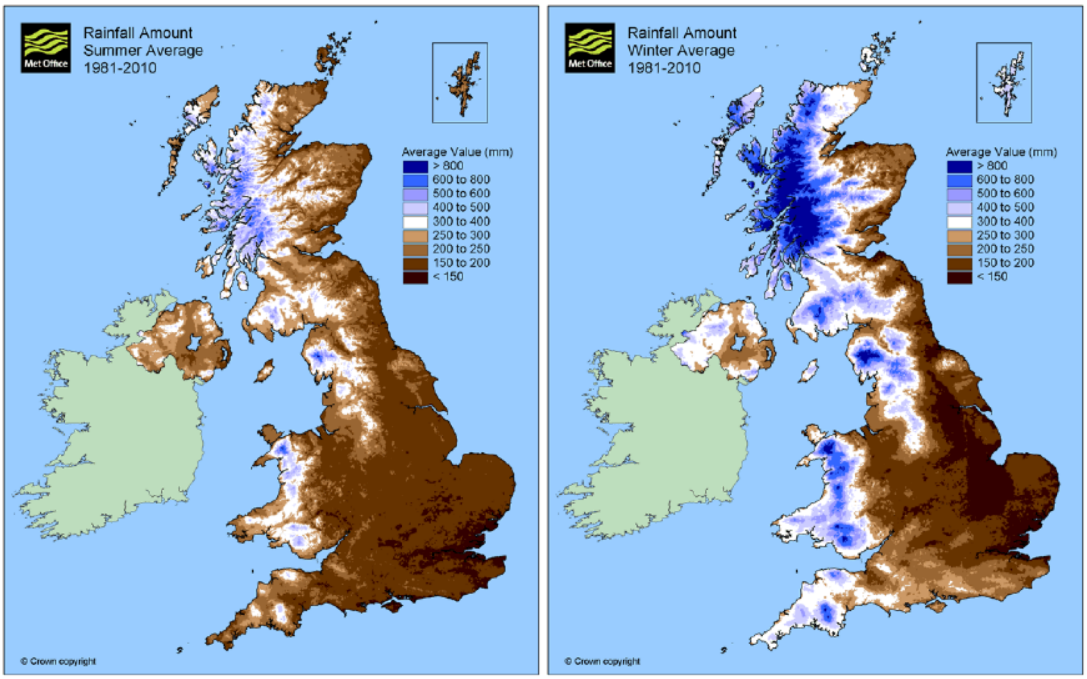
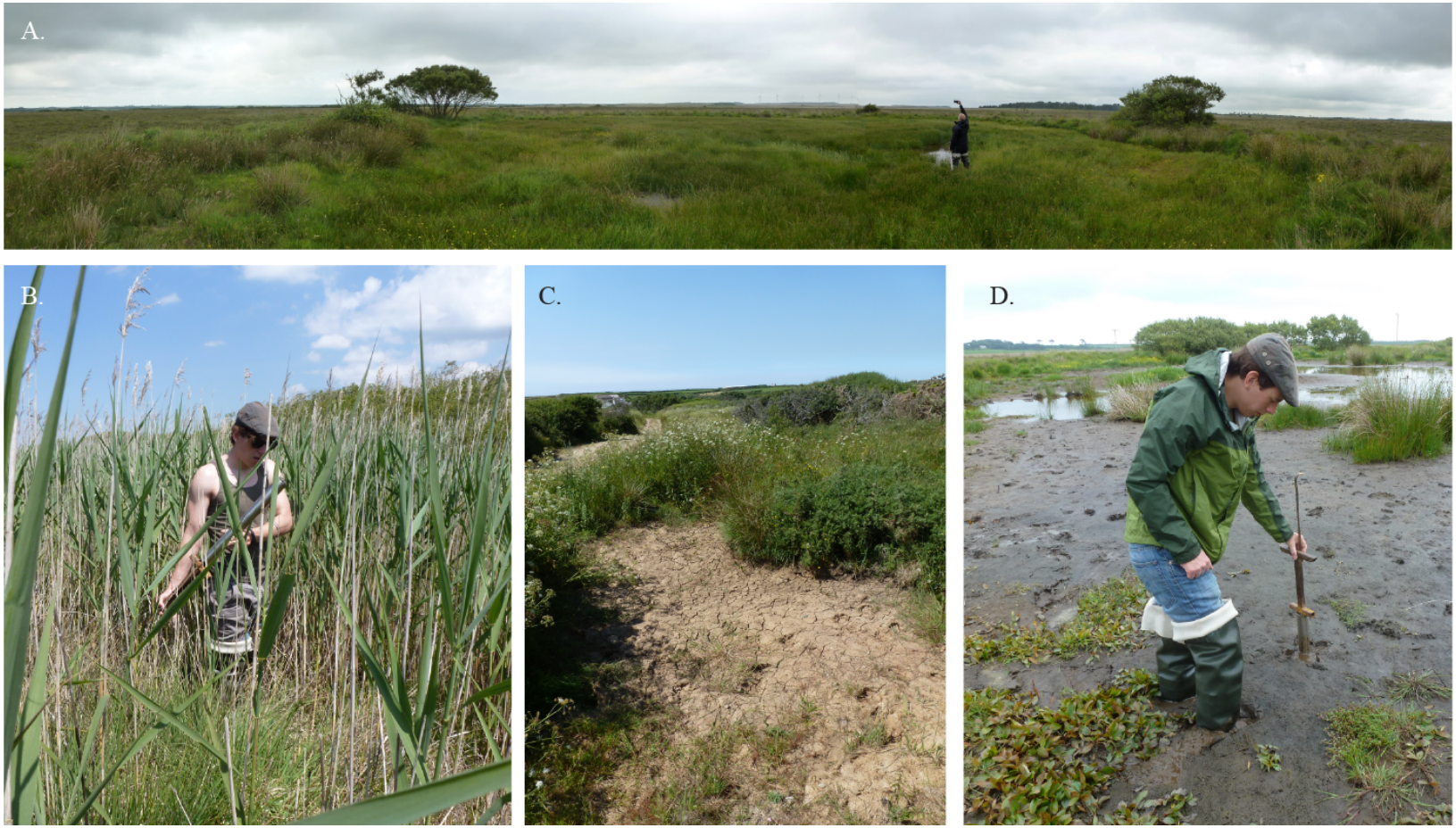


Figure III.4: Aerial images of Cornwall showing (A) Lizard Peninsula, Cornwall, and specific locations: (1) agricultural landscape and (2) heathland.

Figure III.5: Images of ponds on the Lizard Peninsula, Cornwall. (A) Example of a pond (Pond 4) located in amongst heathland; (B) heavily vegetated lowland pond (Pond 1); (C) example of a dried up temporary pond adjacent to ancient cartway; (D) example of the exposed sediment of a heathland pond (Pond 3).



gabbro and schist (Bilton et al., 2009). A network of small (~ 1-3 m^2) temporary ponds populates the heathland and while some originate from peat excavation the majority are hollows left from the construction of small mounds of earth, erected to prevent the landing of enemy gliders during the early 20th C. In addition, temporary pools are abundant along the wet track-ways, gateways and adjacent hedgerows, which house populations of rare plant taxa, the ecological importance of which was first noted in the late 1970s (Hopkins, 1978, 1983). Within this community of wetlands are supported two Habitats Directive Annex I Aquatic Habitats: European Commission (2003); 3140 'Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp.' and 3170 'Mediterranean temporary ponds'. These habitats support more nationally rare flora and fauna taxa than ponds in national surveys, resulting in high conservation value (Bilton et al., 2009).

Initial consultation with reserve managers from Natural England was held to highlight areas with high densities of water bodies and to identify the key broad wetland types in the region. Sediment cores were collected from 5 ponds encompassing both ponds of the heathland (n = 4; Figure III.4.2) and the unimproved grassland (n = 1; Figure III.4.1; See Figure III.5 for pond images). It should be noted that sample collection was conducted in late June 2014; a particularly warm summer with little precipitation resulting in the drying of most ponds. Whilst locations of ponds were identified on maps by reserve managers from Natural England, finding temporary ponds in upland heath without a GPS is somewhat difficult. Equally, due to the desiccation and hardening of sediment in most temporary systems coring was overly challenging. As such it should be noted that the ponds sampled represent the more permanent features of the Lizard Peninsula that hold water for longer than the very temporary features.

3.B. North Yorkshire - Askham Bog Peat Excavation Ponds

Askham Bog (lat: 53.925640, long: -1.124280; Figure III.6-7) is a 43 hectare stretch of ancient fenland, on the southern outskirts of York. The region is typically cool and wet with lower temperatures all year round compared to the other study regions. The base geology of the site is sandstone overlain by glacial clays with a superficial geology of peat (BGS, 2008). Located on the site of a now dried-up 15,000 year old post-glacial lake, drainage from an adjacent terminal moraine has kept the water table relatively high, resulting in the formation of peat bogs across the site. Local communities have utilised this resource since Roman times, with peat excavation leaving a multitude of hollows that have subsequently become ponds. In 1946 the site was purchased by Francis Terry and Arnold Rowntree, sweet manufacturers, who gifted to the Yorkshire Naturalists' (now Wildlife) Trust and has been a site for nature conservation since. The site has been the focus of ecological (Hogg et al., 1995) and historical (Bradshaw et al., 1981) studies. Sediment coring was conducted in February 2015, coupled with an initial survey, guided by a Yorkshire Wildlife Trust

Figure III.6: Images of ponds at Askham Bog, North Yorkshire: (A) & (B) peat excavation ponds situated in amongst the open meadow land (Ponds 2 & 1 respectively); (C) classic wetland mosaic that also occupy the reserve (Pond 4).

Figure III.7: Aerial image of Askham Bog, Yorkshire, and specific pond locations.

member of staff, in order to identify the most suitable ponds for sampling. Sediment cores were collected from 5 ponds, encompassing both the historical peat excavation ponds (n = 3) and the larger wetland mosaic of the site (n = 2).

3.C. Norfolk - Thompson Common Pingo Ponds

A pingo (Inuit for 'hill') is a mound of earth raised by the formation of an ice-lens under the surface and are found in the permafrost of Arctic and Sub-Arctic periglacial landscapes. During warming periods the melting of permafrost and subsequent ice lenses causes collapse of the overlying earth and a depression is formed, creating a *pingo pond*, also known as kettle lakes. It was the post-glacial retreat of the Devensian ice sheet at the end of the last ice age that gave birth to the pingo ponds of Norfolk, dating approximately 11,000 years old (Clay, 2015; Foster, 1993; Walmsley, 2008).

Thompson Common (Figure III.8; lat: 52.530174, long: 0.853147), located approximately 30 km South East of Norwich, is a 2.5 hectare nature reserve consisting of woodland, meadow and wetland, managed by Norfolk Wildlife Trust (NWT). It is one of the most densely populated sites for pingo ponds in Norfolk, with > 400 alone, and is a designated SSSI for both its flora and fauna (Clay, 2015;

Figure III.8: Aerial image of Thompson Common, Norfolk, showing individual pond locations.

Walmsley, 2008). Annual temperatures are generally higher than the other study regions and receives less rainfall making the region comparably hot and dry. The local geological bedrock is chalk with superficial river bed deposits (post-glacial fluvial sands and gravels; includes some glacial sand and gravel and also solifluction deposits; BGS 1999)

Selection of ponds for sampling was conducted under the guidance of the NWT site manager due to the ecologically valuable nature of the SSSI sites, their vulnerability to invasive species, and the current presence of invasive species in certain areas of the reserve. Sediment cores were collected from 5 ponds encompassing both those situated in and amongst woodland (n = 2) and those in open meadow (n = 3; see Figure III.9 for pond images). Due to the SSSI nature of the pingo ponds,

Figure III.9: Images of pingo ponds at Thompson Common, Norfolk: (A) & (B) ponds situated in open meadow land (Ponds 1 and 5 respectively); (C) a woodland pond (Pond 2).

permission for works and removal of sediments was acquired by both Norfolk Wildlife Trust and Natural England prior to sampling. It should also be noted that due to the SSSI designation and ecological value of the pingo ponds at Thompson Common, Norfolk, enhanced biosecurity precautions were requested by both Norfolk Wildlife Trust and Natural England to prevent introduction and spreading of invasive species. This consisted of following the 'Check, Clean, Dry,' procedure outlined by the GB Non-Native Species Secretariat (NNSS 2016).

4. Methodology

4.A. Sampling Strategy and Sediment Core Collection

All sediment cores collected from Cornwall, Yorkshire & Norfolk were collected following the same sampling protocol outlined in Chapter II, Section 4.C.iii. This method was universal in its application, being successful on all pond types, from high moisture content sediments of Askham Bog to the densely compact sediments of temporary ponds in Cornwall.

4.B. Analysis

All sample preparation was conducted at Northumbria University using exactly the same methods as sediment samples from Northumberland as outlined in Chapter II, Section 4.D. C and N analysis and quantification was also conducted using identical methods to those outlined in Chapter II, Section 4.E.i-iii.

4.C. Statistical Calculations: The Use of ANOVA Within this Chapter – National Sediment Survey

As previously, all statistical analysis was conducted using IBM SPSS Statistics 22, with a mixed model Repeat Measures Analysis of Variance (ANOVA), as described in Chapter II, Section 4.E.iv.a. Within this national sediment survey of ponds from Cornwall, Norfolk and Yorkshire, individual ponds were considered to be subjects, and considered to be random factors of the total population of ponds across the landscape. Individual sediment sections over depth were run as repeat measures with an AR[1] covariance structure. Sediment characteristics (i.e., moisture content and DBD) and C measurements (i.e., % C, C:N ratio, and C density) were the dependent variables, with the fixed factors being Regions (Cornwall, Yorkshire and Norfolk) and Sediment Layers (< 5 cm, 5-10 cm, and > 10 cm). Note that additional analysis was conducted in the discussion to compare the data from Chapter II, with Northumberland run as an additional Region in the fixed factors. As described in Chapter II, Section 4.E.iv.b, data was tested for normality using tests and graphical plots (e.g. Shapiro-Wilk test, stem and leaf plots. Tests and plots run in SPSS). As with the sediment data from Chapter II, data were normally distributed for some measures, but not in all cases. However, log-transformation of data was found to have little impact on the statistical analysis produced by ANOVA, and did not change the statistical outcome of the results: as such all statistical analysis was conducted on the un-transformed data for transparency. All statistical significance is reported to 95 % confidence.

5. Results

5.A. Sediment Characteristics

5.A.i. Dry bulk density

The mean DBD (Figure III.10) for all individual sediment sections was 0.59 g cm^{-3} (SD = ± 0.56, range = 0.06-1.97) and increased with depth, being significantly lower (F = 20.1, df = 1, 15, p = < 0.05) in the top 5 cm layer (mean ± SD = 0.41 ± 0.40; range = 0.10-1.34 g cm^{-3}) than the 5-10 cm layer (0.57 ± 0.53; range = 0.09-1.68, p = < 0.05) and sediments > 10 cm depth (0.69 ± 0.60; range = 0.07-1.84, p = < 0.05).

Among ponds from differing regions DBD was highest in sediments from Cornwall (mean ± SD = 1.14 ± 0.59 g cm^{-3}; range = 0.09-1.97), being significantly higher (F = 61.9, df = 1, 19, p = < 0.05) than sediments from Yorkshire (0.14 ± 0.08; range = 0.06-0.51, p = < 0.05) but not significantly different from sediments from Norfolk (0.63 ± 0.42; range = 0.12-1.57, p = > 0.05).

5.A.ii. Moisture content

The mean moisture content (Figure III.11) of all sediment samples from Cornwall, Yorkshire, and Norfolk was 60 % (SD = ± 27; range = 8-93). In contrast to DBD, moisture content decreased with depth, being greater in the upper 5 cm sediment layer (mean ± SD = 67 ± 23 %; range = 22-90) than the 5-10 cm layer (60 ± 26 %; range = 14-90) and sediments > 10 cm depth (57 ± 27 %; range = 15-91), with the upper 5 cm being significantly higher than sediments > 10 cm (F = 88.7, df = 1, 15,1, p = < 0.05).

Moisture content was found to be significantly higher (F = 264.4, df = 16.6, p = < 0.05) in sediments from Yorkshire (mean ± SD = 86 ± 7 %; range = 63-93), than those from Cornwall (32 ± 25 %; range = 8-87, p = < 0.05), and Norfolk (57 ± 19 %; range = 21-90, p = < 0.05), but not significantly different between Cornwall and Norfolk (p = < 0.05).

5.B. Sediment Carbon and C:N Ratios

5.B.i. Carbon concentration

The mean C concentration (Figure III.12) of all sediments collected from Cornwall, Yorkshire and Norfolk was 24.9 % (SD = ± 19.1; range = 0.3-73.1). Sediment C concentrations were significantly higher (F = 133.1, df = 1, 18.1, p = < 0.05) in sediments from Yorkshire (mean ± SD = 45.7 ± 9.3 %; range = 18.1-73.3) than those collected from Norfolk (18.5 ± 11.1 %;

Figure III.10: Dry bulk density measurements of sediment cores from: (A) Cornwall; (B) Yorkshire; (C) Norfolk.

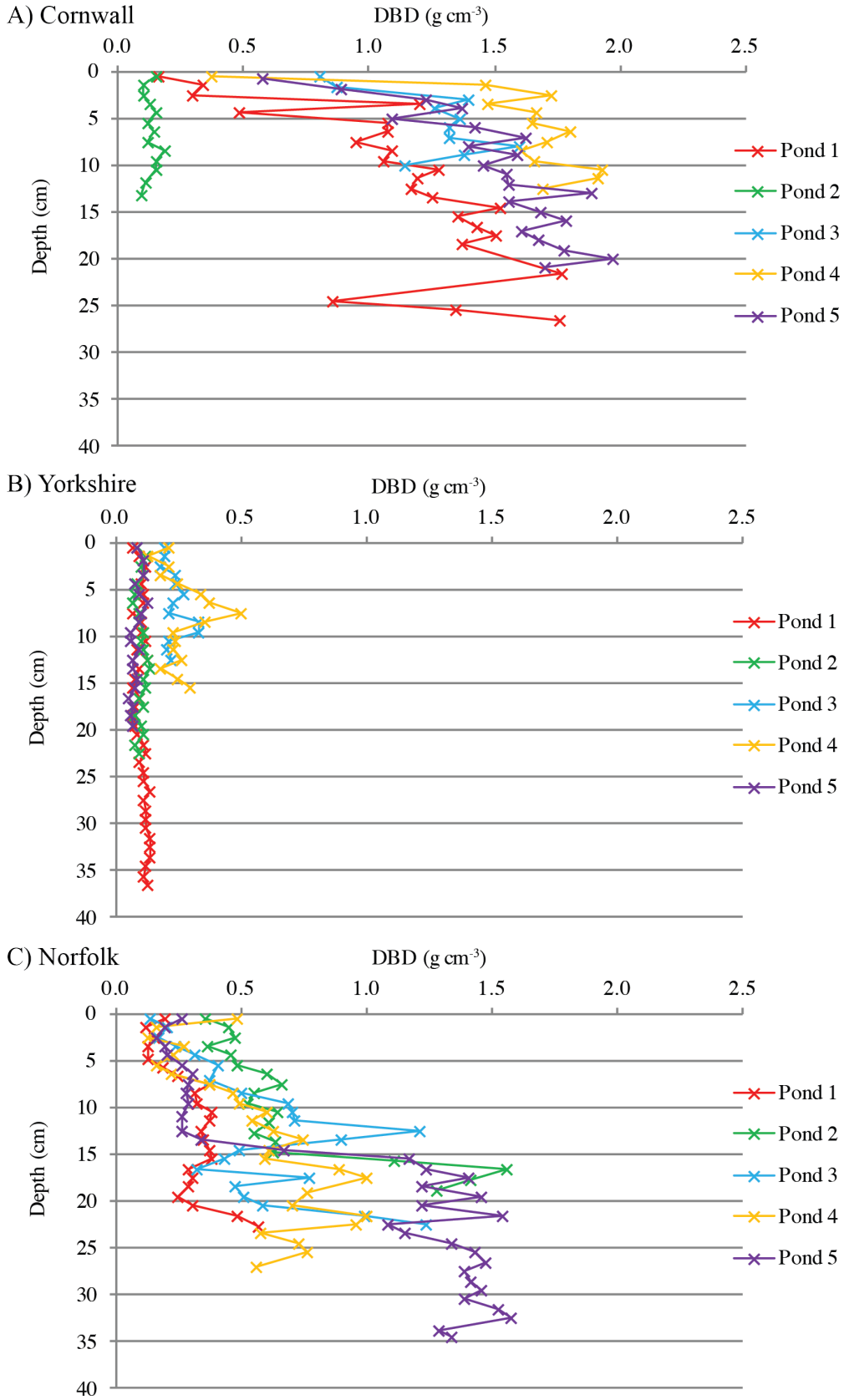


Figure III.11: Moisture content of sediment cores from: (A) Cornwall; (B) Yorkshire; (C) Norfolk.

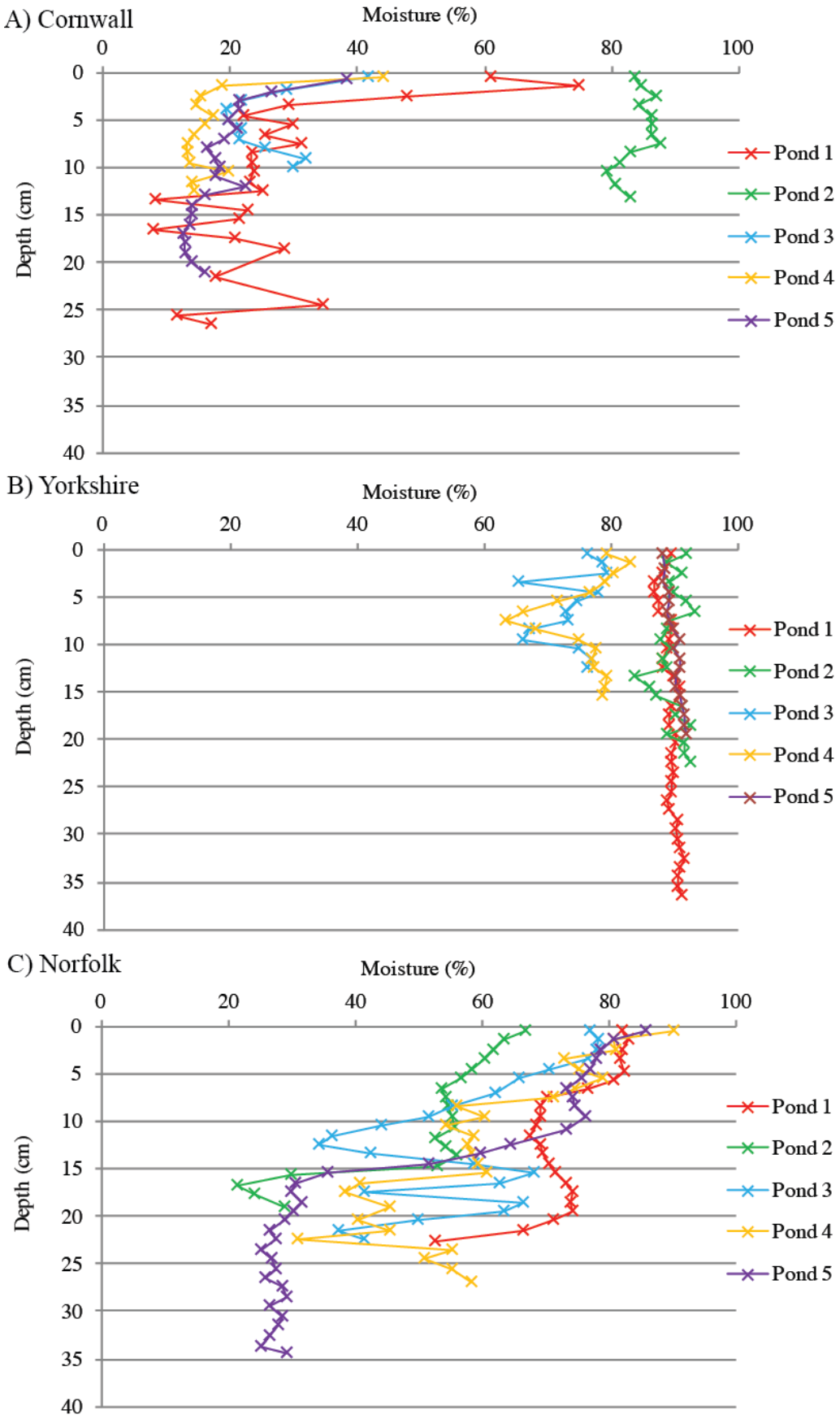
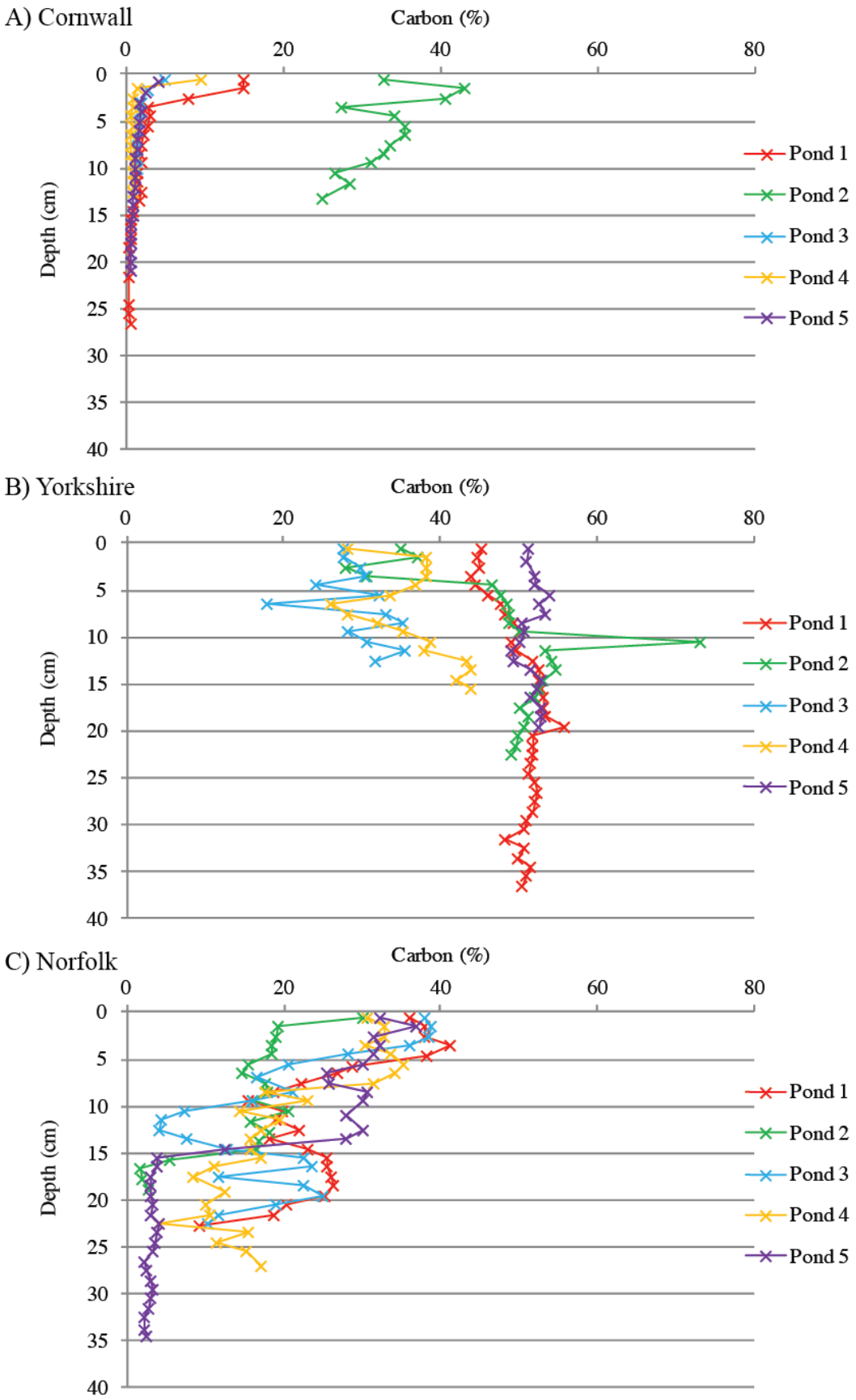


Figure III.12: Carbon content (%) of sediment cores from: (A) Cornwall; (B) Yorkshire; (C) Norfolk.



range = 1.7-41.1, $p = < 0.05$) and those collected from Cornwall (6.9 ± 11.8 %; range = 0.3-43.1, $p = < 0.05$) but not significantly different between Cornwall and Norfolk ($p = > 0.05$).

Overall among sediments, C concentrations decreased over depth, being greater in the upper 5 cm layer (mean ± SD = 26.7 ± 15.1 %; range = 2.3-52.7) than the 5-10 cm layer (24.2 ± 17.1 %; range = 0.7-52.3) and sediments > 10 cm (23.3 ± 19.1 %; range = 0.7-53.4) though no significant difference was observed between layers (F = 32.2, df = 1, 15.1, $p = < 0.05$).

Whilst C concentrations typically decreased with depth in sediments from Cornwall (< 5, 5-10, > 10 cm layer means = 10.3, 7.8, 7.2 %) and Norfolk (32.1, 22.8, 13.4 %), C concentrations in sediments from Yorkshire increased with depth (37.9, 42.0, 46.2 %).

5.B.ii. C:N ratios

The mean C:N ratio (Figure III.13) for all sediments was 21:1 (± SD = 15; range = 2-117), and was significantly higher in sediments from Yorkshire (35:1 ± 18; range = 15-117) than those from Norfolk (14:1 ± 3; range = 3-10, $p = < 0.05$), and from Cornwall (14:1 ± 4; range = 2-20, $p = < 0.05$) but not significantly different among sediments from Norfolk and Cornwall ($p = 1.00$).

C:N ratios of sediments from Yorkshire increased markedly over depth (< 5, 5-10, > 10 cm layers means = 24:1, 33:1, 35:1), compared to the relatively consistent C:N ratios observed over depth in sediments from Cornwall (12:1, 13:1, 15:1) and from Norfolk (14:1, 13:1, 15:1), though no significant differences were observed ($p = > 0.05$).

5.B.iii. C density

The mean C density (Figure III.14) of all sediment samples was 55.3 mg C cm^{-3} (SD = ± 30.8; range = 2.4-148.6). Mean C density was higher in sediments from Norfolk (74.4 ± 26.3 mg C cm^{-3}; range = 25.8-148.6) than Yorkshire (58.9 ± 22.5 mg C cm^{-3}; range = 28.5-143.4) and Cornwall (20.7 ± 12.5 mg C cm^{-3}; range = 2.4-60.0). Sediments from Cornwall were significantly lower (F = 217.4, df = 1, 14.9, $p = < 0.05$) than those from Yorkshire ($p = < 0.05$) and Norfolk ($p = < 0.05$) but were not significantly different among Yorkshire and Norfolk ($p = > 0.05$). Overall, no significant difference was observed in the mean mass of C among sediment layers (< 5, 5-10, > 10 cm layers means = 50.4, 57.8, 54.7 mg C cm^{-3}, $p = > 0.05$)

5.B.iv. Carbon stocks

The mean C stock in the upper 10 cm of all sediments (Figure III.15) was 5.45 kg C $m^{-2}_{< 10\ cm}$ (SD = ± 2.53; range = 1.62-9.04). C stocks were found to be higher in sediments of Norfolk (mean ± SD = 7.7 ± 1.1 kg C $m^{-2}_{< 10\ cm}$; range = 5.8-9.0) than sediments of Yorkshire

Figure III.13: C:N ratio of sediment cores from: (A) Cornwall; (B) Yorkshire; (C) Norfolk.

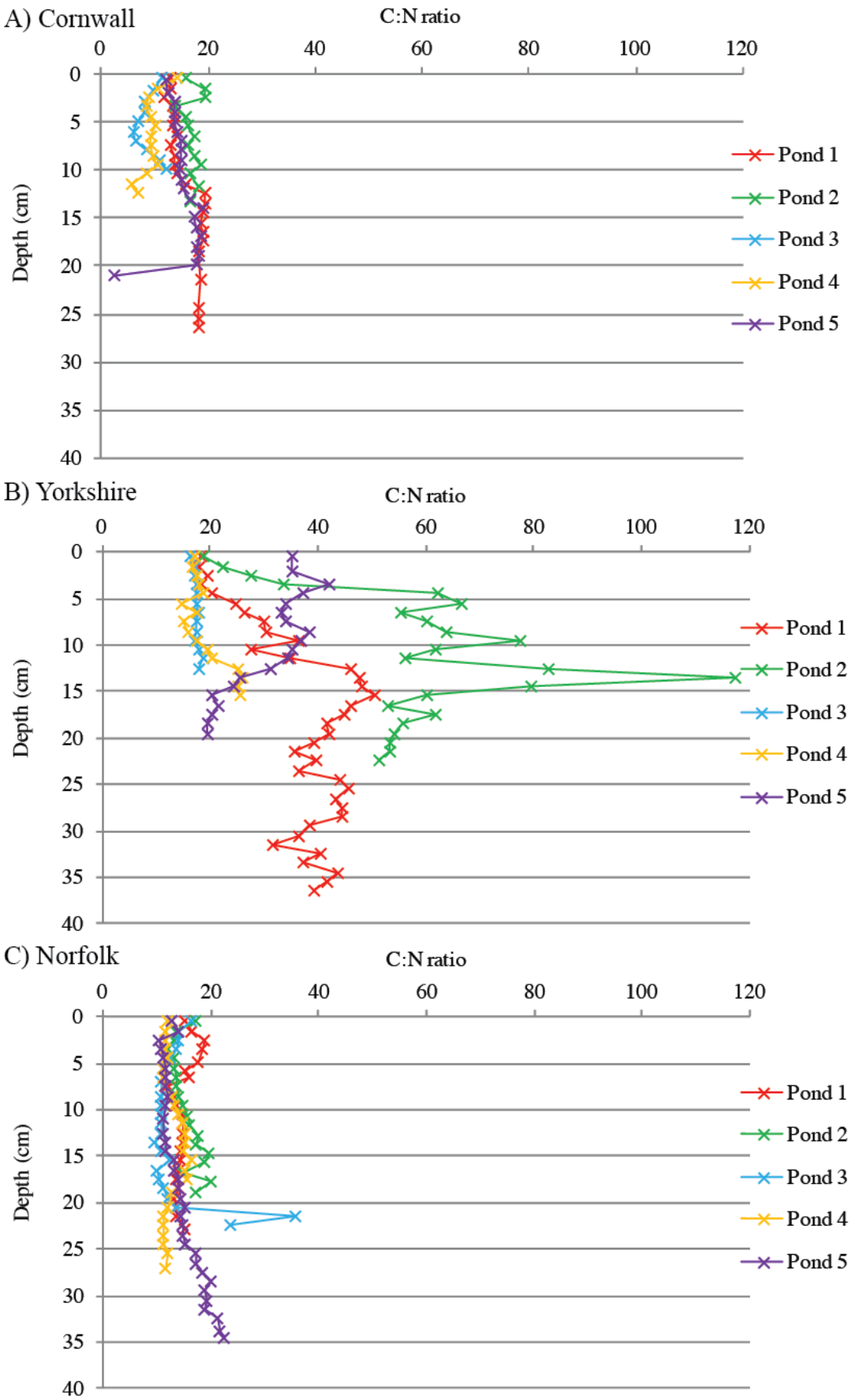
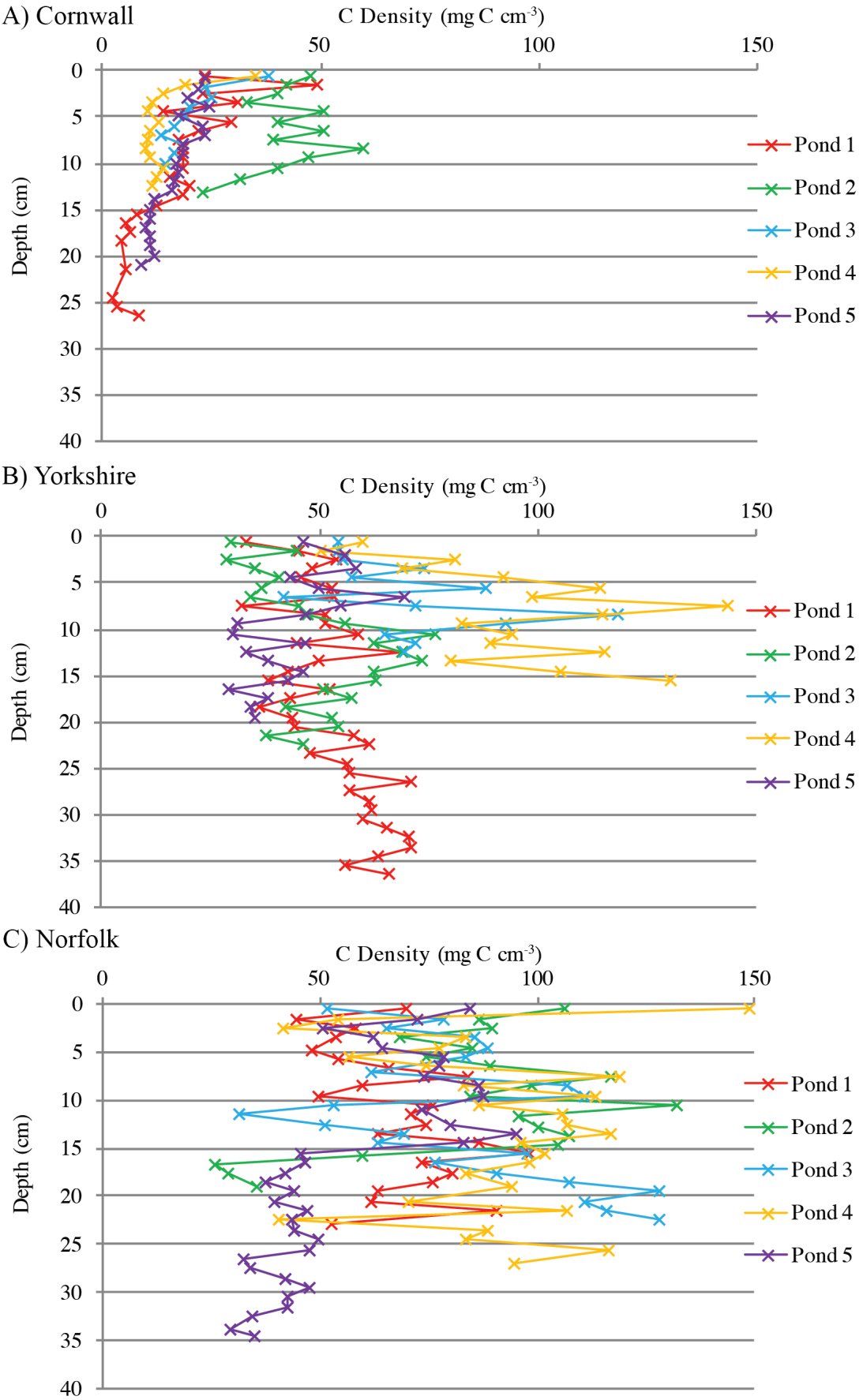


Figure III.14: C density within sediment cores from: (A) Cornwall; (B) Yorkshire; (C) Norfolk.



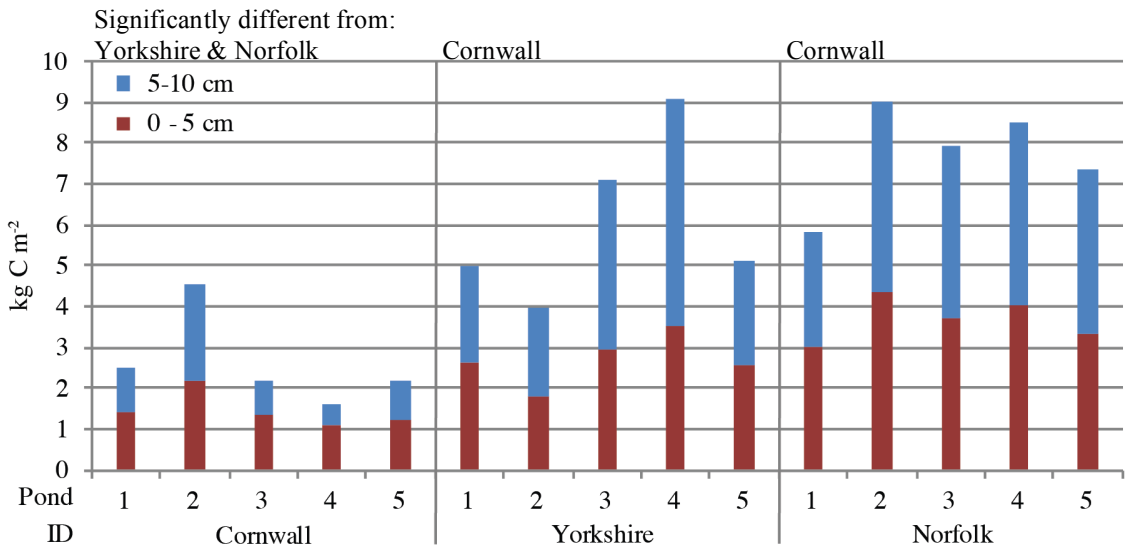


Figure III.15: C stocks within the upper 10 cm of pond sediments from Cornwall, Yorkshire, and Norfolk.

(6.0 ± 1.8 kg C $m^{-2}_{< 10\ cm}$; range = 4.0-9.0) and lowest in sediments from Cornwall (2.6 ± 1.0 kg C $m^{-2}_{< 10\ cm}$; range ± 1.6-4.5). C stocks in sediments from Cornwall were observed to be significantly lower (F = 244.9, df = 1, 15, p = < 0.05) than those from Yorkshire (p = < 0.05) and Norfolk (p = < 0.05), yet not significantly different among sediments from Cornwall and Norfolk (p = > 0.05).

6. Discussion

While C budgets play a large role in climate change mitigation and landscape management, the role of ponds in the C cycle is largely unquantified. Part of the problem surrounding this issue is: (1) a lack of data accurately quantifying the amount of C stored within these systems; (2) and an absence of studies detailing how C stocks vary among ponds from different geographical regions to allow for extrapolations. The large scale sediment survey of Chapter II focused specifically on quantifying both within pond C distributions and variations of C stocks among ponds in a lowland agricultural landscape, helping to identify localised variations and aid in extrapolations from spot measurements. Contrasting this, the survey within this chapter adopts a larger spread of systems, but smaller sample size, quantifying the C stocks of ponds from three climatically and biogeographical distinct regions of England that are populated by characteristically different ponds: temporary ponds of Mediterranean biological classification on the Lizard Peninsula, Cornwall; ancient peat extraction ponds of the lowland Askham Bog, North Yorkshire; and > 10,000 year old post-glacial pingo ponds of Thompson Common, Norfolk. Subsequently this study provides a small insight into the quantity and range of C stored within the sediments of ponds across England.

The mean C concentration of all sediments sampled in this survey (mean ± SD = 24.9 ± 19.1 %; range = 0.3-73.1) is markedly higher than those reported for large mesotrophic lakes of England and Minnesota, USA (means = 7 and 12 % respectively; Dean & Gorham, 1998), small reservoirs of the USA (1.9 %; Ritchie, 1989), marine sediments (0.7-1.5 %; Hedges & Keil, 1995), salt marshes (5.4 %; Duarte et al., 2004) and mangroves (8.5 %; Duarte et al., 2004). They are comparable to values reported for large mesotrophic lakes in Wisconsin (20 %; Brunskill et al., 1971) and slightly lower than those of impoundments reported by Pittman et al. (2013; 38 %). They are also markedly higher than values reported for aquaculture ponds (range = 1-7 %; Boyd et al. 2010; Adhikari et al., 2012), and ponds in lowland agricultural landscape of Northumberland (Gilbert et al., 2014); means range = 3.4-12.9 %) and in Chapter II of this thesis (5.6 %). Equally the mean C density within sediments observed in this study (55.3 mg C cm^{-3}) is higher than those observed within the sediment survey from Northumberland conducted in Chapter II of this thesis (41.2 mg C cm^{-3}).

Calculated C stocks (mean ± SD = 5.45 ± 2.53 kg C $m^{-2}_{< 10\ cm}$; range = 1.62-9.04) are at the upper end of those reported for terrestrial habitats of England (range = 2.9-5.9 kg C $m^{-2}_{< 10\ cm}$; CS2007) and are higher than those observed within the regional survey of Northumberland in Chapter II of this thesis (mean ± SD = 4.36 ± 2.21 kg C $m^{-2}_{< 10\ cm}$; range = 1.17-12.32).

6.A. Variations Among Study Sites

Considerable variations were observed between the three study sites, with the mean C stock (kg C $m^{-2}_{< 10\ cm}$) of sediments from Cornwall being significantly lower than those from Yorkshire and Norfolk, and while no statistically significant difference was observed, the mean C stock of sediments from Norfolk was markedly higher than that of Yorkshire (Figure III.15). This same trend was observed when analysing the C density (mg C cm^{-3}) within individual sediment layers, with those from Cornwall being significantly lower than Yorkshire and Norfolk, and again substantially higher in sediments from Norfolk than Yorkshire, though not significantly different. This difference between sites is partly explained by the change in the C density over depth. While no statistical difference in C density was observed between sediment layers (< 5, 5-10, > 10 cm sections), either nationally or at each individual site, the C density in sediment layers from Norfolk (72.7, 83.2, 75.7 mg C cm^{-3}) and Yorkshire (51.4, 67.0, 64.2 mg C cm^{-3}) increased over depth, being lowest in the upper 5 cm and greatest in the 5-10 cm section, compared to sediments from Cornwall (27.1, 23.1, 16.6 mg C cm^{-3}) which consistently decreased over depth (Figure III.14).

While Norfolk had the highest mean C density and calculated C stocks, Yorkshire had a significantly higher mean C concentration (% C) than sediments from both Norfolk and Yorkshire. This highlights the importance of including DBD to calculate the C density (mg C cm^{-3}) in the sediments as apposed to simply using % C which can lead to misinterpretation of the effectiveness of C storage within sediments.

Similarly to C density, concentrations of C within sediments from Cornwall also decreased over depth, yet so too did those from Norfolk, with the mean % C in the upper 5 cm being significantly higher than the > 10 cm sediment layer at both sites. However, whilst the sediments of Norfolk show a gradual decrease in % C over depth as would be expected with stable decomposition of organic matter over time, sediments from Cornwall decrease abruptly within the upper 5 cm (with the exception of pond 2; Figure III.12.A) indicating shallower sediments. Whilst soils on the Lizard Peninsula are naturally shallow overlying bedrock, it is likely that ponds sampled in Cornwall have shallower sediments than those from Yorkshire or Norfolk due to either: (1) their younger age and chance to accumulate sediment compared to other systems (i.e., Norfolk pingo ponds are > 10,000 years old); (2) slower growth rates of vegetation in temporary ponds and subsequent deposition of organic matter; (3) higher rates of organic matter decomposition due to their temporary nature intermittently creating oxic sediment conditions; (4) or a combination of the above. However in the absence of sediment burial rates or dating of sediment cores this remains speculative.

Contrasting sediments from Norfolk and Cornwall, no significant difference in % C was observed over depth in sediments from Yorkshire, with carbon concentrations remaining highly elevated for the full length of the core. Both the extremely high concentrations of C and lack of change over depth represent a high level of C preservation within the ponds located at Askham Bogs, Yorkshire. However, the high moisture content (mean = 86 %) and C concentration (mean = 46 %) found within cores from Yorkshire are in line with those of peat soils; while exact definitions of peatlands vary, they typical have ~ 90 % moisture content, with the 10 % solid matter being ~ 50 C % (Lindsay, 2010; Mitra et al., 2005). This is especially the case for Yorkshire ponds 1, 2 and 5 (see Figure III.12.B), which also happen to be ponds formed in the hollows left behind after peat extraction. As such, distinguishing between the underlying peat layers and accumulated sediment since the ponds origin is not possible from this data without detailed geochemical analysis, similarly to the issues discussed in Chapter II; Section 6.B.iv.

6.B. Within Site Variations

While sediment cores from Norfolk were relatively consistent with one another several sediment cores from Yorkshire and Cornwall displayed strong differences in their physical and geochemical characteristics from other ponds of same site.

Ponds 1, 3, 4 and 5 from Cornwall all displayed relatively similar trends and values for DBD, moisture content, and % C, in comparison to Pond 2 which was considerably different. Whilst it might be expected that Pond 2 might have obviously different environmental factors to the other ponds that would lead to such differences (e.g., catchment type or underlying geology) this is not the case. Pond 2 was more similar to Ponds 3, 4, and 5 in appearance and surrounding landscape, all being upland heath ponds, compared to Pond 1 which was situated in the lowland landscape. Equally, at the Yorkshire site Ponds 3 and 4 exhibited broadly similar trends and values in DBD, moisture content, % C, and C:N ratios that were considerably different to Ponds 1, 2, and 5. While Pond 4 was a larger, more permanent 'classic' wetland habitat, Pond 3 was a peat excavation pond similar in size and appearance to Ponds 1, 2, and 5, which it would be expected to more closely resemble in its physical and geochemical characteristics. The high C:N ratios that occur throughout the greater depths of sediment cores from ponds 1, 2 and 5 are more in line with those of peat than recently deposited sediment (Meyers and Ishiwatari, 1993) and it is likely that the majority of these cores are sampling the peat that underlays the ponds rather than accumulated sediment. From this, the lower C:N ratios that occur throughout the full depth of cores from ponds 3 and 4 suggest that they represent accumulated sediment that has been stored since the ponds creation. However, as discussed previously, without dating of sediments to establish a base for accumulation it is not possible to state what proportion of the sediment cores collected represent accumulated sediment or underlying soil/peat, again highlighting a discrepancy that exists in many sediment C stock estimates.

The purpose of this study was to elucidate the broad ranges of C distributions among ponds of geographically and climatically different areas across England for the purpose of quantifying overall C stocks, not to pick apart the individual differences among individual ponds. However, this variation highlights the difficulty in selecting ponds for sampling when the physical and geochemical characteristics of a sediment core from an individual pond can more closely resemble that of a wildly different system rather than a pond which is superficially similar in size, appearance and surrounding landscape.

6.C. Comparison to Ponds Sampled in Northumberland

The mean C stock of ponds sampled in Northumberland (4.36 kg C $m^{-2}_{< 10\ cm}$; Figure III.16) was lower than those of ponds sampled in both Norfolk (7.7 kg C $m^{-2}_{< 10\ cm}$) and Yorkshire (6.0 kg C $m^{-2}_{< 10\ cm}$), yet higher than Cornwall (2.6 kg C $m^{-2}_{< 10\ cm}$). The mean C stock of ponds from Cornwall most closely resembled that of Classically vegetated (3.1 kg C $m^{-2}_{< 10\ cm}$) and Arable field (3.2 kg C $m^{-2}_{< 10\ cm}$) pond types in Northumberland, whilst mean C stocks of ponds in Yorkshire were comparable to the Dune slack ponds sampled in Northumberland (6.2 kg C $m^{-2}_{< 10\ cm}$). When grouped together by location, with ponds sampled in Northumberland broken down into Pond Types, no significant difference was observed ($p = > 0.05$) among sites.

Overall, the mean C stock for all ponds sampled across England in both the studies of Chapter II and this study (i.e., Northumberland, Yorkshire, Norfolk, and Cornwall) was 4.65 kg C $m^{-2}_{< 10\ cm}$ (± SD = 2.35, range = 1.17-12.32). This is in the mid-range of values reported during the Countryside Survey (CS, 2007) for habitats in England (range = 3.2-5.9 kg C $m^{-2}_{< 10\ cm}$), being higher than those of coastal margins, agricultural land, grassland, and woodland, yet lower than wetlands, bogs, and fens, marshes and swamps.

Using this mean value and estimates of pond numbers from the Countryside Survey: Pond Report 2007 (Williams et al., 2010) to predict UK pond coverage, assuming an even distribution of ponds within size ranges surveyed, it is estimated that the total C stored within the sediments of ponds (0.0025–2 Ha) across Great Britain is approximately 2.01 Mt (Table III.1). It should be noted that this is based on C stock calculations from the upper 10 cm sediment alone, and is likely to be very conservative. Furthermore, this is only a generalised estimate, requiring much larger datasets over greater geographical regions.

The relatively small size and low volume of ponds renders them susceptible to rapid changes in hydrological regime, especially for systems that lack direct inflow and are dependant upon precipitation for recharge. As a result, many long standing permanent ponds are at risk of either

Figure III.16: C stocks within the upper 10 cm of all ponds surveyed in Northumberland, Cornwall, Yorkshire, and Norfolk.

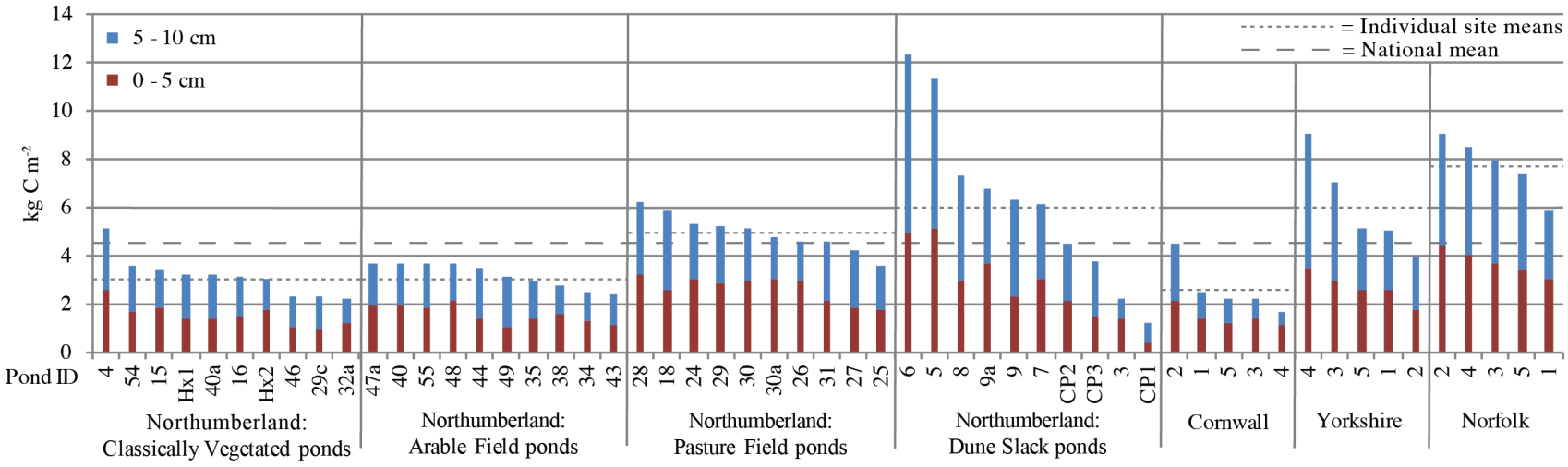


Table III.1: Estimated coverage, and calculated C stocks for ponds across England and Great Britain.

Pond size range (Ha)	**0.0025 - 0.04**	**0.04 - 0.2**	**0.2 - 1**	**1 - 2**	**Sum**
Mean area of ponds[1] (Ha)	0.0213	0.12	0.6	1.5	
Number of ponds in England[2]	158.6	59.1	14.2	2.2	234.1
Total area of ponds in each size class[1] (Ha)	3370	7092	8520	3300	22282
Estimated C stock[3] (Mt C)	0.16	0.33	0.40	0.15	**1.04**
Number of ponds in Great Britain[2]	332.5	117.8	26.5	4.1	480.9
Total area of ponds in each size class[1] (Ha)	7066	14136	15900	6150	43252
Estimated C stock[3] (Mt C)	0.33	0.66	0.74	0.29	**2.01**

[1] = mean pond area and estimated total area of ponds estimated assuming an even distribution of pond sizes within groups. [2] = Data taken from Williams et al., (2010). [3] = Calculated from total area of ponds x mean C stock of 4.65 kg C $m^2_{<10\,cm}$.

seasonal drying or permanent loss, either through anthropogenic activities (e.g., land drainage) or increase in weather extremes as a result of climate change (e.g., drought). This poses a risk of sediment oxidation and C mobilisation in once continually anoxic sediments of permanent pond systems. While the estimated C stock of pond sediments across Great Britain is 2.01 Mt C, when calculated as CO_2e (CO_2 equivalents; based on a conversion factor of 3.67) this equates to 7.38 Mt CO_2e. While this may seem small in comparison to the 2014 UK net CO_2 emission of 514.4 Mt CO_2e, it is comparably substantial when compared to the net intake of 9 Mt CO_2e from land use, land use change and forestry (DECC, 2016). With ponds being so susceptible to environmental changes it is likely that a large portion of this C stock is vulnerable to remobilisation and evasion to the atmosphere, depending on how each environment responds to potential climate changes.

7. Conclusions

This study set out to quantify C stocks from a variety of biogeographical and biodiverse pond habitat types across England. There was marked variation observed among regional study sites, with calculated C stocks being higher in post glacial pingo ponds from Norfolk (mean ± SD = 7.7 ± 1.1 kg C $m^{-2}_{< 10\ cm}$; range = 5.8-9.0), compared to peat excavation ponds in Yorkshire (6.0 ± 1.8 kg C $m^{-2}_{< 10\ cm}$; range = 4.0-9.0), and significantly higher than shallow temporary ponds of Mediterranean classification in Cornwall (2.6 ± 1.0 kg C $m^{-2}_{< 10\ cm}$; range ± 1.6-4.5). This regional difference in sediment C stocks may be part explained by the permanent, long standing nature of the Norfolk pingo ponds (> 10,000 years old) compared to the shallow and temporary nature of the ponds on the Lizard Peninsula, Cornwall. Moreover, as well as marked variation among individual sites, the overall range of sediment C stocks observed within the national survey of this Chapter (1.62-9.04 kg C $m^{-2}_{< 10\ cm}$) is comparable to the large variation observed within the detailed singular regional study of Northumberland (Chapter II; range = 1.17-12.32 kg C $m^{-2}_{< 10\ cm}$) suggesting large variation both among and within biogeographically different regions. It is evident that C sequestration and storage are not the same for all ponds, and there is a clear need to differentiate between distinct habitat types and their systems processes in order to fully integrate ponds into national C budgets.

Yet without accurate sediment depth measurements and burial rates it is is impossible to determine both the full extent of sediment C stocks and burial rates; a key knowledge gap that is missing from literature and preventing a comprehensive integration of ponds in national C budgets.

When coupled with those ponds surveyed in Northumberland during Chapter II, the overall mean C stocks for ponds in England (± SD = 4.65 ± 2.35 kg C $m^{-2}_{< 10\ cm}$, range = 1.17-12.32) was comparable to the mid-range of values reported by the countryside survey (range = 3.2-5.9 kg C $m^{-2}_{< 10\ cm}$; CS, 2007) being higher than those of coastal margins, agricultural land, grassland, and woodland, yet lower than wetlands, bogs, and fens, marshes and swamps. Using pond numbers and aerial coverage (Williams et al. 2010), and the mean sediment C stock reported in this survey, it is estimate that pond sediments of Great Britain alone hold 2.01 Mt C. When calculated as CO_2e, this equates to 7.38 Mt CO_2e, comparable to the net intake of 9 Mt CO_2e by land use, land use change and forestry during 2014 (DECC, 2016) highlighting the true volume of C stored within pond sediments. Given predicted increases in climatic variability and rainfall (IPCC, 2014) there is increasing likelihood for the creation of new pond environments, especially if properly integrated into land management practices (i.e., UK policy for Ecological Focus Areas on 5 % of arable land; DEFRA, 2013), and given the results of this national sediment survey they have considerable potential as future sinks of atmospheric CO_2.

Yet without accurate sediment depth measurements and burial rates it is simply impossible to determine the full extent of sediment C stocks, and burial rates that are critical to accurately quantifying actual stocks; an issue that is not addressed in the majority of academic reviewed literature. Perhaps the most unique insight that comes from this study is the potential for constructed ponds to rapidly sequester and store atmospheric carbon. Therein lies potential possibilities in relation to Carbon Capture, and exists an opportunity to be integrated into conservationists/policy makers perspectives; as well as addressing a key knowledge gap that is missing from academic and IGO (Inter-Governmental Organisation) literature.

Chapter IV. CO_2 Emission from Temporary Ponds

1. Introduction

Whilst temporary ponds are known by a range of diverse regional names or technical definitions (e.g., seasonal, ephemeral, playa or vernal; Keeley & Zedler, 1998), they are internationally important terrestrial habitats, ubiquitous in all climatic and ecological zones across the globe (Allende and Mataloni, 2013; Catalán et al., 2014; Gallagher and Huissteden, 2011; Jonai and Takeuchi, 2014; Mozley, 1937). While their presence is frequently overlooked both in natural landscapes such as grassland or temperate forest, and in intensively modified landscapes such as arable or grazing agriculture (Williams et al., 2001) recent studies are beginning to document their widespread occurrence (Jeffries, 2012, 2015).

The key feature that unifies such systems is that they exhibit seasonal changes in their hydrological regimes resulting in periodic dry phases, exposure of the base substrate, and often desiccation of upper sediment layers. For temporary ponds in Northeast England recharge is typically rainfall dependent, and as such this change in hydro-period is dependent on the balance between evaporation rates and net rainfall over short periods. In the UK unreliable summer rainfall (Fowler & Kilsby, 2002) often results in several drying and re-wetting cycles over short periods of time, with rainfall variations from year to year further complicating the quantification and modelling of their ecosystem processes. This problem could be compounded by the likely increase in climate variability. A particular uncertainty arises from new extremes of rainfall or temperature (Ovens, 2015), which will subject wetlands and their wildlife, to novel stresses which may alter existing rates of geochemical processing and species' distributions (Jeffries, 2010; Jones, 2013).

Whilst small lakes and ponds have been highlighted as having high carbon burial rates (Downing, 2010; Gilbert et al., 2014), it is the low water volume of temporary ponds that renders them vulnerable to drying and sediment desiccation during periods of reduced rainfall, greatly impacting sediment carbon stability. Sediment conditions quickly change from anoxic to oxic, permitting aerobic microbial activity in the surface substrate, resulting in higher mineralization rates of organic matter and subsequent CO_2 efflux (Fromin et al., 2010). Furthermore, in exposed sediments, CO_2 release is no longer hindered by the water column, through which CO_2 must usually diffuse before release to the atmosphere at the surface boundary layer (Catalán et al., 2014). Elucidating CO_2 effluxes of temporary ponds in response to rapid changes in seasonal drying cycles is crucial to quantifying their role in the global carbon cycle. While research is beginning to be reported on CO_2 flux rates from temporary systems (Catalán et al., 2014; Fromin et al., 2010; von Schiller et al.,

2014), they are relatively limited in both their spatial and temporal resolution, focusing upon temporary systems in Mediterranean climates. This component of research is intended to address the lack of detailed flux rates for ponds in the academic literature by monitoring the temporal and spatial heterogeneity of CO_2 fluxes among 26 small experimental ponds in Northeast England.

2. Aims & Objectives

The aim of this chapter is to quantify the *F* CO_2 from a group of constructed temporary ponds in Druridge Bay, Northumberland, UK. The study was designed to assess both the short-term (i.e., between days) and long-term (i.e., between seasons) temporal variations that occur in response to hydrological changes in pond permanence. The study was specifically designed to capture changes over relatively fine grained spatial and temporal scales; ponds just metres apart, continually over a period of days, and within rapid drying and rewetting periods. Furthermore, the spatial heterogeneity among ponds on individual sampling days based on different hydrological classifications and vegetation types among ponds was assessed. Specific objectives include:

1) Quantify net CO_2 flux rates from small, temporary ponds.
2) Assess the influence of differing vegetation types and hydrological permanence on the spatial heterogeneity of CO_2 flux rates among ponds.
3) Assess both the short and long term temporal heterogeneity of CO_2 flux rates from ponds in response to hydrological changes.

3. Site Description

3.A. Study Region

This section focuses on a group of small ponds ($n = 26$) at the northern end of Druridge Bay, Northumberland, UK; the same region that comprised Chapter II. The region forms part of the Northumberland coastal plain in Northeast England, a lowland landscape dominated by intensive arable and livestock agriculture. The climate is relatively cool but also dry due to a rain shadow from hills to the west (Lunn, 2004). Despite the relatively low rainfall the area is rich in ponds, especially shallow, temporary habitats associated with sand dunes or land subsidence over old coal mines. This mosaic of wetland environments occupies ~ 2 % of the landscape, and while the majority of ponds are < 400 m^2, several large lakes have been created from open-cast coal mining for nature conservation (M. Jeffries, 2012; for further details see Chapter II, Section 3.A.).

3.A.i. Hauxley Nature Reserve

The ponds surveyed in this study are all located within a small field of unimproved open meadow grassland, which is part of Hauxley Nature Reserve, and is owned and managed by Northumberland Wildlife Trust (Figure IV.1, 55°19'04.1"N 1°33'22.1"W). While the site now appears completely natural, it was previously part of the large open-cast mine which was remediated for nature conservation when mining ceased in the 1980s (See Figure IV.2). The site was covered with plastic sheeting, topped with a rough clay backfill, and finished with approximately 50 cm of soil. Note that as part of the sediment survey discussed in Chapter II two larger semi natural ponds were sampled from this same field, though they are not part of the ponds or data included within this chapter.

Located within the field at Hauxley Nature Reserve there is a total of 30 experimental ponds, constructed in 1994 for the purpose of monitoring the spatial and temporal heterogeneity in the development of invertebrate and macrophyte communities (Jeffries, 2008; Zealand and Jeffries, 2009). More recent studies have also focused on carbon burial within the ponds on the site (Gilbert et al., 2014). To meet the purpose of the initial ecological studies each pond was constructed to the same dimensions, all being approximately 1 m^2 x 30 cm depth. Set out in a triangular array across approximately 30 x 30 m the ponds are in very close proximity to one another (~ 1-3 m apart) rendering them exposed to the same environmental conditions. In relation to the life cycle of ponds, as discussed in Chapter I, Section 4.D, the ponds surveyed at Hauxley Nature Reserve are in a mid/late successional stage, dominated by rooted emergent macrophyte communities with some encroachment of terrestrial grasses. Of the original 30 ponds, 26 were selected and monitored in this study; the integrity of the remaining ponds had been compromised due to previous sediment disruption during other C research studies (Scott, 2013; Taylor, 2012) and were therefore excluded from this study.

Figure IV.1: Aerial image showing the location of Hauxley Nature Reserve within: (A) the UK; (B) North Druridge Bay; (C) the lower field of Hauxley Nature Reserve.

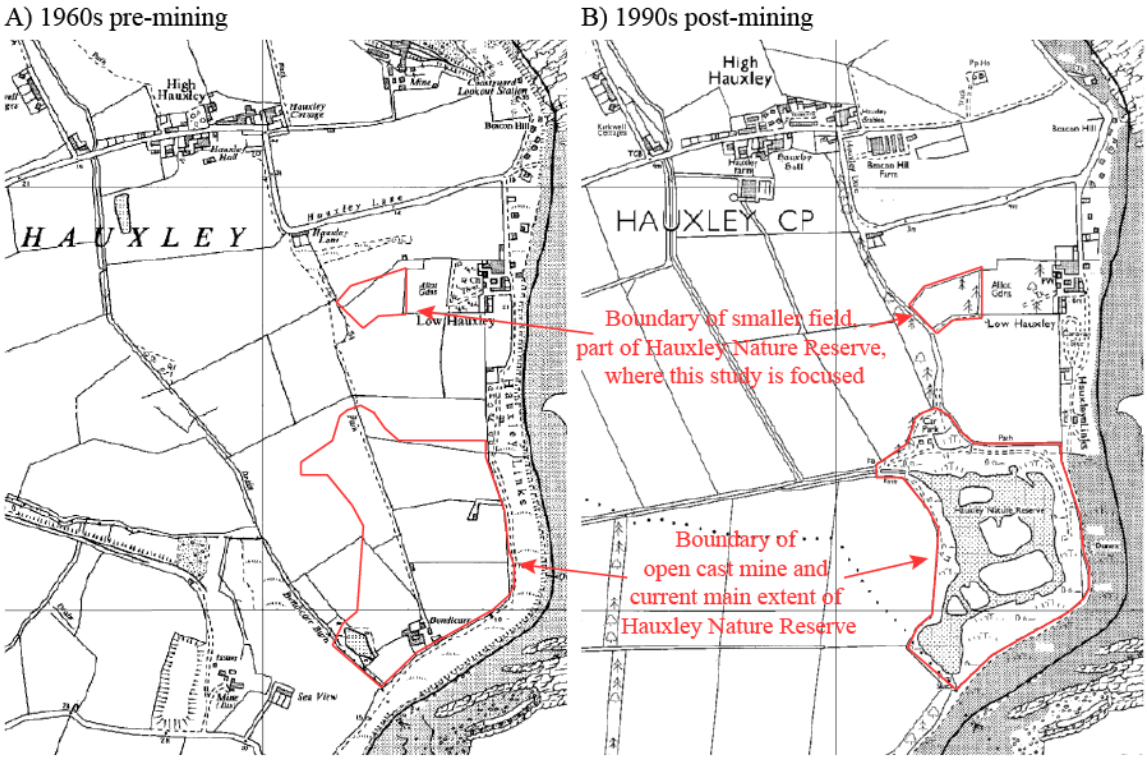


Figure IV.2: Historical maps of Hauxley Nature Reserve from: (A) the 1960s before mining commenced; (B) the 1990s after mining and restoration.

The clay layer which underlies the site is impermeable and as such the ponds are dependent on direct precipitation, subsequent surface run off, and horizontal through-flow in the topsoil layer for recharge. While barely visible to the eye, a slight gradient across the site runs north east to south west causing subtle hydrological variations among the ponds, which in turn has influenced the vegetation. The south west portion of the site is typically marshier and dominated by spike rush, *Eleocharis palustris* (L.) Roem. & Schult., with ponds here being the first to fill during rainfall events, yet also the first to dry during rainfall absence. Contrastingly the north east portion of the site is less marshy, dominated by grasses such as marsh foxtail, *Alopecurus geniculatus* L., and glaucus sedge, *Carex glauca* Schreb., and the ponds hold water for longer. Despite their close proximity and identical history, the ponds have developed a diverse set of plant and animal communities and hydrological patterns typical of the ponds and wetlands through the coastal plain lowland region (Jeffries, 2008, 2010).

4. Methodology

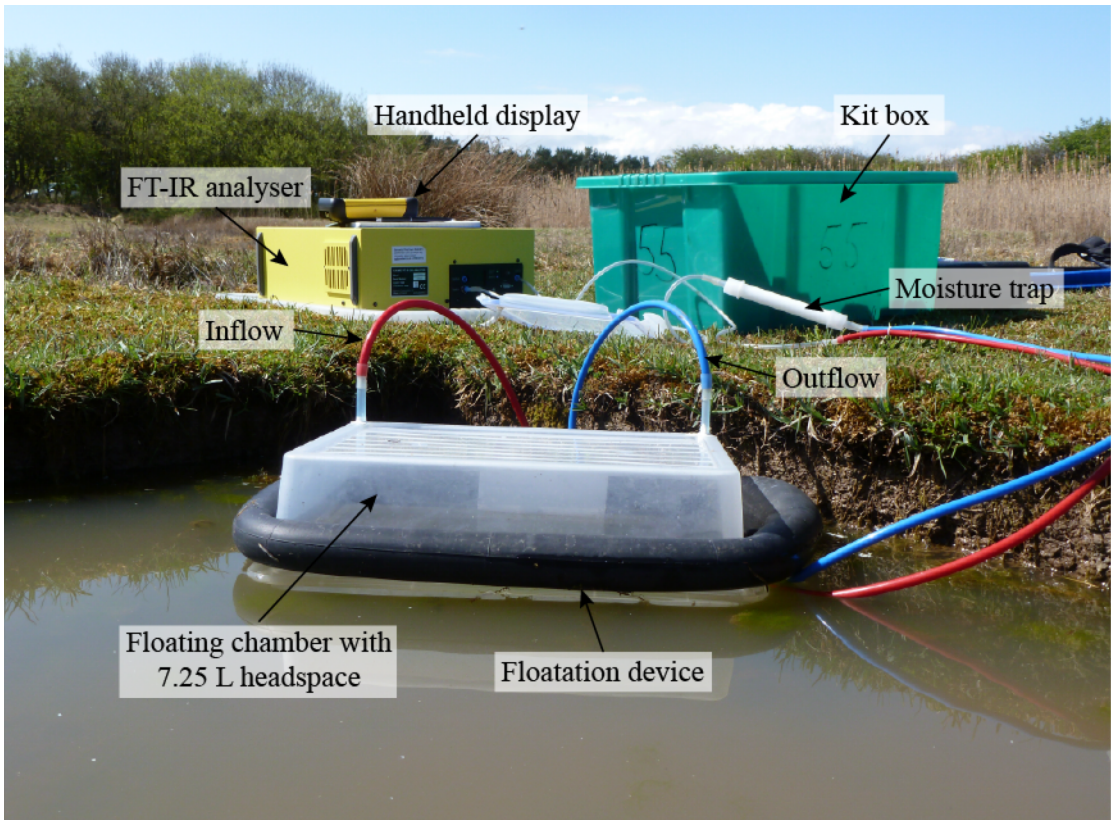
4.A. CO_2 Fluxes

4.A.i. In-situ CO_2 flux monitoring: floating chamber method

Flux rates of CO_2 (F CO_2) were monitored for 26 ponds over several weeks within two separate monitoring campaigns. The first monitoring phase was conducted over 11 days, monitoring on 24th August, 27th August, 1st September, and 4th September, 2014; these dates are referred to as days 0, 3, 8, and 11, representing the number of days passed from the beginning of the sampling phase. The second monitoring phase was conducted two months later, on 24th October, 29th October, 5th November, and 11th November, 2014; these dates are referred to as days 0, 5, 12, and 18.

Fluxes of CO_2 were measured using a floating chamber method. The chamber consisted of an upturned container (length = 37 cm; width = 24.5 cm; height = 8 cm) of 7.25 L volume with attached floatation device (see Figure IV.3). Whilst ponds were in an inundated phase the chamber was placed gently on the surface of the water in each pond to avoid disturbance and allowed to float freely. For systems that had dried out the chamber was placed directly on vegetation within the centre of the pond and sealed with plastic sheeting. Inflow and outflow tubes connected the chamber to an in-situ Gasmet FT-IR (Fourier Transform - Infra Red) analyser pumping at a rate of 2 L per minute to allow

Figure IV.3: Image showing the floating chamber method used for monitoring of CO_2 fluxes.



continual circulation. Prior to each sampling session, the Gasmet FT-IR analyser was run for 30 minutes and flushed with N_2 (pure nitrogen), and zero calibrated to assure accurate baseline measurements. Gas concentrations (ppm) within the chamber were automatically recorded at 20 second intervals over a five-minute period, which was found to be the optimum time to achieve a reliable r^2 (> 0.8) yet not too long so that temperature or pressure changes impacted flux rates within the chamber. In-situ air temperature was also recorded to allow for calculation of in-situ molar volume, necessary for converting from recorded CO_2 concentrations (ppm) to mass (mg m^{-3}). In between each flux measurement the chamber was flushed with air until readings returned to atmospheric concentrations. Every 10th pond was measured in triplicate to assess the precision of the analysis. Monitoring of flux rates for all ponds took approximately 6 hours, from 10 am to 4 pm, and whilst this represents an element of systematic error, as *F* CO_2 are known to vary throughout the day, it is this central period at which flux rates are most stable (Chanda et al., 2013). Attempts were made to monitor the diurnal variations in *F* CO_2, however these proved unsuccessful; methodological difficulties are discussed further in Section 4.D.

4.A.ii. Calculating the flux of CO_2

Calculations for the *F* CO_2 were based on Equation IV.1 (Gonzalez-Valencia et al., 2013), where: Δ (delta) represents total change; ΔC is change in CO_2 concentration; Δ*t* is change in time; Vc is volume of the chamber; and Ac is area of the chamber. Essentially, this equation calculates *F* CO_2 from floating chambers based on the concentration gradient within the chamber of over time (ΔC/Δ*t*), and accounts for the volume and area of the chamber headspace within this equation (Vc/Ac). Equation IV.1 was adapted to Equation IV.2 in order to specifically fit the data and methods of this project.

Equation IV.1:

$$F\ \mathrm{CO_2} = \frac{\Delta \mathrm{C}}{\Delta t} \times \frac{\mathrm{Vc}}{\mathrm{Ac}}$$

Equation IV.2:

$$F\ \mathrm{CO_2} = \text{Concentration gradient} \times \frac{\text{Volume of chamber}}{\text{Area of chamber}} \times 1440$$

Or:

$$\mathrm{mg\ C\ m^{-2}\ d^{-1}} = \mathrm{mg\ C\ m^{-3}\ min^{-1}} \times \frac{0.245 \times 0.37 \times 0.08}{0.245 \times 0.37} \times 1440$$

The multiplication by 1440 is to convert from minutes to day, and the calculation of the concentration gradient is expanded in detail in Equation IV.3. The change in concentration of CO_2 within the floating chamber headspace was calculated from the linear regression over time using the Microsoft Excel, Data Analysis, Regression tool pack. An example of a typical flux gradient can be seen in

Figure IV.4. While it was aimed for each linear regression to achieve $r^2 = > 0.8$ it should be noted that occasionally this was not possible; when *F* CO_2 was minor, changes in concentration within the chamber between 20 second measurements were lower than the accuracy of the FT-IR analyser (1 ppm), resulting in a poor r^2 (< 0.8). However, in this situation *F* CO_2 were negligible in comparison to the high flux rates from the other ponds. Concentration gradients of CO_2 were then converted from ppm to mg m^{-3} min^{-1} based on calculation of the molar volume and in-situ air temperature (Equation IV.3). To allow for comparison with other ecosystems all flux values reported have been adjusted to standard temperature and pressure (STP; 0 °C and 1 atmosphere of pressure) using Equation IV.3, where molar volume at STP is 22.414 L, and according to the ideal gas law stated for reference in Equation IV.4, where: *P* is pressure; *V* is volume; *n* is number of moles; *R* is the ideal gas constant (0.08206 L atm mol^{-1} K^{-1} stipulated in Equation 3); *T* is temperature (Kelvin).

Equation IV.3:

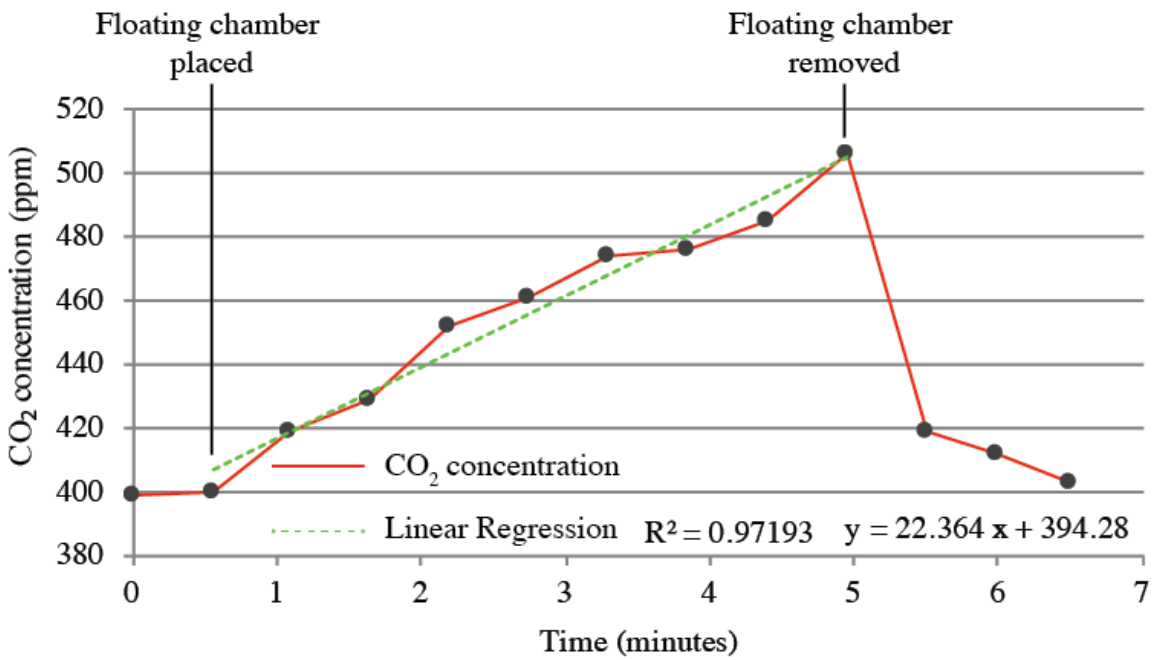
$$\text{Concentration gradient} = \frac{\text{Change in } CO_2 \text{ concentration}}{\text{Length of time}} \times \frac{\text{Molar mass}}{\text{Molar volume in-situ}}$$

Or:

$$\text{mg C m}^{-3}\ \text{min}^{-1} = \frac{\text{Change in } CO_2\ (\text{ppm})}{\text{Length of time (min)}} \times \frac{44.01}{1 \times (0.08206 \times (273.15 + \text{in-situ temp}))}$$

Equation IV.4:

Figure IV.4: Example of a concentration gradient within the headspace of the floating chamber and linear regression. In this particular example the increasing concentration represents an emission of CO_2 from the pond to the atmosphere



$$PV = nRT$$

Negative values for the *F* CO_2 reported herein represent an intake of atmospheric CO_2 by the pond systems whilst positive values represent an emission to the atmosphere. When referring to the averages across all ponds on the site the term ecosystem flux rate (E-*F* CO_2) is used to refer to the ponds as a collective.

4.B. Supplementary Data

4.B.i. Hydrological classification

In order to assess the influence of hydro period on the *F* CO_2 from pond systems the hydrological condition of each pond was characterised by personal observation during each site visit, grouping into one of three categories:

1) Aquatic Phase - Ponds contained standing water that covered the substrate, though with occasional emergent vegetation.
2) Transitional Phase - Ponds contained no standing water with the base layer exposed. However, the sediment and vegetative layer were still saturated and moist to touch.
3) Dry Phase - Ponds contained no standing water with sediment and vegetation now dry to touch.

This technique of categorising pond hydrological status at the time of sampling provides a simple but effective method for grouping ponds during analysis to assess the influence of hydro period on the *F* CO_2 during drying and flooding events.

4.B.ii. Vegetation classification.

Macrophyte vegetation of each pond was recorded by Dr. M. Jeffries during spring/summer 2014 as part of ongoing ecological monitoring of the ponds (Jeffries, 2008, 2010). Macrophyte surveying was conducted using standard methods for UK National Vegetation Survey, quantifying the abundance of each taxon using quadrat identification and the DOMIN scale. Specifically, a 1 m^2 quadrat grid with cross wires every 10 cm was used to record plant species under each intersection of the cross wires to give a % cover for each species (for plant survey methods see Jeffries 2008).

The vegetation data was subject to a two-tier TWINSPAN analysis (run on CAP 3.1) to classify the ponds based on key indicator species, resulting in four groups of ponds with distinct plant communities. Similar to the hydrological status, ponds of the same vegetation classification were grouped during analysis to assess the influence of vegetation on the spatial heterogeneity in *F* CO_2 among ponds.

4.B.iii. Meteorological Data

Wind speed, precipitation and temperature data were used to assess the impact of climatic variability on the *F* CO_2 from the ponds. Meteorological data was obtained from Boulmer Weather Station, approximately 12 km north of Hauxley Nature Reserve. Meteorological data from Boulmer has been shown to be closely correlated with those at Newcastle Weather Centre and Albermarle, both ~ 60 km south, suggesting that Boulmer provides representative meteorological data for the region (Jeffries, 2015).

4.C. Data Analysis - ANOVA

To assess both spatial and temporal variations in the *F* CO_2 among ponds, statistical analysis was conducted using a mixed model Repeat Measures Analysis of Variance (ANOVA) using IBM SPSS statistics 22, as described in Chapter II, Section 4.E.iv.a. Individual ponds across the site were considered to be the subjects, and run as a random factor of the total population of ponds. Individual *F* CO_2 measurements over time were considered to be repeat measures with an AR[1] covariance structure that assumes the relationship between measurements changes in a systematic way, i.e., *F* CO_2 on Day 0 is more closely related to that of Day 4 than Day 12. Measurements of *F* CO_2 were the dependent variables, with the fixed factors being Vegetation Type as grouped by TWINSPAN (Groups 1.1, 1.2, 2.1, and 2.2) and Hydrological status at the time of sampling (Aquatic phase, Transitional phase, or Dry). Post-hoc comparisons were performed using Bonferroni test and all statistical significance is reported to 95 % confidence. As with the data in Chapters II and III, data was tested for normality using tests and graphical plots (e.g. Shapiro-Wilk test, stem and leaf plots. Tests and plots run in SPSS). In the majority of cases data were normally distributed, but not in all cases (e.g., for some of the *F* CO_2 measurements among ponds on individual sampling days). Again, as with Chapters II and III, log-transformation of data was found to have little impact on the statistical analysis produced by ANOVA, and did not change the statistical outcome of the results and as such all statistical analysis was conducted on the un-transformed data for transparency. All statistical significance is reported to 95 % confidence.

4.D. Method Development

While the analysis within this chapter focuses upon the spatial, and short term temporal variations in *F* CO_2 rates among ponds, it is noted that a key limitation of the data is the relatively brief time period covered (only two sampling phases), restricting the ability to calculate mean annual flux rates and extrapolate findings to assess with certainty whether the ponds surveyed act as net sources or sinks of CO_2 over the course of a full year. It was initially intended that sampling would be conducted across all four seasons to provide full annual measurements, however, extensive method development was required, ultimately resulting in time restrictions for actual sampling. A brief discussion of sampling difficulties and method development is provided here.

4.D.i. Construction of the floating chamber

Calculation of *F* CO_2 is dependent on acquiring a clear concentration gradient, determined by the magnitude of the flux rate itself, and subsequent change in concentration within the headspace of the chamber, dependent on its volume. As few studies had been conducted on CO_2 flux rates from ponds, the broad rates of emissions to be expected were unknown, and subsequently the volume of the floating chamber required to detect any change in CO_2 concentration needed to be determined. Three floating chambers were tested, with volumes of 7.25, 16 and 32 L, in order to assess which gave the quickest, but also clearest, change in concentration of CO_2. The smallest chamber (7.25 L) proved the most successful, providing a clearly defined concentration gradient within a five-minute sampling period, and was subsequently used for the rest of the study.

4.D.ii. Analytical drift

The Gasmet FT-IR analyser is designed to be used as a portable instrument that can run on battery power, making it ideal for the in-situ measurements required for this study. However, whilst sampling atmospheric CO_2 concentrations it was observed that there was a decrease in the detection of the instrument, dropping considerably below atmospheric concentrations (< 300 ppm CO_2). It was determined that this drop in detection was linked to the decrease in battery power, and while the batteries typically last for 3 hours of continuous running, a decrease in accuracy was observed within the first 30 minutes. The decrease in detection was not observed when running the Gasmet on mains power, and was likely a result of the age of the batteries, yet due to the in-situ requirements of the study, an alternative was necessary. This problem was overcome by using a car battery and converter stored within a departmental sampling vehicle, connected to the Gasmet in the field via a 30 m extension cable, achievable only due to the pond's close proximity to the road. This solution provided a more reliable power source allowing for continual monitoring of pond fluxes throughout the day. The battery life was extended to ~ 10 hours with little to no decrease in detection.

4.D.iii. Diurnal measurements

As with all systems where CO_2 uptake is dependent on photosynthetic activity, a natural respiration cycle is observed throughout the day, with *F* CO_2 often being highest at night, and potentially counteracting any uptake of atmospheric CO_2 throughout the day (Chanda et al., 2013). Just as it is important to acquire a full seasonal range of data, it is equally important to acquire nocturnal measurements if an accurate quantification of *F* CO_2 from ponds is to be acquired. Acquiring diurnal measurements was within the original scope of this study, with the aim being to monitor three ponds nine times over a 24 hour period. Sampling times were not set to specific times, but adjusted on each date to match the hours of sunlight throughout the day (i.e., following peaks of photosynthetic activity), sampling at: midday; halfway between midday and sunset; sunset; halfway between sunset and midnight; midnight; halfway between midnight and sunrise; sunrise; halfway between sunrise

and noon; and noon once again. Multiple attempts were made to acquire a full range of diurnal flux measurements to assess the level of respiration once photosynthetic activity had stopped, however this proved considerably more difficult than anticipated.

As stated above battery power was a considerable limitation within this study, and whilst the initial complication of a decrease in detection was resolved with the use of a car battery and converter, this was still limited to ~ 10 hours: each of the diurnal sampling points would take ~ 1 hour with a further 30 minutes required to zero calibrate the Gasmet. Furthermore, while the Gasmet is portable, it is not weather proof, and finding a sampling period without rainfall was considerably more difficult than anticipated: a note for not just the diurnal measurements, but for daytime measurements as well. A second key issue encountered in nocturnal sampling was that it was dark, very dark, without any surrounding light (e.g., street lights) meaning all processes had to be conducted with torches. Floodlights could not be used as this may trigger photosynthetic activity.

Whilst this chapter focuses on $F\,CO_2$ across eight different days, > 20 days of further sampling were attempted (for both seasonal variations and diurnal measurements) that proved unsuccessful. While this lack of diurnal measurements does not detract from the importance of this study (i.e., establishing the spatial and temporal variations in $F\,CO_2$ among ponds) it does however limit the ability to extrapolate with certainty whether the ponds act as net sources or sinks of CO_2.

The difficulties experienced in the method development stages of this study highlight a key methodological difficulty in acquiring a full set of accurate, robust, diurnal and seasonal flux measurements for aquatic systems. In-situ monitoring proves difficult and is a key reason for the limited data of CO_2 flux rates available for aquatic systems which restricts their inclusion in regional or national C budgets. It is in these situations where automated data loggers prove most useful, yet their application for chamber measurements on aquatic systems is limited. Eddy-covariance systems can be used to monitor $F\,CO_2$ on larger water bodies, however their detection footprint is too large for small aquatic systems without also detecting emissions from the surrounding terrestrial environment (von Schiller et al., 2014). Automated systems where a chamber is mechanically placed on the water surfaces and $F\,CO_2$ is measured by in-situ analysers do exist, however these are expensive and not widely available. In choosing between manual and automated systems a trade-off must be made between spatial or temporal resolution respectively (Savage and Davidson, 2003): in this study, a higher spatial resolution was chosen.

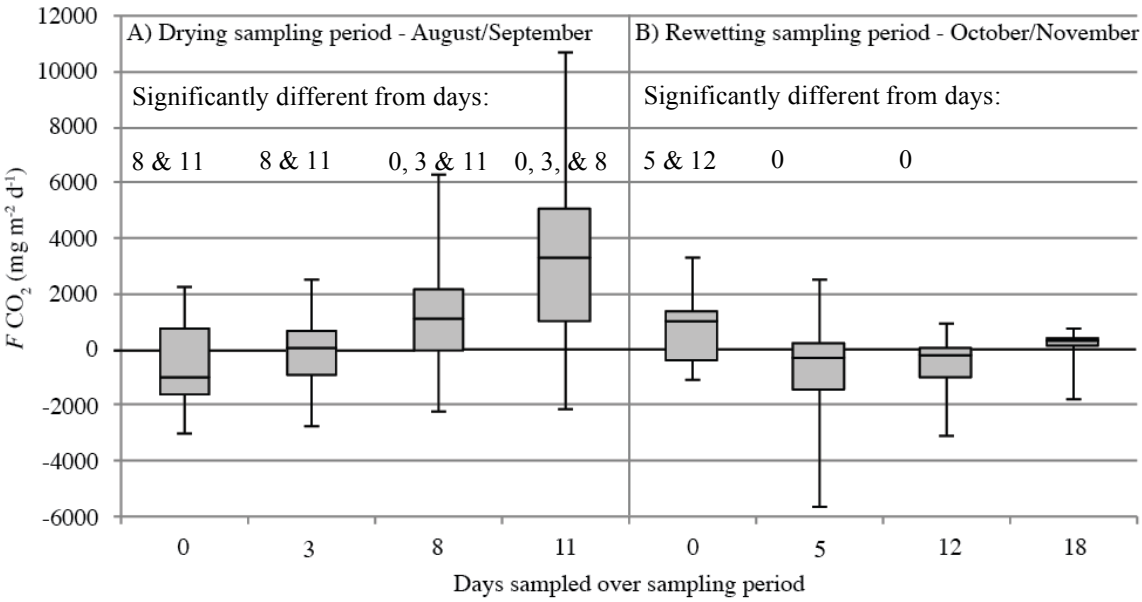
5. Results

The overall mean ecosystem-flux (E-*F*) CO_2 observed for all ponds in this study was 429 mg m^{-2} d^{-1} (± SD = 2041, range = -5651 to 10658) being a net emission of CO_2. The mean E-*F* CO_2 was significantly higher during the drying monitoring phase than the rewetting phase (F = 14, df = 1,70, p = 0.00), with the ponds acting as a net source of CO_2 to the atmosphere during the drying phase (900 ± 2472 mg m^{-2} d^{-1}, range = -2994 to 10658) compared to a net sink in the rewetting phase (-43 ± 1331 mg m^{-2} d^{-1}, range = -5651 to 3330).

5.A. Drying Monitoring Period

Mean flux rates of ponds in the drying phase varied markedly among the four sampling days (Figure IV.5.A), with mean E-*F* CO_2 switching from a net intake on Day 0 (mean ± SD = -641 ± 1490 mg m^{-2} d^{-1}, range = -2294 to 2263), to near neutral on Day 3 (-1 ± 1421 mg m^{-2} d^{-1}, range = -2793 to 2513), and markedly higher net emissions on Day 8 (1184 ± 1854 mg m^{-2} d^{-1}, range = -2229 to 6300), and Day 11 (3058 ± 2975 mg m^{-2} d^{-1}, range = -2154 to 10658). The *F* CO_2 from ponds on the four sampling days varied significantly (F = 9, df = 1, 52, p = < 0.01) with pairwise comparisons being statistically different between days 0-8 (p = < 0.01) and 0-11 (p = < 0.01), days 3-8 (p = 0.05) and 3-11 (p = < 0.01), and days 8-11 (p = < 0.01). Only flux rates on days 0 and 3 were not significantly different (p = > 0.05).

Figure IV.5: Daily ecosystem flux of CO_2 over both the drying and rewetting sampling phases.



Boxplots show the median, upper and lower quartiles, and the minimum and maximums. Negative values represent a net intake of CO_2 by the ponds whilst positive values represent a net emission to the atmosphere.

5.B. Rewetting Monitoring Period

Mean flux rates in the rewetting sampling period also varied among the four sampling days (Figure IV.5.B.), with mean E-*F* CO_2 dropping from a net emission on Day 0 (mean ± SD = 765 ± 1291mg m^{-2} d^{-1}, range = -1090 to 3330), to net intake on Day 5 (-623 ± 1767 mg m^{-2} d^{-1}, range = -5651 to 2543), and Day 12 (-461 ± 906 mg m^{-2} d^{-1}, range = -3061 to 983), and back to a net emission by Day 18 (147 ± 528 mg m^{-2} d^{-1}, range = -1778 to 793). Significant difference was observed among flux rates across the four sampling days (F = 11, df = 1, 77, p = 0.00), with *F* CO_2 on Day 0 being significantly higher than Day 5 (p = 0.00) and Day 12 (p = 0.00), though no other significant differences were observed among alternative pairwise comparisons.

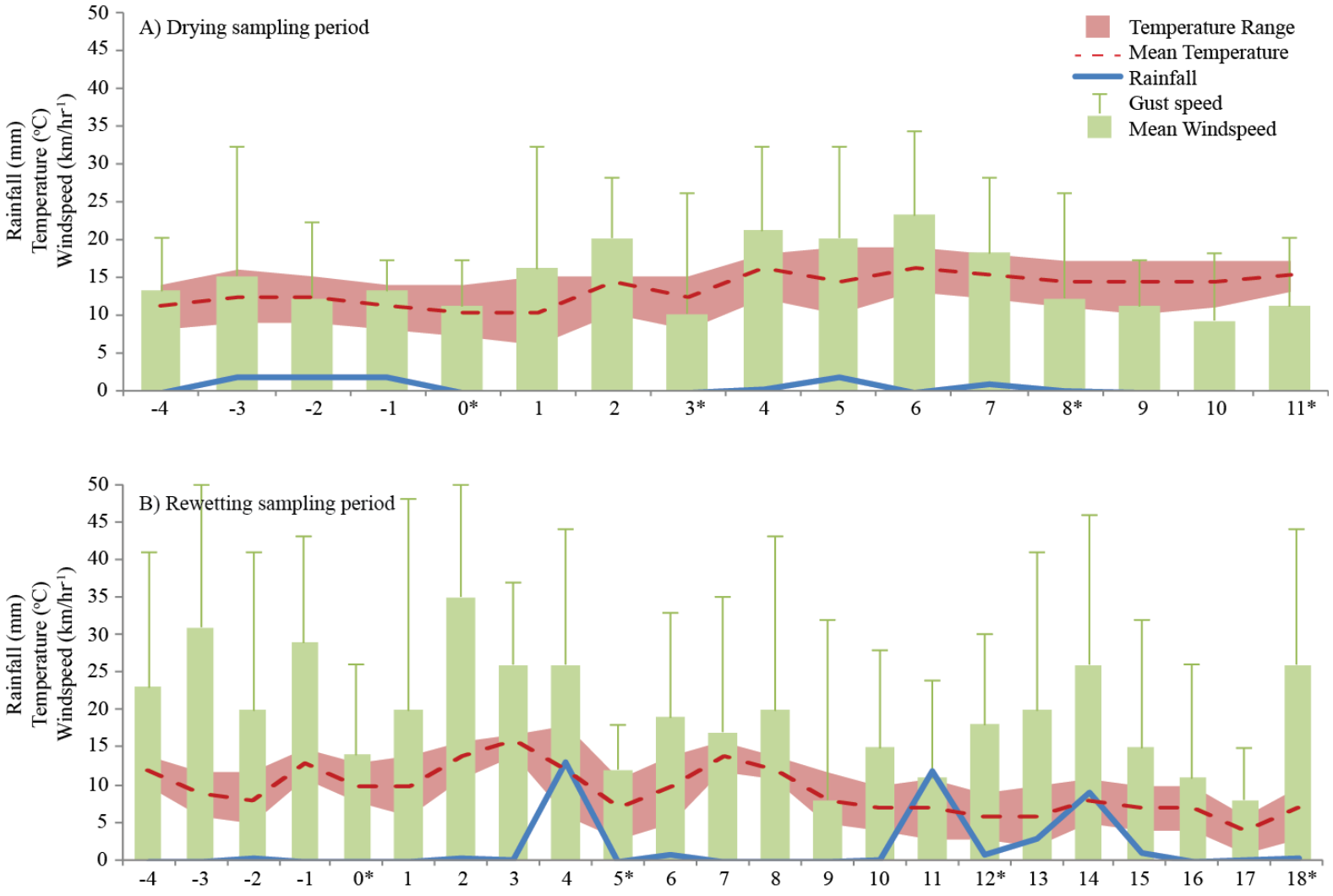
It should be noted that in the Repeat Measures ANOVA used to compare mean daily flux rates over the sampling period, Mauchly's test indicated that the assumption of sphericity had been violated (x^2 [5] = 12.85, p = < 0.05): i.e., there was a degree of non-independence of data due to the repeated measures. Therefore, degrees of freedom were corrected using Huynh-Feldt estimates of sphericity. This is not merely a necessary statistical adjustment but also reveals an important outcome, indicating a significant degree of variability within the data associated with the individual pond being monitored.

5.C. Climatic Variations

The weather patterns during the drying and rewetting monitoring periods were typical of the times of year, with wind speed, precipitation, and temperature being in line with seasonal averages. Mean daily temperature throughout the drying monitoring period was higher than that of the rewetting (13 °C and 9 °C respectively), whilst mean wind speed (15 km hr^{-1} and 20 km hr^{-1} respectively) and precipitation (0.6 mm and 1.80 mm respectively) were lower in the drying period than the rewetting period.

During the drying monitoring period there was no major change in weather over the sampling period, with wind speed and atmospheric temperature remaining relatively constant (Figure IV.6.A.). The only notable aspect of the weather over the actual sampling period was the absence of precipitation. The total rainfall in the three weeks preceding sampling was 39.37 mm with the last substantial rainfall (6.10 mm) being 11 days prior to sampling on Day 0. With only 3.6 mm of rainfall over the course of the sampling period itself the ponds quickly dried up, with the vegetation in many of the ponds wilting by the end of the sampling period.

Figure IV.6: Meteorological data over: (A) drying monitoring phase; (B) rewetting monitoring phase. Meteorologial data is from Boulmer weather station, approximately 12 km north of Hauxley Nature Reserve. * Days *F* CO_2 monitored.



Contrasting the drying monitoring period, the weather during the rewetting monitoring phase was considerably more variable with both wind speed and temperature being markedly higher during the first half of the monitoring period compared to the second half (Figure IV.6.B.). Three large precipitation events occurred over the course of the sampling period, resulting in a total precipitation of 41.0 mm, which was considerably higher than the 14.3 mm in the two weeks preceding the monitoring period.

5.D. Influence of Hydrology

The changes in precipitation rates over the two sampling periods, as well as the overall differences between the two seasons, resulted in shifts in the hydrological regimes of the pond systems. During the drying monitoring phase the prolonged absence of rainfall led to a drying out of the pond systems, with the number of ponds in the Dry Phase increasing from 5 to 13, and those in an Aquatic Phase decreasing from 8 to 0 (Figure IV.7.). Contrasting this, increased rainfall during the rewetting monitoring period resulted in all ponds being in an Aquatic Phase by Days 11 and 18, compared to mostly Transitional Phase on Days 0 and 4 (Figure IV.8).

Statistical difference was observed in $F\ CO_2$ among ponds when grouped by their hydrological status ($F = 41$, $df = 2, 155$, $p = < 0.01$), with ponds that were Dry at the time of sampling (mean ± SD = 2824 ± 2238 mg m^{-2} d^{-1}, range = 56 to 10658) being significantly higher than those in a Transitional Phase (57 ± 1723 mg m^{-2} d^{-1}, range = -4914 to 4823, $p = < 0.01$) and those in an Aquatic Phase (-439 ± 1039 mg m^{-2} d^{-1}, range = -5651 to 983, $p = < 0.01$), but not between those in the Transitional and Aquatic phases ($p = > 0.05$).

5.E. Influence of Vegetation

Subtle variations in the hydrology of the site have led to marked differences in the vegetation between the ponds. Thirty-six species of macrophyte were recorded in total. *Eleocharis palustris* was the indicator species in Groups 1.2 and 2.1, totalling 20 of the 26 ponds. *Glyceria fluitans* (L.) R. Br. and *Carex otrubae* Podp. were indicator species in Group 1.1, whilst *Carex glauca* and *Ranunculus aquatilis* L. were the indicator species in Group 2.2. Figure IV.9 shows the location of each pond grouped by the four vegetation groups and their mean daily E-$F\ CO_2$ shown in Figure IV.10.

Figure IV.7: Fluxes of CO_2 during the drying monitoring phase. (A) shows the flux CO_2 from each individual pond grouped by hydrological status at the time of sampling. (B) shows the flux CO_2 for each pond as they are situated across the site; area of bubbles represents the flux CO_2 relative to one another, with hollow bubbles representing negative values.

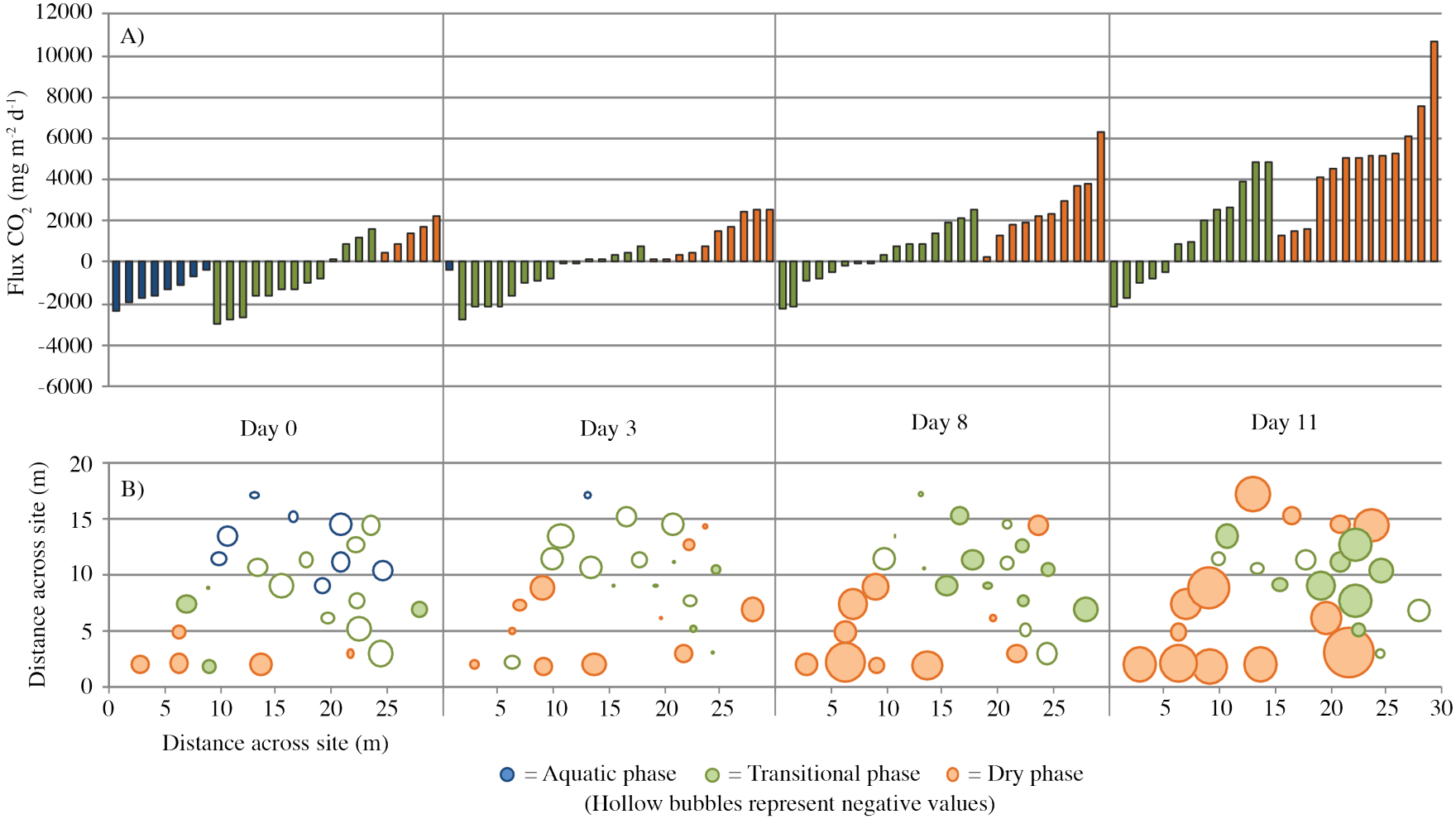
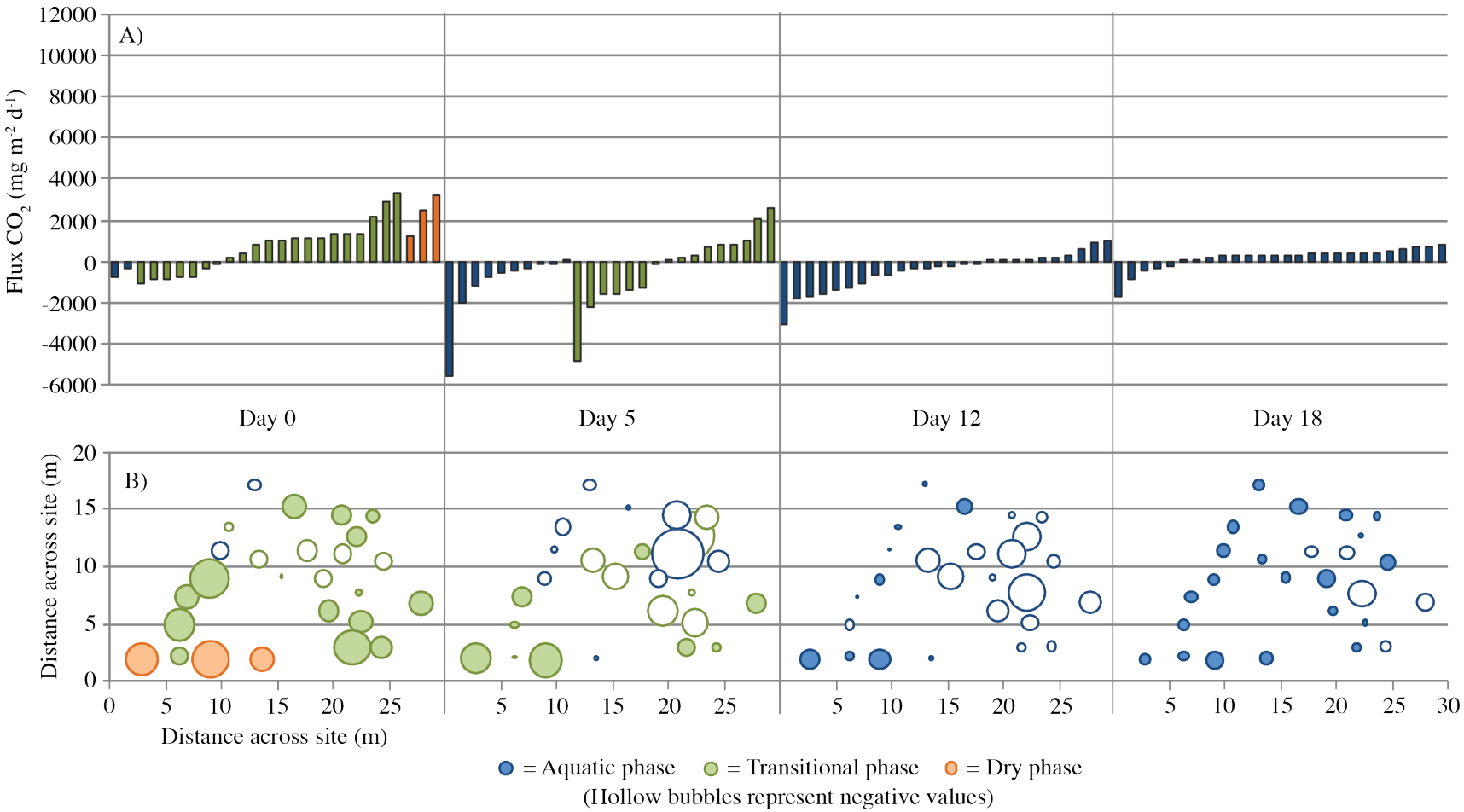


Figure IV.8: Fluxes of CO_2 during the rewetting monitoring phase. (A) shows the flux CO_2 from each individual pond grouped by hydrological status at the time of sampling. (B) shows the flux CO_2 for each pond as they are situated across the site; area of bubbles represents the flux CO_2 relative to one another, with hollow bubbles representing negative values.



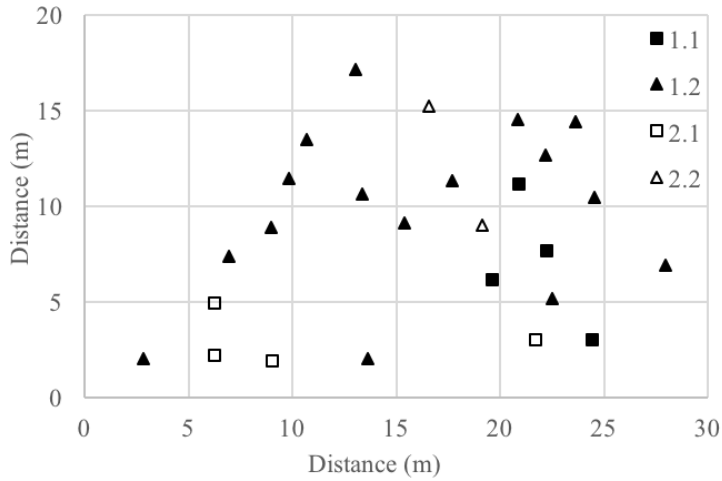
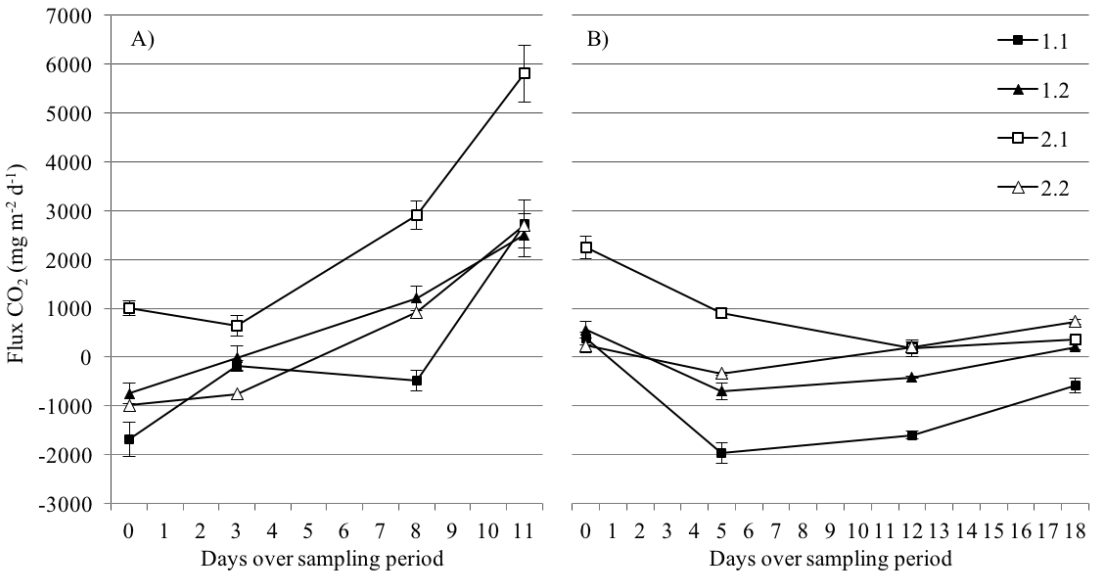


Figure IV.9: Location of each pond across the site, grouped by their vegetation type as identified by TWINSPAN.

Significant difference was observed in the mean $F\,CO_2$ among vegetation groups across both sampling seasons (F = 4, df = 1, 61.7, $p = 0.01$), being significantly higher in ponds from Group 2.1 (mean ± SD = 1753 ± 1428 mg m^{-2} d^{-1}, range = -414 to 4025) than ponds from Group 1.1 (-424 ± 1267 mg m^{-2} d^{-1}, range = -1824 to 1550, $p = 0.01$) and Group 1.2 (322 ± 867 mg m^{-2} d^{-1}, range = -725 to 1529, $p = 0.05$), and higher, but not significantly, than Group 2.2 (337 ± 931 mg m^{-2} d^{-1}, range = -664 to 1713, $p = 0.51$). No significant difference was observed among other pairwise comparisons. When the two sampling seasons were separated the mean $F\,CO_2$ was highest from ponds in Group 2.1 and lowest in ponds of Group 1.1 in both the drying and rewetting sampling periods.

Figure IV.10: Mean daily flux CO_2 for ponds within each vegetation group during: (A) drying monitoring phase; (B) rewetting monitoring phase. Error bars show the standard error.



6. Discussion

The aim of this study was to quantify fluxes of CO_2 for small temporary ponds; a habitat type previously overlooked in most C flux models. Short term variations were monitored over several weeks to assess the response of fluxes to rapid hydrological shifts, whilst inter seasonal sampling provides an indication of the annual variation. The 26 experimental ponds monitored were the same size, age and located in the same field, providing insights into the spatial heterogeneity of flux rates among superficially similar ponds. The data provides evidence of spatial and temporal heterogeneity of CO_2 fluxes among small aquatic systems.

The overall mean E-*F* CO_2 observed for all ponds in this study (mean ± SD = 429 ± 2041 mg C m^{-2} d^{-1}, range = -5651 to 10658) indicates that on average these systems act as a net source of CO_2 to the atmosphere. While the overall range is in line with those observed for shallow terrestrial aquatic systems (-11734 to 26745 mg m^{-2} d^{-1}; Torgersen & Branco 2007), the mean is lower than that reported for larger ponds (1002 mg m^{-2} d^{-1}, Torgersen & Branco 2008) and temporary ponds during drying events (5325 mg m^{-2} d^{-1}, Catalán et al. 2014).

6.A. Temporal Variation in CO_2 Flux Rates

6.A.i. Long term temporal variation between seasons

Whilst the overall mean observed in this study was a net ecosystem emission of CO_2 to the atmosphere, significant differences were observed between the two study periods, with the mean E-*F* CO_2 in the drying monitoring period being a net emission (mean ± SD = 900 ± 2472 mg m^{-2} d^{-1}), and a net sink during the rewetting period (-43 ± 1331 mg m^{-2} d^{-1}). The significant difference between the two seasons indicates that there is a large temporal variation in CO_2 flux rates throughout the year, yet without full annual measurements it is impossible to state from this dataset whether these systems act as net sinks or sources of CO_2. Similar shifts in the direction of *F* CO_2 have been reported for other temporary aquatic systems during drying periods; i.e., ponds, river courses, wetland, and tidal regions (Catalán et al., 2014; Fromin et al., 2010; Schiller et al., 2014). With flux rates varying so significantly it is crucial that annual ecosystem flux estimations are based on a full range of seasonal measurements rather than measurements from a single season.

6.A.ii. Short term temporal variation between days

As well as the long term temporal variations in E-*F* CO_2 observed between seasons, considerable short term variation was observed among sampling days within seasons. In the drying sampling period the E-*F* CO_2 was a net sink on Day 0, yet this had shifted to a net source of CO_2 to the atmosphere only 3 days later, and reached a 9-fold increase in CO_2 efflux by the end of the two weeks sampling period. Equally, whilst the range of daily mean E-*F* CO_2 in the rewetting period was

not as large as that of the drying period, significant difference was observed among days, further highlighting the short term temporal variation within seasons. Similarly to the long term seasonal variations, this short term variation in fluxes highlights the risk of extrapolating to seasonal estimations from flux measurements acquired on individual days alone, where fluxes may not be representative of the overall mean. Furthermore, the acquisition of night time measurements is crucial for accurately quantifying the net diurnal $F\ CO_2$ within ponds systems, and as discussed in Section 4.D.iv., these can prove difficult to acquire when using manual monitoring programs. While this study provides a high resolution insight into the spatial variation among ponds, it may be that automated monitoring systems are the only reliable technique to gather the significant data required to accurately quantify the temporal variations in $F\ CO_2$.

6.B. Hydrology as a Factor of Temporal Variation

The hydrological regime of temporary aquatic systems plays a key role in determining flux rates, with $F\ CO_2$ being significantly higher in ponds when dry, resulting in considerable spatial variation among systems where hydrological status differs; i.e., wet or dry. Recharge of the ponds in this study is largely dependant upon precipitation and, due to their small volume, changes in rainfall over short time scales can result in a rapid shift of their hydrological status from wet to dry or vice-versa. Subsequently, variations in precipitation rates and hydrological regime can be a major contributor to the temporal variation in $F\ CO_2$.

This influence of precipitation and hydrological regime on $F\ CO_2$ was observed in the temporal variations of both the short term monitoring within seasons, and long term between seasons. During the drying monitoring period the ponds exhibited a shift from a net intake of atmospheric CO_2, to a net emission. Moreover the $F\ CO_2$ continued to increase throughout the monitoring period long after the initial drying event. A factor that might have exacerbated CO_2 emissions between Days 3, 8, and 11 was the precipitation on Days 5 and 7 (Figure IV.6.A.). Whilst only small and not enough to fully rehydrate the ponds, rewetting of sediments or soils after dry periods is known to increases C lability and microbial activity resulting in rapid release of CO_2, also known as 'hotspot' events (Fromin et al., 2010; Schiller et al., 2014). Equally, along with an initial peak in $F\ CO_2$ at the beginning of the drying processes, substrate induced respiration from microbial activity has been shown to peak after 3 weeks, with emissions decreasing in the remaining drought period (Fromin et al., 2010). While it is likely that $F\ CO_2$ beyond this survey period may decrease when all ponds enter a stable drought phase, this study highlights the high $F\ CO_2$ emissions during the initial drying phase. Furthermore, it is likely that temporary wetlands in temperate climates are especially vulnerable to such flux peaks and hotspot events as they often undergo several wetting and drying cycles during summer: subsequently net summer wetland E-$F\ CO_2$ may be greater in temperate climates than those of warmer climates where temporary aquatic habitats typically remain dry for the entire season.

The hydrological regime of the rewetting monitoring period mirrors that of the drying, with considerable precipitation on days 4, 11, and 14 resulting in all ponds becoming inundated. While sampling occurred on the following days (i.e., days 5 and 12) no clear 'hotspot' event was observed. Furthermore, the spatial variability in $F\,CO_2$ among ponds (i.e., the range of values observed on individual days) was lowest on Day 18 when all ponds had been inundated for at least 7 days. This suggests that both spatial and temporal variations in $F\,CO_2$ are lowest when ponds are in a steady, stable state of inundation, and with the predicted likely increase in precipitation their potential role as sinks of atmospheric CO_2 could increase. This study however, does not take into account the influence of hydrology on CH_4 production and emission which is highest when ponds remain permanently inundated and anoxic. Considering the importance of CH_4 as a GHG it is important that it is included in future investigations.

The short term changes in hydrological regime observed in this study mimics the broader seasonal changes that occur, highlighting the need for both short-term and long-term monitoring programmes in order to accurately quantify the $F\,CO_2$ from temporary aquatic environments.

6.C. Influence of Vegetation on Spatial Heterogeneity of CO_2 Fluxes

While the differences within and among the drying and rewetting monitoring periods highlights the temporal variation in the $F\,CO_2$, there is still considerable variation among ponds on individual sampling days. The 26 experimental ponds monitored are the same size, age and located in the same field, making them as close to field replicates as possible. However, subtle variation in drainage across the site has resulted in different water retention times among ponds, and subsequently, different vegetation groups have developed.

In both the drying and rewetting monitoring periods, when grouped by TWINSPAN analysis of their vegetation the mean E-$F\,CO_2$ was lowest in ponds within Group 1.1 (-424 ± 1267 mg m^{-2} d^{-1}, range = -1824 to 1550), and highest in Group 2.1 (mean ± SD = 1753 ± 1428 mg m^{-2} d^{-1}, range = -414 to 4025). With significant difference observed ($p = 0.01$) and the fact that one vegetation type acts as a net sink whilst another acts as a net source of CO_2 indicates that vegetation may play a roll in determining CO_2 fluxes from small aquatic systems. The implications from this for the management of ponds to increase their potential CO_2 capture is clear, and the role of vegetation types should be investigated further to elucidate their exact influence on CO_2 release rates.

Personal observations revealed that ponds with thick vegetation layers held in moisture longer during the drying period, slowing the processes of sediment desiccation compared to ponds with a looser, thinner, vegetation coverage. The sediments underneath the vegetation were conspicuously darker,

damper and appeared anoxic compared to the few ponds lacking extensive vegetation cover. It may be that during brief periods of low rainfall and potential sediment desiccation, these vegetation mats act as a moisture 'barrier' preventing complete sediment desiccation and limiting CO_2 mobilisation. Equally this may be one factor contributing to the continued increase in CO_2 flux rates over the drying monitoring period as the swards of grasses and moss overlying the bottom of the ponds continued to dry out during the switch from transitional phase to dry phase, permitting oxic sediment conditions.

While significant difference was observed in the *F* CO_2 among ponds of differing vegetation types it is important to note that the relationship does not specify a cause, and that vegetation type may have no direct influence on CO_2 flux rates. It may be that an underlying control dictating vegetation groups, such as pond permanence, also influences CO_2 flux rates. As the vegetation communities are a consequence of the subtle differences in hydrological patterns among ponds, it may be that the differences in *F* CO_2 observed among vegetation groups is simply due to differences in hydrology at the time of sampling, with vegetation as a co-variant. Equally, vegetation communities in temporary systems change regularly depending on annual climate variations (Jeffries, 2008) and as such it may be that the previous macrophyte communities, which now comprise the sediment layer, result in differences in organic matter lability, and subsequently may be more important than the growth of current plant communities. Again, the ponds in this survey are in a mid/late successional stage of a ponds life cycle with their vegetation communities changing over the course of a ponds lifecycle: it may also be that *F* CO_2 vary throughout a ponds life cycle as the vegetation communities shift.

The large degree of spatial heterogeneity among ponds poses complications for accurately quantifying the E-*F* CO_2 on a landscape scale where a complete inversion of the flux rate estimates may occur if too few ponds, or an unrepresentative group are chosen. The use of eddy covariance for monitoring terrestrial net ecosystem exchange over comparably large areas (> 100 m^2) provides a useful comparison to the flux from individual ponds (Abnizova et al., 2012) or for monitoring *F* CO_2 from larger water bodies (Fromin et al., 2010). However, the use of eddy covariance on a landscape scale can easily overlook the influence of individual ponds, especially during wet and dry cycles. More effort is needed to underpin the constraints of hydrology on the frequency of drying and rewetting cycles and their impact on *F* CO_2 among ponds across the landscape if accurate regional extrapolations of these small systems are to be acquired.

7. Conclusion

This study set out to monitor spatial and temporal changes to CO_2 flux rates in small, temporary ponds in a typical lowland European landscape during both drying and rewetting periods. While the overall mean E-*F* CO_2 observed in this study (mean ± SD = 429 ± 2041 mg C m^{-2} d^{-1}, range = -5651 to 10658) was a net emission, considerable long-term temporal change was observed among sampling periods, being significantly higher during the drying sampling period than the rewetting period. Furthermore, the results showed striking short-term temporal change in E-*F* CO_2 linked to hydrological changes, with ponds exhibiting a nine-fold difference in flux rates over a two week drying period alone. These results indicate that the hydrological status of temporary ponds can have a considerable influence in determining their CO_2 flux direction. Equally, significant difference was also observed in the spatial heterogeneity of *F* CO_2 among ponds when grouped by their dominant vegetation types, indicating that the individual plant communities within ponds themselves may also have considerable influence in determining the direction of CO_2 fluxes from temporary ponds. These results show that small scale spatial and temporal changes can result in large variations in CO_2 fluxes to the atmosphere from temporary ponds.

The organic carbon content of sediments from other ponds in the area is high compared to adjacent non-wetland habitat indicating a net accumulation of carbon within temporal aquatic systems (Gilbert et al., 2014). Yet the data presented here highlights the interaction of carbon fluxes with the atmosphere. Taken together the sediment storage and flux rates suggest that these ecosystems have the potential to be highly active sequesters of atmospheric carbon if hydrated during summer months as illustrated by the net intake of CO_2 on Day 0 of the drying monitoring period. However, this high degree of temporal variability in the *F* CO_2 over such a short period of time poses serious complications for extrapolations of measurements to seasonal averages from singular measurements alone and highlights the need for more comprehensive surveys when trying to form accurate estimations.

Without complete annual and diurnal flux measurements and carbon burial rates it would be inappropriate to extrapolate any study to state whether small aquatic systems act as a net sources or net sinks to the atmosphere. However, it is impossible to ignore the fact that these constructed ponds have in-filled almost completely with ~ 30 cm of sediment over a 15 year period since their creation, and therefore appear to be sinks. Nonetheless, the flux rates observed in this study indicate that whilst temporary ponds may act as sources of CO_2 during drying periods, when inundated they have the potential to act as sinks of atmospheric CO_2. These results also suggest important practical outcomes, notably the potential for small ponds as CO_2 sinks, if the frequency of inundation periods and vegetation communities are managed. Management of land use and soil/vegetation interactions has

been proposed for the sequestration of atmospheric CO_2 (Manning and Renforth, 2013) and there is potential for similar approaches to ponds. However, a key management outcome from our study is the need to get the design of pond environment right to maximize their effectiveness in the face of natural climate variations and the threat of greater climatic variation.

Chapter V. Conclusions and Outlook

1. Overview

This thesis investigated the processing and storage of C within small ponds, primarily in Northumberland but also with smaller datasets from selected regions across England. This study not only provides new data on pond sediment C stocks and the variations among biogeographically and ecologically diverse systems, but is also the first study of its kind to document the response in *F* CO_2 to hydrological changes with a high degree of both spatial and temporal resolution. A further uniqueness of this study is that it focuses upon small natural ponds (< 10,000 m^2), including those of a temporary nature; a habitat type wholly missed from C budgets, presenting new data for a currently understudied aquatic habitat type and providing new evidence for the importance of small ponds in the C cycle.

1.A. Pond Sediment C Stocks

1.A.i. The variations of C stocks among ponds

The studies in Chapters II and III together comprise one of the most comprehensive surveys of pond sediment C stocks to date (> 1500 individual sediment samples from 55 ponds) from a variety of biogeographical and biodiverse pond habitat types across England. Marked variation was observed among regional study sites, with calculated C stocks being higher in post glacial pingo ponds from Norfolk (mean ± SD = 7.7 ± 1.1 kg C $m^{-2}_{< 10\ cm}$, range = 5.8-9.0), compared to peat excavation ponds in Yorkshire (6.0 ± 1.8 kg C $m^{-2}_{< 10\ cm}$, range = 4.0-9.0), agricultural lowlands of Northumberland (4.4 ± 2.21 kg C $m^{-2}_{< 10\ cm}$, range = 1.17-12.32), and shallow temporary ponds of Mediterranean classification in Cornwall (2.6 ± 1.0 kg C $m^{-2}_{< 10\ cm}$, range ± 1.6-4.5). Moreover, as well as marked variation among biogeographically distinct regions, the large spatial variation in C stocks observed among ponds of differing ecological classifications within the same region, as well as among those of the same ecological classification (i.e., the regional study of Northumberland, Chapter II), highlights the extent to which C stocks can vary among ponds within localised regions. This large variation has considerable implication for the accurate quantification and extrapolation of C stocks from studies that are limited in both number of ponds surveyed and geographical spread. Yet a useful conclusion from the variation in results is that ponds located in dune slacks and dominated by *Agrostis stolonifera* are best suited to high levels of C storage, a useful outcome for the construction and management of ponds for the purpose of sequestering atmospheric CO_2. Certainly it is evident that C stocks are not the same for all ponds, and there is need to elucidate the C storage and

processing rates among different types of ponds if they are to be successfully integrated into C budgets.

1.A.ii. National estimates of pond sediment C stocks

The overall mean C stocks of pond sediments surveyed across England from Northumberland, Yorkshire, Norfolk, and Cornwall (± SD = 4.65 ± 2.35 kg C $m^{-2}_{< 10\ cm}$, range = 1.17-12.32) was directly comparable to the mid-range of values reported by the countryside survey (range = 3.2-5.9 kg C $m^{-2}_{< 10\ cm}$; CS, 2007) being higher than those of coastal margins, agricultural land, grassland, and woodland, yet lower than wetlands, bogs, and fens, marshes and swamps. With pond sediment C stocks being directly comparable to these major land use types it highlights the importance of achieving their inclusion in national C budgets. Furthermore, when extrapolating this mean value of 4.65 kg C $m^{-2}_{< 10\ cm}$ to national C stocks based on UK pond numbers (Williams et al., 2010) it is estimated that pond sediments of Great Britain alone hold 2.01 Mt C. However, there are two key outcomes from the sediment surveys conducted in this thesis, neither of which are addressed in the majority of academic and IGO literature: (1) the need to establish clear sediment depths to allow quantification of absolute C stocks, and; (2) the acquisition of accurate sediment accumulation and C burial rates, without which it is impossible compare the true C storage capability of two contrasting systems (e.g., the similar C stock values reported for sediment and soils in this study). It is crucial that future efforts are focused upon quantifying sediment burial rates within small natural ponds, so that their rates of C accumulation can be accurately quantified and their full importance in the C cycle determined.

Regardless, given that the estimated sediment C stock of ponds in Great Britain (2.01 Mt C) is based solely on the upper 10 cm of sediment, this figure is likely to be extremely conservative, and will no doubt increase with further studies of pond C stocks. Furthermore, when calculated as CO_2e, this equates to 7.38 Mt CO_2e; roughly equivalent to ~ 1.4 % of the 2014 total UK greenhouse gas emissions (514.4 Mt CO_2e), and directly comparable to the net intake of 9 Mt CO_2e by land use, land use change and forestry (DECC, 2016) highlighting the true volume of C stored within pond sediments. Given predicted increases in climatic variability and rainfall there is increasing likelihood for the creation of new pond environments, especially if properly integrated into land management practices (i.e., UK policy for Ecological Focus Areas on 5 % of arable land; DEFRA, 2013), and given the results of this national sediment survey they have considerable potential as future sinks of atmospheric CO_2.

1.B. CO_2 Flux Rates from Ponds

Inland waters have been shown to be major sites for remobilisation of terrestrial C to the atmosphere, yet data for small, especially temporary, aquatic features is sparse. This thesis provides a unique high

resolution study on the spatial and temporal variability of *F* CO_2 for small, temporary ponds. Mean CO_2 flux rates for ponds were significantly higher when dry than when in a transitional or inundated phase, with *F* CO_2 being comparable to both the lower and upper flux rates reported for other ecosystems (Raymond et al., 2013). Moreover, small temporary ponds are susceptible to rapid changes in hydrology, with strikingly short term temporal change in E-*F* CO_2 observed during changes in hydrological status; a nine-fold difference in flux rates was observed over a two-week period as the ponds shifted from inundated to dry. Equally, large variation was observed among ponds on individual days, highlighting the spatial variability in C processing rates among ponds. The high degree of spatial and temporal variability in the *F* CO_2 over such small distance and time poses serious complications for extrapolations of measurements to seasonal or annual means from singular measurements alone. Only a handful of recent studies have documented CO_2 flux rates for temporary ponds and streams yet these are often compounded by a low spatial and temporal resolution within the data. The variability observed in this study highlights the need for more spatially and temporally comprehensive surveys in order to form accurate estimations of the *F* CO_2 for small, temporary ponds.

Without complete annual and diurnal flux measurements and carbon burial rates it would be inappropriate to state from this study whether small aquatic systems act as net sources or net sinks of C to the atmosphere. Yet the spatial variability among ponds in the data presented in this thesis shows that pond sediment C stocks can be highly elevated above other ecosystems, and that during the correct conditions they can act as active sinks for atmospheric CO_2. An important practical outcome is evident; notably the potential for management or construction of small ponds in order to enhance their C capture capability and storage, and act as net sinks of atmospheric CO_2. Practical manipulation of pond hydrology and vegetation is crucial to get the design of wetland right and maximise their effectiveness in the face of natural climate variations and the threat of greater climatic change.

2. Reflections: 'what does it all mean?'

Small aquatic systems, specifically small ponds, are ignored from global carbon cycles and inventories. There exists a clear need to integrate the micro-scale 'field' processes and observations, such as those reported here, with the top down approaches, like global circulation models, if accurate climate change predictions are to be made. From recent research there is no doubt that small aquatic systems have significant combined global coverage and disproportionately high biochemical processing rates when compared to other terrestrial ecosystems. Yet it is envisaged that a tremendous amount of research would be required just to accurately quantify C stocks at regional and national levels. From the $F\ CO_2$ observed here it is abundantly clear that they are highly dynamic systems, responding almost 'instantaneously' to changes in hydrological conditions, giving them the future potential to be either significant sinks or sources of carbon depending on future changes in climate. However, the sink/source issue is a long way from being established, and perhaps more important is the need to develop a critical understanding of the feedback mechanisms, and their relative importance, requiring a comprehensive evaluation of all pond processes. Only then could a meaningful linkage be made to the global circulation models.

The findings here highlight that ponds can be tremendously heterogeneous, both at the national level and in close proximity. However, a large uncertainty remains in the time it has taken for observed C stocks to accumulate. Equally, whilst soil samples were collected for comparison purposes, and expectations were of lower carbon levels, it was initially surprising that in many cases they were very similar. This specifically highlights the need to acquire accurate burial rates for pond small aquatic systems. Even though C stocks may be similar among ponds and compared to terrestrial ecosystems the question of how long they take to establish is critical, and again largely ignored in the 'pond' literature.

However, what does stand out is that even without quantified sediment burial rates, it is evident that many recent pond systems have accumulated substantial amounts of organic matter. For example, the constructed micro-ponds at Hauxley Nature Reserve (as surveyed for $F\ CO_2$ in Chapter IV) have accumulated > 20 cm of sediment depth over their brief ~ 15 year lifespan. Furthermore, it must be recognised that even these exceptionally high burial rates are conservative, in that, the majority of the sediment would have been accumulated within a peak window of the early/mid successional phase of the ponds lifecycle. The potential for these constructed systems to be further enhanced and utilised in carbon capture is evident. With recent changes to Government policy on agricultural subsidies for biodiversity this may provide a realistic option, giving the necessary biodiversity but also sequester atmospheric carbon more rapidly than any other ecosystem in the UK.

So how important are small ponds? In the author's mind they will prove to be a key mechanism in the carbon cycle, and one of potentially high importance. Aquatic systems are the nexus point between atmospheric and terrestrial processes, and small ponds will be the first and most dynamic of these to respond to climatic change. However, the true extent and importance of these wonderful little ecosystems will only be established after many years of future research.

References

Abnizova, A., J. Siemens, M. Langer, and J. Boike. 2012. Small Ponds with Major Impact: The Relevance of Ponds and Lakes in Permafrost Landscapes to Carbon Dioxide Emissions. *Global Biogeochemical Cycles* 26(2)

Abril, G., J. Martinez, F. Artigas, P. Moreira-Turcq, M. Benedetti, L. Vidal, T. Meziane, J. Kim, M. Bernardes, N. Savoye, J. Deborde, E. Souza, P. Albéric, M. Landim de Souza, F. Roland. 2014. Amazon River Carbon Dioxide Outgassing Fuelled by Wetlands. *Nature* 505(7483):395–98.

Adhikari, S., R. Lal, and B. Sahu. 2012. Carbon Sequestration in the Bottom Sediments of Aquaculture Ponds of Orissa, India. *Ecological Engineering* 47:198–202.

Allen, R. (ed). 1990. *Concise Oxford Dictionary of Current English*. Clarendon Press: Oxford.

Allende, L., T. Guillermo, H., Zagarese, A. Torremorell, G. Perez, J. Bustingorry, R. Escaray, I. Izaguirre. 2009. Phytoplankton and Primary Production in Clear-Vegetated, Inorganic-Turbid and Algal-Turbid Shallow Lakes from the Pampa Plain (Argentina). *Hydrobiologia* 624:45–60.

Allende, L., and G. Mataloni. 2013. Short-Term Analysis of the Phytoplankton Structure and Dynamics in Two Ponds with Distinct Trophic States from Cierva Point (maritime Antarctica). *Polar Biology* 36(5):629–44.

Alonso, I., K. Weston, R. Gregg, and M. Morecroft. 2012. Carbon Storage by Habitat: Review of the Evidence of the Impacts of Management Decisions and Condition of Carbon Stores and Sources. *Natural England Research Report NERR043*. 1-58

Ashworth, W. 1991. *The Encyclopedia of Environmental Studies*. Facts on File: New York

Aufdenkampe, A., E. Mayorga, P. Raymond, J. Melack, S. Doney, S. Alin, R. Aalto, K. Yoo. 2011. Riverine Coupling of Biogeochemical Cycles between Land, Oceans, and Atmosphere. *Frontiers in Ecology and the Environment* 9(1):53–60.

Ballantyne, A., C. Alden, J. Miller, P. Tans, and J. White. 2012. Increase in Observed Net Carbon Dioxide Uptake by Land and Oceans during the Past 50 Years. *Nature* 488:70–72.

Bastviken, D., J. Cole, M. Pace, and L. Tranvik. 2004. Methane Emissions from Lakes: Dependence of Lake Characteristics, Two Regional Assessments, and a Global Estimate. *Global Biogeochemical Cycles* 18(4):GB4009.

Battin, T., S. Luyssaert, L. Kaplan, A. Aufdenkampe, A. Richter, L. Tranvik. 2009. The Boundless Carbon Cycle. *Nature Geoscience* 2(9):598–600.

Batzer, D., S. Dietz-Brantley, B. Taylor, and A. DeBiase. 2005. Evaluating Regional Differences in Macroinvertebrate Communities from Forested Depressional Wetlands across Eastern and Central North America. *Journal of the North American Benthological Society* 24(2):403–14.

Beebee, T. 1991. *Pondlife*. Whittet Books: London.

Della-Bella, V., M. Bazzanti, M. Dowgiallo, and M. Iberite. 2007. Macrophyte Diversity and Physico-Chemical Characteristics of Tyrrhenian Coast Ponds in Central Italy: Implications for Conservation. *Hydrobiologia* 597(1):85–95.

Biggs, J., P. Williams, M. Whitfield, P. Nicolet, and A. Weatherby. 2005. 15 Year Pond Assessment in Britain: Results and Lessons Learned from the Work of Pond Conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems* 15:693–714.

Biggs, J., A. Corfield, and D. Walker. 1994. New Approaches to the Management of Ponds. *British Wildlife* 5(5).

Bilton, D. L. McAbendroth, P. Nicolet, A. Bedford, S. Rundle, A. Foggo, and P. Ramsay. 2009. Ecology and Conservation Status of Temporary and Fluctuating Ponds in Two Areas of Southern England. *Aquatic Conservation: Marine and Freshwater Ecosystems* 19:134–46.

Boix, D., J. Biggs, R. Céréghino, A. Hull, T. Kalettka, and B. Oertli. 2012. Pond Research and Management in Europe: "Small Is Beautiful". *Hydrobiologia* 689(1):1–9.

Bolin, B. 1983. The Carbon Cycle. In: *The Major Biogeochemical Cycles and their Interactions.* B. Bolin and R. Cook (eds.): 980-987. John Wiley & Sons, New York, USA.

Boven, L., R. Stoks, L. Forró, and L. Brendonck. 2008. Seasonal Dynamics in Water Quality and Vegetation Cover in Temporary Pools with Variable Hydroperiods in Kiskunság (Hungary). *Wetlands* 28(2):401–10.

Boyd, C., C. Wood, P. Chaney, and J. Queiroz. 2010. Role of Aquaculture Pond Sediments in Sequestration of Annual Global Carbon Emissions. *Environmental Pollut.* 158(8):2537–40.

Bradshaw, R., P. Coxon, J. Greig, and A. Hall. 1981. New Fossil Evidence for the Past Cultivation and Processing of Hemp (Cannabis Sativa L.) in Eastern England. *New Phytologist* 89(3):503–10.

Bridgham, S., H. Cadillo-Quiroz, J. Keller, and Q. Zhuang. 2013. Methane Emissions from Wetlands: Biogeochemical, Microbial, and Modeling Perspectives from Local to Global Scales. *Global change biology* 19(5):1325–46.

British Geological Survey (BGS). 1975. Lizard. England and Wales Sheet 359. Drift Geology. 1:50 000 Provisional Series. (Keyworth, Nottingham: British Geological Survey).

British Geological Survey (BGS). 2008. Selby. England and Wales Sheet 71. Bedrock and superficial deposits. 1:50 000 Geology Series. (Keyworth, Nottingham: British Geological Survey).

British Geological Survey (BGS). 2009. Swaffham. England and Wales Sheet 160. Solid and Drift Geology. 1:50 000 Provisional Series. (Keyworth, Nottingham: British Geological Survey).

Brown, A. 1971. *Ecologyy of Fresh Water. Heinmann: London*

Brown, A., and B. Wake. 2012. Certainty about Uncertainty. *Nature Climate Change* 2:395.

Brunskill, G., D. Povoledo, B. Graham, and M. Stainton. 1971. Chemistry of Surface Sediments of Sixteen Lakes in the Experimental Lakes Area, Northwestern Ontario. *Journal of the Fisheries Research Board of Canada* 28(2):277–94.

Calhoun, A., T. Walls, S. Stockwell, and M. McCollough. 2003. Evaluating Vernal Pools as a Basis for Conservation Strategies: A Maine Case Study. *Wetlands* 23(1):70–81.

Catalán, N., D. von Schiller, R. Marcé, M. Koschorreck, L. Gomez-Gener, B. Obrador. 2014. Cabon Dioxide Efflux during the Flooding Phase of Temporary Ponds. *Limnetica* 33(2):349–60.

Céréghino, R., D. Boix, H. Cauchie, K. Martens, and B. Oertli. 2013. The Ecological Role of Ponds in a Changing World. *Hydrobiologia* 723(1):1–6.

Chanda, A., A. Akhand, S. Manna, S. Dutta, I. Das, S. Hazra, K. Rao, and V. Dadhwal. 2013. Measuring Daytime CO2 Fluxes from the Inter-Tidal Mangrove Soils of Indian Sundarbans. *Environmental Earth Sciences* 72(2):417–27.

Clay, P. 2015. The Origin of Relic Cryogenic Mounds at East Walton and Thompson Common, Norfolk, England. *Proceedings of the Geologists' Association* 126(4-5):522–35.

Clegg, J. 1952. *The Freshwater Life of the Birtish Isles*. Frederick Warne, London.

Clegg, J. 1974. *Freshwater Life.* F. Warne: London.

Coker, R. 1968. *Streams, Lakes, Ponds*. Harper and Row: New York.

Cole, J., Y. Prairie, N. Caraco, W. McDowell, L. Tranvik, R. Striegl, C. Duarte, P. Kortelainen, J. Downing, J. Middelburg, and J. Melack. 2007. Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget. *Ecosystems* 10(1):172–85.

Collinson, N., J. Biggs, A. Corfield, M. Hodson, D. Walker, M. Whitfield, and P. Williams. 1995. Temporary and Permanent Ponds: An Assessment of the Effects of Drying out on the Conservation Value of Aquatic Macroinvertebrate Communities. *Biological Conservation* 74(2):125–33.

Couwenberg, J. 2009. Methane Emissions from Peat Soils (organic Soils, Histosols). Wetlands International.

Crisman, T., L. Chapman, and C. Chapman. 1998. Predictors of Seasonal Oxygen Levels in Small Florida Lakes: The Importance of Color. *Hydrobiologia* 368(1-3):149–55.

Countryside Survey (CS). 2007. *Countryside Survey: England Results from 2007 (published 2009)*. NERC/Centre for Ecology & Hydrology, Department for Environment, Food and Rural Affairs, Natural England. 119 (CEH Project Number: C03259).

Davies, B., J. Biggs, P. Williams, J. Lee, and S. Thompson. 2007. A Comparison of the Catchment Sizes of Rivers, Streams, Ponds, Ditches and Lakes: Implications for Protecting Aquatic Biodiversity in an Agricultural Landscape. *Hydrobiologia* 597(1):7–17.

Dean, W., E. Gorham, and D. Swaine. 1993. Geochemistry of Surface Sediments of Minnesota Lakes. Pp. 115–34 in Elk Lake Minnesota: Evidence for rapid climate change in the north-

central United States: Geological Society of America Special Paper 276, edited by J. Bradbury and W. Dean.
Dean, W. 1993. Physical Properties, Mineralogy, and Geochemistry of Holocene Varved Sediments from Elk Lake, Minnesota. *Geochmical Society of America Special Papers* 276:135–58.
Dean, W. and E. Gorham. 1998. Magnitude and Significance of Carbon Burial in Lakes Reservoirs and Peatlands. *Geology* 26:535–38.
Department for Environment, Food, and Rural Affairs (DEFRA). 2013. *Consultation on the Implementation of CAP Reform in England: Summary of Responses and Government Response.*
Department of Energy & Climate Change (DECC). 2016. *2014 UK Greenhouse Gas Emissions, Final Figures*.
Dodson S., S. Arnott, and K. Cottingham. 2000. The relationship in lake communities between primary productivity and species richness. *Ecology*. 81(10):2662-2679
Downing, J., Y. Prairie, J. Cole, C. Duarte, L. Tranvik, R. Striegl, W. McDowell, P. Kortelainen, N. Caraco, and J. Melack. 2006. The Global Abundance and Size Distribution of Lakes, Ponds, and Impoundments. *Limnology and Oceanography* 51(5):2388–97.
Downing, J., J. Cole, J. Middelburg, R. Striegl, C. Duarte, P. Kortelainen, Y. Prairie, and K. Laube. 2008. Sediment Organic Carbon Burial in Agriculturally Eutrophic Impoundments over the Last Century. *Global Biogeochemical Cycles* 22:1–10.
Downing, J. 2009. Global Limnology: Up-Scaling Aquatic Services and Processes to Planet Earth. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie* 30:1149–66.
Downing, J. and C. Duarte. 2009. Abundance and Size Distribution of Lakes, Ponds, and Impoundments. Pp. 469–78 in *Encyclopedia of inland waters*, edited by G. Likens. Elsevier: Oxford, UK.
Downing, J. 2010. Emerging Global Role of Small Lakes and Ponds: Little Things Mean a Lot. *Limnetica* 29(1):9–24.
Duarte, C., J. Kalff, and R. Peters. 1986. Patterns in Biomass and Cover of Aquatic Macrophytes in Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 43(10):1900–1908.
Duarte, C., J. Middelburg, and N. Caraco. 2004. Major Role of Marine Vegetation on the Oceanic Carbon Cycle. *Biogeosciences* 2:1–8.
Duarte, C., J. Middleburg, N. Caraco. 2005. Major Role of Marine Vegetation on the Oceanic Carbon Cycle. Biogeosciences. 2:1-8
Edina. 2015. British National Grip. Scale 1:2,500. Using: EDINA Ancient Roam 1920s, 1950s, 1960s, 1990s. (http://digimap.edina.ac.uk/roam/historic) Accessed: December 2015.
Elmberg, J., P. Nummi, H. Poysa, and K. Sjoberg. 1994. Relationships Between Species Number, Lake Size and Resource Diversity in Assemblages of Breeding Waterfowl. *Journal of biogeography* 21(1):75–84.
Elton, C. and R. Miller. 1954. The Ecological Survey of Animal Communities: With a Practical System of Classifying Habitats by Structural Characters. *Journal of Ecology* 42:460–96.
European Commission DG Environment. 2003. *Interpretation Manual of European Union Habitats*. EUR25
Ewald, N., T. Kalettka, and L. Brendonck. 2012. Eyes of the Landscape – Value, Conservation and Management of European Ponds. *Limnologica - Ecology and Management of Inland Waters* 42(4):251–53.
Fairchild, G., C. Robinson, A. Brainard, and G. Coutu. 2013. Historical Changes in the Distribution and Abundance of Constructed Ponds in Response to Changing Population Density and Land Use. *Landscape Research* 38(5):593–606.
Fitter R., R. Manuel. 1986. *A Guide to the Freshwater Life of Britain and North-west Europe.* Collins: London.
Forster, J. 1771. A Letter from Mr. John Reinhold Forster, F. A. S. to the Hon. Daines Barrington, Vice - Pres. R. S. on the Management of Carp in Polish Russia. *Philosophical Transactions (1683-1775)* 61:310–25.
Foster, G. 1993. Pingo Fens, Water Beetles and Site Evaluation. *Antenna.* 17:184-195

Fowler, H. and C. Kilsby. 2002. Precipitation and the North Atlantic Oscillation: A Study of Climatic Variability in Northern England. *International Journal of Climatology* 22(7):843–66.

Friedlingstein, P., R. Houghton, G. Marland, J. Hackler, T. Boden, T. Conway, J. Canadell, M. Raupach, P. Ciais, and C. Le Quere. 2010. Update on CO2 Emissions. *Nature Geoscience* 3:811–12.

Fromin, N., G. Pinay, B. Montuelle, D. Landais, J. Ourcival, R. Joffre, and R. Lensi. 2010. Impact of Seasonal Sediment Desiccation and Rewetting on Microbial Processes Involved in Greenhouse Gas Emissions. *Ecohydrology* 3(3):339–48.

Fryer, G. 1993. *The Freshwater Crustacea of Yorkshire*. Titus Wilson & Son: Kendal, UK.

Gala, T. and A. Melesse. 2012. Monitoring Prairie Wet Area with an Integrated LANDSAT ETM+, RADARSAT-1 SAR and Ancillary Data from LIDAR. *CATENA* 95:12–23.

Gallagher, A. and K. van Huissteden. 2011. Increased Greenhouse Gas Emission from Ponds in Siberian Arctic Tundra on Continuous Permafrost. *Geophyiscal Research Abstracts* 13.

George, D., S. Maberly, and D. Hewitt. 2004. The Influence of the North Atlantic Oscillation on the Physical, Chemical and Biological Characteristics of Four Lakes in the English Lake District. *Freshwater Biology* 49(6):760–74.

Gilbert, P. S. Taylor, D. Cooke, M. Deary, M. Cooke, and M. Jeffries. 2014. Variations in Sediment Organic Carbon among Different Types of Small Natural Ponds along Druridge Bay, Northumberland, UK. *Inland Waters* 4:57–64.

Gonzalez-Valencia, R., A. Sepulveda-Jauregui, K. Martinez-Cruz, J. Hoyos-Santillan, L. Dendooven, and F. Thalasso. 2013. Methane Emissions from Mexican Freshwater Bodies: Correlations with Water Pollution. *Hydrobiologia* 721(1):9–22.

Goody, R., and J. Walker. 1972. *Atmospheres*. Prentice-Hall. Englewood Cliffs, NJ, USA. 160 pp.

Gorham, E., J. Lund, J. Sanger, and W. Dean. 1974. Some Relationships between Algal Standing Crop, Water Chemistry, and Sediment Chemistry in the English Lakes. *American Society of Limnology and Oceanography*. 19(4):601-617

Halbfaß, W. 1922. Die Seen Der Erde. Peterm. Mitteilungen. Ergungzungshaft. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* 185(1-6):1–169.

Hallett, T., T. Coulson, J. Pilkington, T. Clutton-Brock, J. Pemberton, and B. Grenfell. 2004. Why Large-Scale Climate Indices Seem to Predict Ecological Processes Better than Local Weather. *Nature* 430(6995):71–75.

Hedges, J. and R. Keil. 1995. Sedimentary Organic Matter Preservation: An Assessment and Speculative Synthesis. *Marine Chemistry* 49(2-3):81–115.

Heimann, M. and M. Reichstein. 2008. Terrestrial Ecosystem Carbon Dynamics and Climate Feedback. *Nature* 451(17):289–92.

Herdendrof, C. 1984. Inventory of the Morphometric and Limnological Characteristics of the Large Lakes of the World. Ohio State University, Ohio Sea Grant College Program. 1-54

Hogg, P., P. Squires, and A. Fitter. 1995. Acidification, Nitrogen Deposition and Rapid Vegetational Change in a Small Valley Mire in Yorkshire. *Biological Conservation* 71(2):143–53.

Hopkins, J. 1978. The Conservation Status of the Inland Heathlands and Other Vegetational Types and Their Associated Rare and Uncommon Species at the Lizard District, Cornwall. Report to the Nature Conservancy Council, Peterborough

Hopkins, J. 1983. Historical Ecology, Vegetation and Flora of the Lizard District, Cornwall, with Particular Reference to Heathland. University of Bristol.

Horne, A., C. Goldman. 1994. *Limnology*. McGraw-Hill: New York.

Huttunen, J., T. Väisänen, M. Heikkinen, S. Hellsten, H. Nykänen, O. Nenonen, and P. Martikainen. 2002. Exchange of CO2, CH4 and N2O between the Atmosphere and Two Northern Boreal Ponds with Catchments Dominated by Peatlands or Forests. *Plant and Soil* 242:137–46.

Intergovernmental Panel on Climate Change (IPCC). 2001. IPCC Third Assessment Report: Climate Change 2001 (TAR).

Intergovernmental Panel on Climate Change (IPCC). 2007. IPCC Fourth Assessment Report: Climate Change 2007 (AR4).

Intergovernmental Panel on Climate Change (IPCC). 2014. IPCC Fifth Assessment Report (AR5).

Jaggs, E. 2008. Northumberland Biodiversity Action Plan.

Jeffries, M. and D. Mills. 1990. *Freshwater Ecology, Principles and Applications*. Belhaven: London.

Jeffries, M. 1998. Pond Macrophyte Assemblages, Biodisparity and Spatial Distribution of Ponds in the Northumberland Coastal Plain, UK. *Aquatic Conservation: Marine and Freshwater Ecosystems* 8:657–67.

Jeffries, M. 2008. The Spatial and Temporal Heterogeneity of Macrophyte Communities in Thirty Small, Temporary Ponds over a Period of Ten Years. *Ecography* 31(6):765–75.

Jeffries, M. 2010. The Temporal Dynamics of Temporary Pond Macroinvertebrate Communities over a 10-Year Period. *Hydrobiologia* 661(1):391–405.

Jeffries, M. 2012. Ponds and the Importance of Their History: An Audit of Pond Numbers, Turnover and the Relationship between the Origins of Ponds and Their Contemporary Plant Communities in South-East Northumberland, UK. *Hydrobiologia* 689(1):11–21.

Jeffries, M. 2015. Flood, Drought and the Inter-Annual Variation to the Number and Size of Ponds and Small Wetlands in an English Lowland Landscape over Three Years of Weather Extremes. *Hydrobiologia*.

Jonai, H. and W. Takeuchi. 2014. Comparison between Global Rice Paddy Field Mapping and Methane Flux Dataa from GOSAT. *Geoscience and Remote Sensing Sypossium (IGARSS), 2014 IEEE International* 2098–2101.

Jones, I. 2013. *The Impact of Extreme Events on Freshwater Ecosystems*. British Ecological Society: London.

Jones, K., Y. Lanthier, P. van der Voet, E. van Valkengoed, D. Taylor, and D. Fernández-Prieto. 2009. Monitoring and Assessment of Wetlands Using Earth Observation: The GlobWetland Project. *Journal of environmental management* 90(7):2154–69.

Kalff, J. 2002. Limnology : Inland Water Ecosystems. Prentice Hall.

Keeley, J. and P. Zedler. 1998. Characterization and Global Distribution of Vernal Pools. Pp. 1–14 in Ecology, Conservation, and Management of Vernal Pool Ecosystems - Proceedings from a 1996 conference. California Native Plant Society, Sacramento, vol. 22230, edited by C. Witham, E. Bauder, D. Belk, W. Ferren, and R. Ordnuff.

Kling, G., G. Kipphut, and M. Miller. 1991. Arctic Lakes and Streams as Gas Conduits to the Atmosphere: Implications for Tundra Carbon Budgets. *Science (New York, N.Y.)* 251(4991):298–301.

Knight, J. and S. Harrison. 2013. The Impacts of Climate Change on Terrestrial Earth Surface Systems. *Nature Climate Change* 3:24–29.

Kortelainen, P., M. Rantakari, J. Huttunun, T. Mattasson, J. Alm, S. Juutinen, T. Larmola, J. Silvola, P. Martikainen. 2006. Sediment Respiration and Lake Trophic State Are Important Predictors of Large CO_2 Evasion from Small Boreal Lakes. *Global Change Biology* 12(8):1554–67.

Kutschker, A., L. Epele, and M. Miserendino. 2014. Aquatic Plant Composition and Environmental Relationships in Grazed Northwest Patagonian Wetlands, Argentina. *Ecological Engineering* 64:37–48.

Lehner, B. and P. Döll. 2004. Development and Validation of a Global Database of Lakes, Reservoirs and Wetlands. *Journal of Hydrology* 296(1-4):1–22.

Lindsay, R. 2010. Peat Bogs and Carbon: A Critical Synthesis. RSPB Scotland.

López-Blanco, J. and L. Zambrano. 2002. Characterization of Small Shallow Ponds with Color Video Imagery in Central Mexico. *Hydrobiologia* 177–85.

Lunn, A. 2004. *Northumberland*. Harper Collins, London.

Mackey, B., C. Prentice, W. Steffen, J. House, D. Lindenmayer, H. Keith, and S. Berry. 2013. Untangling the Confusion around Land Carbon Science and Climate Change Mitigation Policy. *Nature Climate Change* 3:552–57.

Macrae, M., R. Bello, and L. Molot. 2004. Long-Term Carbon Storage and Hydrological Control of CO_2 Exchange in Tundra Ponds in the Hudson Bay Lowland. *Hydrological Processes* 18(11):2051–69.

Macan, T., E. Worthington. 1972. *Life in Lakes and Rivers*. Colins: London.

Macan, T. 1973. *Ponds and Lakes.* George Allen & Unwin: London.

Manning, D., P. Renforth. 2013. Passive Sequestration of Atmospheric CO_2 through Coupled Plant-Mineral Reactions in Urban Soils. *Environmental Science & Technology.* 47:135-141

Minestry of Agriculture, Fisheries, and Food (MAFF). 1985. *Survey of Environmental Topics on Farms, England and Wales: 1985.* Prepared by the Government Statistical Service. Ministry of Agriculture, Fisheries, and Food, London.

Melack, J., L. Hess, M. Gastil, B. Forsberg, S. Hamilton, I. Lima, and E. Novo. 2004. Regionalization of Methane Emissions in the Amazon Basin with Microwave Remote Sensing. *Global Change Biology* 10(5):530–44.

Met Office. 2016. UK Climate Average Maps. Available at: www.metoffice.gov.uk/public /weather/climate. Accessed: February 2016.

Meybeck, M. 1995. Global Distribution of Lakes. Pp. 1–35 in *Physics and Chemistry of Lakes*, edited by A. Lerman, D. Imboden, and J. Gat.

Meyers, P. and R. Ishiwatari. 1993. Lacustrine Organic Geochemistry - an Overview of Indicators of Organic Matter Sources and Diagenesis in Lake Sediments. *Organic Geochemistry* 20(7):867–900.

Michmerhuizen, C., R. Stiegel, and M. McDonald. 1996. Potential Methane Emission from North-Temperate Lakes Following Ice Melt. *Limnology and Oceanography* 41(5):985–91.

Middelboe, A. and S. Markager. 1997. Depth Limits and Minimum Light Requirements of Freshwater Macrophytes. *Freshwater Biology* 37(3):553–68.

Mitra, S., R. Wassmann, and P. Vlek. 2005. An Appraisal of Global Wetland Area and Its Organic Carbon Stock. *Current Sciences* 88(1):25–35.

Morgan., A. 1930. *Field Book of Ponds and Streams, an Introduction to the Life of Fresh Water.* Putnam: London.

Morris, E., S. Flecha, J. Figuerola, E. Costas, G. Navarro, J. Ruiz, P. Rodriguez, and E. Huertas. 2013. Contribution of Doñana Wetlands to Carbon Sequestration. *PloS one* 8(8).

Moss, B., 1988. *Ecology of Fresh Waters. Man and Medium.* Blackwell: Oxford

Mozley, A. 1937. The Ponds, Lakes and Streams of the Kirghiz Steppe. *Scottish Geographical Magazine* 53(1):1–10.

Mulholland, P. and J. Elwood. 1982. The Role of Lake Reservoir Sediments as Sinks in the Perturbed Global Carbon Cycle. *Tellus* 34:490–99.

Munsiri, P., C. Boyd, and B. Hajek. 1995. Physical and Chemical Characteristics of Bottom Soil Profiles in Ponds at Auburn, Alabama, USA and a Proposed System for Describing Pond Soil Horizons. *Journal of the World Aquaculture Society* 26(4):346–77.

Natchinmuthu, S., B. Panneer Selvam, and D. Bastviken. 2014. Influence of Weather Variables on Methane and Carbon Dioxide from a Shallow Pond. *Biogeochemistry* 119:403–13.

New, M., D. Liverman, H. Schroder, and K. Anderson. 2011. Four Degrees and beyond: The Potential for a Global Temperature Increase of Four Degrees and Its Implications. *Philosophical Transactions of the Royal Socitey A* 369:6–19.

Non Native Species Secretariat (NNSS) GB. 2016. Check, Clean, Dry. (http://www.nonnativespecies.org/checkcleandry/index.cfm).

Ntengwe, F. and M. Edema. 2008. Physico-Chemical and Microbiological Characteristics of Water for Fish Production Using Small Ponds. *Physics and Chemistry of the Earth, Parts A/B/C* 33(8-13):701–7.

Oertli, B. 2009. Pond Conservation: From Science to Practice. *Hydrobiologia* 1:1–9.

Ometto, J., A. Nobre, H. Rocha, P. Artaxo, and L. Martinelli. 2005. Amazonia and the Modern Carbon Cycle: Lessons Learned. *Oecologia* 143(4):483–500.

Ovens, C. 2015. *The Vulnerability of Broad Vegetation Community Types to Climate and Land Use Change within Northumberland National Park*. Doctorla Thesis, Norhtumbria University.

Pacheco, F., F. Roland, and J. Downing. 2013. Eutrophication Reverses Whole-Lake Carbon Budgets. *Inland Waters* 41–48.

Pitt, A., R. Baldwin, D. Lipscomb, R. Brown, J. Hawley, C. Allard-Keese, and P. Leonard. 2011. The Missing Wetlands: Using Local Ecological Knowledge to Find Cryptic Ecosystems. *Biodiversity and Conservation* 21(1):51–63.

Pittman, B., J. Jones, J. Millspaugh, R. Kremer, and J. Downing. 2013. Sediment Organic Carbon Distribution in 4 Small Northern Missouri Impoundments : Implications for Sampling and Carbon Sequestration. *Inland Waters* 3:39–46.

Porter, V. 1988. *The Pond Book.* Christopher Helm: London.

Prinn, R., S. Paltsev, A. Sokolov, M. Sarofim, J. Reilly, and H. Jacoby. 2011. Scenarios with MIT Integrated Global Systems Model: Significant Global Warming regardless of Different Approaches. *Climate Change* 104:515–37.

Probert, C. 1989. Pearls in the Landscape: The Conservation and Management of Ponds. Farming Press: Ipswich, UK.

Raupach, M. 2011. Pinning down the Land Carbon Sink. *Nature Climate Change* 1.

Raymond, P., J. Hartmann, R. Lauerwald, S. Sobek, C. McDonald, M. Hoover, D. Butman, R. Striegl, E. Mayorga, C. Humborg, P. Kortelainen, H. Dürr, M. Meybeck, P. Ciais, P. Guth. 2013. Global Carbon Dioxide Emissions from Inland Waters. *Nature* 503(7476):355–59.

Ritchie, J. 1989. Carbon Content of Sediments of Small Reservoirs. *Journal of the American Water Resources Association* 25(2):301–8.

Saarnio, S., W. Winiwarter, and J. Leitão. 2009. Methane Release from Wetlands and Watercourses in Europe. *Atmospheric Environment* 43(7):1421–29.

Savage, K. and E. Davidson. 2003. A Comparison of Manual and Automated Systems for Soil CO_2 Flux Measurements: Trade-Offs between Spatial and Temporal Resolution. *Journal of Experimental Botany* 54(384):891–99.

Scheffer, M., G. van Geest, K. Zimmer, E. Jeppesen, M. Søndergaard, M. Butler, M. Hanson, S. Declerck, L. De Meester. 2006. Small Habitat Size and Isolation Can Promote Species Richness: Second-Order Effects on Biodiversity in Shallow Lakes and Ponds. *Oikos* 112(1):227–31.

von Schiller, D., R. Marcé, B. Obrador, L. Gómez-Gener, J. Casas-Ruiz, V. Acuña, and M. Koschorreck. 2014. Carbon Dioxide Emissions from Dry Watercourses. *Inland Waters* 4(4):377–82.

Schlesinger, W. 1997. *Biogeochemistry: An Analysis of Global Change*. Academic Pres. San Diego, California, USA.

Schimel, D., I. Enting, M. Heimann, T. Wigley, D. Raynaud, D. Alves, and U. Siegenthaler. 1995. CO_2 and the Carbon Cycle. In: *Climate Change: Radiative Forcing of Climate Change and an Evaluation of the IPCC IS92 Emission Scenarios*, (ed.) J. Houghton: 35-71. Cambridge University Press: Cambridge, UK.

Schmidt, M., M. Torn, S. Abiven, T. Dittmar, G. Guggenberger, I. Janssens, M. Kleber, I. Kögel-knabner, J. Lehmann, D. Manning, P. Nannipieri, D. Rasse, S. Weiner, S. Trumbore. 2011. Persistence of Soil Organic Matter as an Ecosystem Property. *Nature.* 478(7367):49-56.

Schuiling, R. 1977. Sources and Composition of Lake Sediments. *In*. Interaction Between Sediments and Freshwater. Proceedings of an International Symposium Held at Amsterdam, the Netherlands, September 6-10 1976. Pp. 12–18. [Ed]. H. Golterman

Scott, S. 2013. *How Effective are Small Constructed Ponds in Carbon Sequestration?* Undergrauate Thesis, Northumbria University.

Seekell, D. and M. Pace. 2011. Does the Pareto Distribution Adequately Describe the Size-Distribution of Lakes? *Limnology and Oceanography* 56(1):350–56.

Shiklomanov, I. and J. Rodda. 2004. World Water Resources at the Beginning of the Twenty-First Century. Cambridge University Press.

Shotbolt, L., A. Thomas, and S. Hutchinson. 2005. The Use of Reservoir Sediments as Environmental Archives of Catchment Inputs and Atmospheric Pollution. *Progress in Physical Geography* 29:337–61.

Simpson, J., and E. Weiner. 1989. The Oxford English Dictionary. Clarendon Press: Oxford.

Søndergaard, M., E. Jeppesen, J. Jensen. 2005. Pond or Lake: Does it Make any Difference? *Archieve für Hydrobiologie.* 162:143-165.

Stallard, R. 1998. Terrestrial Sedimentation and the Carbon Cycle: Coupling Weathering and Erosion to Carbon Burial. *Global Biogeochemical Cycles* 12(2):231–57.

Stephens, B., K. Gurney, P. Tans, C. Sweeney, W. Peters, L. Bruhwiler, P. Ciais, M. Ramonet, P. Bousquet, T. Nakazawa, S. Aoki, T. Machida, G. Inoue, N. Vinnichenko, J. Lloyd, A. Jordan, M. Heimann, O. Shibistova, R. Langenfelds, P. Steele, R. Francey, and A. Denning. 2007. Weak Northern and Strong Tropical Land Carbon Uptake from Vertical Profiles of Atmospheric CO_2. *Science (New York, N.Y.)* 316(5832):1732–35.

Sterry, P. 1982. *Pond Watching.* Severn House: Lonodn

Straskraba, M. 1980. The Effects of Physical Variables on Freshwater Production: Anlysis Based on Models. Pp. 13–84 in *The functioniong of freshwater ecosystems*, edited by E. D. Le Cren and R. H. Lowe-McConnell.

Taylor, S. 2012. *How Effective are Small Constructed Ponds Permanently Sequestering Organic Carbon?* Undergraduate Thesis. Northumbria University

Thienemann, A. 1925. *Die Binnengewässer Mittleuropas*. Schweizerbart, Stuttgart.

Torgersen, T. and B. Branco. 2008. Carbon and Oxygen Fluxes from a Small Pond to the Atmosphere: Temporal Variability and the CO_2 /O_2 Imbalance. *Water Resources Research* 44(2).

Torgersen, T. and B. Branco. 2007. Carbon and Oxygen Dynamics of Shallow Aquatic Systems: Process Vectors and Bacterial Productivity. *Journal of Geophysical Research* 112(G3):G03016.

Tranvik, L., J. Downing, and J. Cotner. 2009. Lakes and Reservoirs as Regulators of Carbon Cycling and Climate. *Limnology and Oceanographylimnology* 54(6):52–68.

United States Climate Change Science Program (USCCSP). 2003. Vision for the Program and Highlights of the Scientific Strategic Plan.

Vanni, M., W. Renwick, A. Bowling, M. Horgan, and A. Christian. 2011. Nutrient Stoichiometry of Linked Catchment Lakes-Systems along a Gradient of Land Use. *Freshwater Biology* 56:791–811.

Verpoorter, C., T. Kutser, D. Seekell, and L. Tranvik. 2014. A Global Inventory of Lakes Based on High-Resolution Satellite Imagery. *Geophysical Research Letters* 41(18):6396–6402.

Verstraeten, G. and J. Poesen. 2002. Using Sediment Deposits in Small Ponds to Quantify Sediment Yield from Small Catchments: Possibilities and Limitations. *Earth surface processes and landforms* 27:1425–39.

Walmsley, A. 2008. The Norfolk 'Pingo' Mapping Project.

Wehril, B. 2013. Conduits of the Carbon Cycle. *Nature* 503:346–47.

Weisner, S. 1991. Within-Lake Patterns in Depth Penetration of Emergent Vegetation. *Freshwater Biology* 26(1):133–42.

Welch, P. 1952. *Limnology*. McGraw-Hill: New York

West, J., H. Close, W. Whitmore, T. Robins, J. Gregory, W. Tugwell, R. Comely, J. Wagstaffe, T. Hoy, W. Laurie, J. Harriot, A. Young. 1786. Papers in Agriculture. *Transactions of the Society, Insititued in London, for the Encouragement of Arts, Manufactures, and Commerce* 4:3–106.

Wetzel, R. 2001. *Limnology, Third Edition: Lakeand River Ecosystems*. Academic Pres. San Diego, California, USA.

White, C. 1868. The Lakes of Iowa; Past and Present. *The American Naturalist* 2(3):143–55.

Williams, W. 1983. *Life in Inland Waters.* Blackwell: Melbourne

Williams, P., J. Biggs, G. Fox, P. Nicolet, and M. Whitfield. 2001. History, Origins and Importance of Temporary Ponds. *Freshwater Forum* 17(1).

Williams, P., M. Whitfield, J. Biggs, S. Bray, G. Fox, P. Nicolet, and D. Sear. 2004. Comparative Biodiversity of Rivers, Streams, Ditches and Ponds in an Agricultural Landscape in Southern England. *Biological Conservation* 115(2):329–41.

Williams, P., J. Biggs, A. Crowe, J. Murphy, P. Nicolet, A. Weatherby, and M. Dunbar. 2010. *Countryside Survey: Ponds Report from 2007*.

Xinglong, J. and C. Boyd. 2006. Relationship between Organic Carbon Concentration and Potential Pond Bottom Soil Respiration. *Aquacultural Engineering* 35(2):147–51.

Zealand, A. and M. Jeffries. 2009. The Distribution of Pond Snail Communities across a Landscape: Separating out the Influence of Spatial Position from Local Habitat Quality for Ponds in South-East Northumberland, UK. *Hydrobiologia* 632:177–87.

Appendices

Appendix A: Gilbert et al. (2016)

Hydrobiologia
DOI 10.1007/s10750-016-2855-y

SMALL WATER BODIES

Quantifying rapid spatial and temporal variations of CO_2 fluxes from small, lowland freshwater ponds

Peter J. Gilbert · David A. Cooke · Michael Deary · Scott Taylor · Michael J. Jeffries

Received: 22 December 2015 / Revised: 31 May 2016 / Accepted: 1 June 2016

Abstract Small ponds comprise a substantial portion of the total area of the Earth's inland waters. They can be powerful carbon sinks or sources, potentially significant processors of organic carbon. Our understanding of their role is constrained by the absence of information regarding their CO_2 fluxes (F CO_2) and how these change with wet or dry phases and across distinct pond plant communities. We monitored the F CO_2 from 26 neighbouring small ponds over a 2-week drying period in late summer in 2014. The mean F CO_2 on day 1 (-641 ± 1490 mg m^{-2} day^{-1}) represented a net intake across the site. As ponds dried they switched to becoming CO_2 sources resulting in a net site emission of CO_2 by day 12 (3792 ± 2755 mg m^{-2} day^{-1}) although flux rates did not vary systematically between plant communities. Significant variability in the F CO_2 was observed amongst adjacent ponds on individual sampling days, resulting in marked spatial heterogeneity in CO_2 processing. This large degree of temporal and spatial heterogeneity across short time periods and small distances highlights the variability in the F CO_2 from temporary systems, making it hard to generalize their role in carbon cycle models.

Keywords Carbon flux · Temporary pond · Small wetland

Guest editors: Mary Kelly-Quinn, Jeremy Biggs & Stefanie von Fumetti / The Importance of Small Water Bodies: Insights from Research

P. J. Gilbert · D. A. Cooke · M. Deary · S. Taylor · M. J. Jeffries (✉)
Northumbria University, Ellison Building, Newcastle upon Tyne NE1 8ST, UK
e-mail: michael.jeffries@northumbria.ac.uk

Introduction

In recent years, the role that small ponds play in global geochemical processes has received increasing interest (Cole et al., 2007; Battin et al., 2009; Downing, 2010). Estimations of their cumulative global coverage have suggested that they are comparable in area to the Earth's largest lakes (Downing et al., 2006), although more recent estimates have been more conservative (Seekell & Pace, 2011; Verpoorter et al., 2014). Equally these systems support disproportionately intense processes for their size, when compared to larger water bodies (Torgersen & Branco, 2008; Downing, 2010; Catalán et al., 2014). This makes them ideal cyclers of atmospheric carbon (here after C), accounting for a substantial portion of the missing C budget from which small ponds and wetlands are frequently omitted.

The absence of small ponds from C budgets is in part due to a lack of robust data quantifying their rates and processes. Biogeochemical processing in ponds remained relatively understudied until the late twentieth century as limnological research focused on

Published online: 08 June 2016

larger lakes and river systems, where it was assumed that the dominant inland aquatic processing of *C* occurred (Downing, 2010). However, just as ponds were once overlooked as wildlife habitats but are now known to be disproportionately rich in species and rarities compared to streams, rivers and lakes (Williams et al., 2004; Davies et al., 2008), their potential significance for ecosystem services, such as *C* sequestration, is being increasingly recognized (Cole et al., 2007; Tranvik et al., 2009; Downing, 2010; Cereghino et al., 2014; Gilbert et al., 2014). The number of studies on *C* cycling within ponds is rapidly increasing (Downing, 2010; Boix et al., 2012; Ewald et al., 2012). Within this overall biome of ponds, temporary systems are receiving increasing interest (Torgersen & Branco, 2008; Fromin et al., 2010; Catalán et al. 2014; von Schiller et al., 2014).

Temporary ponds are known by a range of diverse regional names or technical definitions: e.g. seasonal, ephemeral, playa or vernal (Keeley & Zedler, 1998). Recognized as ecologically valuable they support a specialist flora and fauna, which adds a significant contribution to γ biodiversity on the landscape scale, that is able to withstand drought through resistant propagules or by rapid re-colonization (Collinson et al., 1995; Jeffries, 1998, 2010). They are internationally important terrestrial habitats, ubiquitous in all climatic zones across the globe including extreme desert and polar environments, including thaw ponds in Arctic Tundra (Gallagher & Huissteden, 2011), temporal pools in Mediterranean and desert biomes (Catalán et al., 2014), constructed rice paddies in equatorial tropics (Jonai & Takeuchi, 2014), to melt pools in Antarctica (Allende & Mataloni, 2013). They are also typical of temperate biomes such as south American grasslands (e.g. *mallines*, Kutschker et al., 2014), prairie potholes and woodland vernal ponds in North America (Batzer et al., 2005; Gala & Melesse, 2012), across the riverine plains of Europe (e.g. tributaries of the Danube in Hungary; Boven et al., 2008), through into the Asian steppes (Mozley, 1937; an unusual example of a rare early appreciation of their value). Temporary habitats can also be historically long-lived features in the landscape, for example the pingo ponds of East England (Foster, 1993; Williams et al., 2001), so that their geochemical impact will also play out over many years. However, their presence is frequently overlooked both in natural landscapes such as grassland or temperate forest, and in intensively modified landscapes such as arable or grazing agriculture (Williams et al., 2001).

The key feature that unifies such systems is that they exhibit seasonal changes in their hydrological regimes resulting in periodic dry phases, exposure of the base substrate and often desiccation of sediment layers. Typically in temperate climates, including the ponds in this study, recharge is rainfall dependent, and as such this change in hydro-period is dependent on the balance between evaporation rates and net rainfall over short periods. In the UK unreliable summer rainfall (Fowler & Kilsby, 2002) often results in several drying and rewetting cycles over short periods of time, with rainfall variations from year to year further complicating the quantification and modelling of their ecosystem processes. This problem is compounded by the likely increase in climate variability caused by climate change. A particular uncertainty arises from new extremes of rainfall or temperature, which will subject ponds and their wildlife, to novel stresses which may alter existing rates of geochemical processing and species' distributions (Jeffries, 2010; Jones, 2013).

Whilst *C* burial rates in ponds are amongst the highest of all ecosystems (Downing, 2010), ironically it is the low water volume of temporary ponds that renders them vulnerable to drying and sediment desiccation during periods of low rainfall, greatly impacting sediment *C* stability. Sediment conditions quickly change from anoxic to oxic, permitting aerobic microbial activity in the surface substrate, resulting in higher mineralization rates of organic matter and subsequent CO_2 efflux (Fromin et al., 2010). Furthermore, in exposed sediments, CO_2 release is no longer hindered by the water column, through which CO_2 will usually diffuse before release to the atmosphere at the surface boundary layer (Catalán et al., 2014). Assessing CO_2 effluxes of temporary ponds in response to rapid changes in seasonal drying cycles is crucial to quantifying their role in the global *C* cycle.

In this study we begin to address this by monitoring the temporal and spatial heterogeneity of CO_2 fluxes amongst 26 small experimental ponds in Northeast England in response to desiccation during a summer dry period. The study was intentionally designed to capture changes over relatively fine-grained spatial and temporal scales; ponds just metres apart, and over a period of days within a two-week summer drying phase.

Site description

This study took place on a set of 26 experimental ponds. Constructed in 1994, all ponds are the same age, approximately the same size (1 m^2 × 30 cm deep) and set out in an array across an area of approximately 30 × 30 m. Their close proximity renders them exposed to the same regional environmental conditions; they are as close to replicate ponds as is possible under natural conditions. The ponds' 20 years history of hydrological and ecological monitoring provided data on potentially important drivers of variation in gas exchange, e.g. wet and dry phases or vegetation (Bouchard et al., 2007; Fromin et al, 2010; Turetsky et al., 2014; Catalán et al., 2014; Mo et al., 2015. For detailed site description see Jeffries, 2008, 2010). The region in which they are located forms part of the Northumberland Northeast coastal plain in northeast England, a lowland landscape dominated by intensive arable and livestock agriculture. The climate is relatively cool but also dry due to the rain shadow from hills to the west (Lunn, 2004). Despite the relatively low rainfall the area is rich in ponds, especially shallow, temporary habitats associated with sand dunes or land subsidence over old coal mines (Jeffries, 2012).

The ponds are situated within a meadow field (55°19′04.1″N 1°33′22.1″W), owned by Northumberland Wildlife Trust, at the northern end of Druridge Bay, Northumberland, UK. Originally an opencast coal mine which was restored when mining ceased around 50 years ago, the site is now topped with a rough clay lining and approximately 50 cm topsoil. The clay lining is impermeable and, as such, the ponds are dependent on precipitation, subsequent surface run-off and horizontal throughflow in the topsoil layer for recharge. A slight gradient across the site runs northeast to southwest causing subtle hydrological variations amongst the ponds. The southwest portion of the site is typically marshier and dominated by spike rush, *Eleocharis palustris* (L.) Roem. & Schult., with ponds here being the first to fill during rainfall events, yet also the first to dry during rainfall absence. Contrastingly, the northeast portion of the site is less marshy, dominated by grasses such as marsh foxtail, *Alopecurus geniculatus* L., and glaucous sedge, *Carex glauca* Schreb., yet the ponds hold water for longer. Despite their close proximity and superficial similarity, the ponds have developed a diverse set of plant and animal communities and their hydrological patterns are typical of the ponds and wetlands through the region (Jeffries, 2008, 2010).

Methods

Site description

To quantify CO_2 flux rates and how these change (a) on a fine-grained spatial and temporal scale between adjacent ponds over a few days and (b) in response to drying out of ponds, flux rates of CO_2 (F CO_2) were monitored for all 26 ponds over a 2-week period from 24th August to 4th September 2014, monitoring on days 1, 4, 9 and 12.

Fluxes of CO_2 were measured using a floating chamber method. Our chamber consisted of an upturned 7252 cm^3 container (length = 37 cm; width = 24.5 cm; height = 8 cm) with attached floatation device around the base of the chamber which assured that the volume of the chamber was above the water's surface. The chamber was placed gently on the surface of the water in each pond to avoid disturbance and the inflow and outflow tubes which connected the chamber to the flux metre tethered the chamber in position. The small area of the ponds combined with water levels considerably below the top of the ponds' edges created a sheltered environment. Headspace volume did not change because of varying water level or being lifted by vegetation. For systems that had dried out the chamber was placed directly on vegetation within the centre of the pond and sealed by placing plastic sheeting around the base of the chamber to assure minimal interaction with the atmosphere. It should be noted that the chamber was transparent and allowed for photosynthesis, and as such, fluxes represent the respiration rate of the system as a whole.

Inflow and outflow tubes connected the chamber to an in situ FT-IR (Fourier Transform-Infra Red) analyser pumping at a rate of 2 l per min to allow continual circulation from and back to the chamber. Gas concentrations within the chamber were recorded repeatedly at 20 s intervals over a 5 min period, which was found to be the optimum time to achieve a reliable r^2 (>0.8) yet not too long so that pressure changes would affect flux rates. In between each flux measurement, the chamber was flushed until readings returned to atmospheric concentrations. Note that the volume of the air space within the FT-IR analyser and

tubing was also accounted for in calculations of head space volume and changes in CO_2 concentrations. Every 6th pond was measured in triplicate to assess the precision of the analysis. Monitoring of flux rates for all ponds took approximately 6 h, from 10 am to 4 pm, with each pond sampled in the same order and at roughly the same time on each of the survey days to focus on how the fluxes changed from individual ponds across the sample period. Whilst fluxes from systems are known to vary throughout the day it is this central period at which flux rates are most stable (Chanda et al., 2013).

Flux rates were calculated from the linear regression of the change in concentration over the 5 min period, aiming for $r^2 = >0.8$, and all flux rates were corrected for temperature and atmospheric pressure. Negative values reported herein represent an intake of CO_2 from the atmosphere by the system whilst positive values represent an emission. It should be noted that in several instances changes in concentration within the chamber were lower than the accuracy of the FT-IR analyser (1 ppm), resulting in a poor r^2 (<0.8). However, in this situation values reported are negligible in comparison to high flux rates from active ponds. When referring to the averages across all ponds the term ecosystem flux rate (*E–F* CO_2) is used to refer to the ponds as a collective.

Hydrological classification

The hydrological condition of each pond was characterized by personal observation during each site visit, grouping each pond into one of three categories:

1. Aquatic Phase—Ponds contained standing water that covered the base, though with occasional emergent vegetation.
2. Transitional Phase—Ponds contained no standing water with base layer exposed. However, the sediment and vegetative layer were still saturated and moist to touch.
3. Dry Phase—Ponds contained no standing water with sediment and vegetation now dry to touch.

Vegetation classification

Macrophyte vegetation of each pond was surveyed during spring/summer 2014 using a 1 m^2 quadrat grid with cross wires every 10 cm. The plant species under each intersection of the cross wires was recorded, to give a % cover for each species (for details on plant survey methods see Jeffries, 2008). TWINSPAN analysis was used to classify the ponds by the plant data, resulting in 4 distinct plant communities. TWINSPAN was run on CAP 3.1.

Data analysis

To assess the changes in CO_2 flux rates over the sampling period a mixed model Repeated Measures Analysis of Variance (ANOVA) was performed. Flux rates from the four sampling days were considered as within-subject variables, factoring in each pond as the subjects of the repeated measurements across the four sampling days. The four types of ponds differing in vegetation classification as defined by the TWINSPAN analysis were used as between-subject factors, to assess any variations in flux rates between broad vegetation types. All statistical analysis was performed using IBM SPSS Statistics 22.

Results

Flux rates of CO_2 amongst ponds varied markedly over the four sampling days (Fig. 1), with mean ($\pm$ standard deviation, SD) *E–F* CO_2 switching from a net intake on Day 1 of -641 ± 1490 mg m^{-2} day^{-1}, to a net emission of 190 ± 1286 mg m^{-2} day^{-1} by Day 4, 1354 ± 1805 mg m^{-2} day^{-1} by Day 9, and 3792 ± 2755 mg m^{-2} day^{-1} by Day 12. The precision of triplicate measurements were mostly <20 % relative standard deviation (RSD) with the exception of three triplicate sets (35, 38 and 49 % RSD) suggesting occasional quick variations in flux rates.

Mean daily flux rates between the four sampling days varied significantly ($F = 41.94$, df 3, 69, $P = <0.00$. NB all tests included interactions terms and ponds as random subjects which reduces the error dfs slightly whilst we only report the results for the main factors) with flux rates being statistically different between days 1–9 ($P = <0.00$) and 1–12 ($P = <0.00$), days 4–9 ($P = 0.04$) and 4–12 ($P < 0.00$), and days 9–12 ($P < 0.00$). Only flux rates on days 1 and 4 were not significantly different ($P > 0.05$).

Note that in the Repeated Measures ANOVA used to compare mean daily flux rates over the sampling

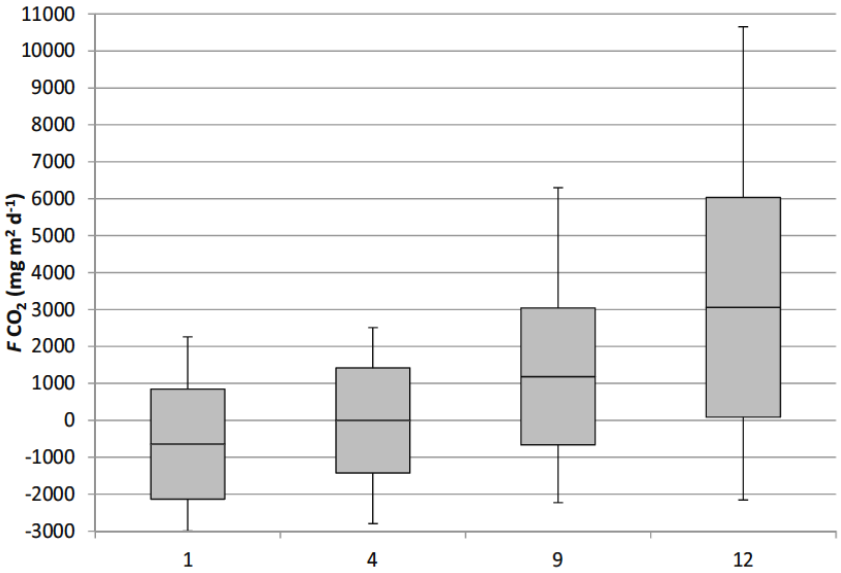


Fig. 1 Box plot of CO_2 flux rates from the 26 ponds surveyed on the four sampling days. The boxplot represents the minimum, lower quartile, median, upper quartile and maximum values. Positive values represent an emission of CO_2 from the ponds to the atmosphere whilst negative values represent an intake

period, Mauchly's test indicated that the assumption of sphericity had been violated ($x^2 = 12.85$, df 5, $P = <0.05$), i.e. there was a degree of non-independence of data from individual ponds. Therefore, degrees of freedom were corrected using Huynh–Feldt estimates of sphericity. This is not merely a necessary statistical adjustment but also reveals an important outcome, indicating a significant degree of variability within the data associated with the individual pond being monitored.

There were no major weather variations over the sampling period, with wind speed and atmospheric temperature remaining relatively constant (Fig. 2). The only notable aspect of the weather over the period was the absence of precipitation. The total rainfall in the 3 weeks preceding sampling was 39.37 mm with the last substantial rainfall (6.10 mm) being 11 days prior to sampling on Day 1. With <5 mm rainfall during the middle of the sampling period the ponds quickly dried up, with the softer vegetation in many of the ponds wilting by the end of the sampling period.

Figures 3 and 4 show the F CO_2 on the four sampling days for each of the ponds characterized by their hydrology at the time of sampling. Flux rates were markedly higher in ponds that were dry, and as ponds dried out over the survey period, their flux rates shifted from intake to emission. Not only did the fluxes shift from an intake to emission but the flux also continued to increase with increasing absence of rainfall.

Subtle variations in the hydrology of the site have led to marked differences in the vegetation between the ponds. Figure 5 shows the location of each pond grouped by the four divisions of the TWINSPAN analysis and their mean daily E–F CO_2 is shown in Fig. 6. Thirty-six species of macrophytes were recorded. Differences between the four groups are subtle with many species found in most ponds, in particular amphibious grasses e.g. *Agrostis stolonifera* L., the rush *Juncus articulatus* L. and the moss *Leptodictyum riparium* (Hedw.) Warnst. Differences between groups resulted from the dominance of particular species. Group 1 was dominated by *Glyceria fluitans* (L.) R. Br. and *Carex otrubae* Podp. and Group 2 characterized by dense *Eleocharis palustris* (L.) Roem. & Schult. Group 3 was a slightly wetter set of pools with *E. palustris* and the filamentous algae *Spirogyra* sp., whilst Group 4 lacked the *E. palustris*. No significant differences were found between the F CO_2 for the four groups of ponds characterized by their plant community communities ($F = 2.24$, df 3, 66, $P = >0.05$). However, the overall design is very unbalanced with the majority of ponds in just one plant community group which may explain the lack of differences between the four communities.

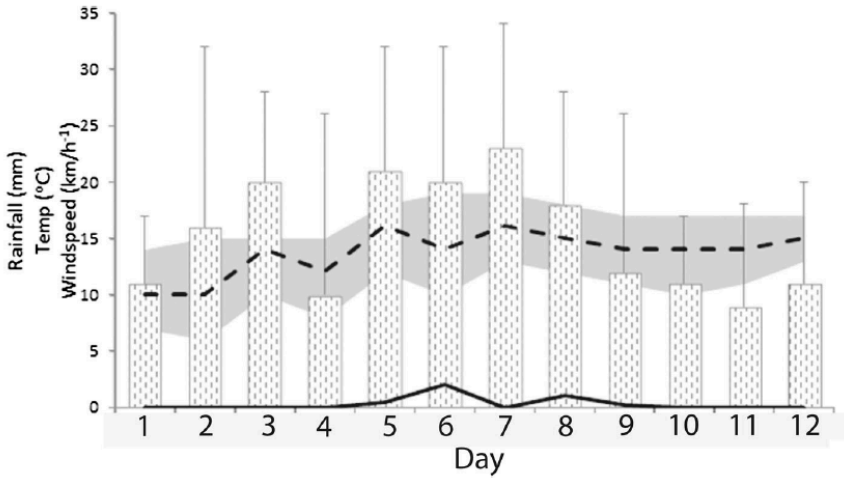


Fig. 2 Climatic conditions over the sampling period. Weather data are from the UK Met Office Boulmer Weather Station located approximately 12 km north from Hauxley Nature Reserve. Rainfall is the *solid line*. The *dashed line* is mean temperature, the shading either side maximum and minimum. The columns are mean wind speed with maximum *bars* above

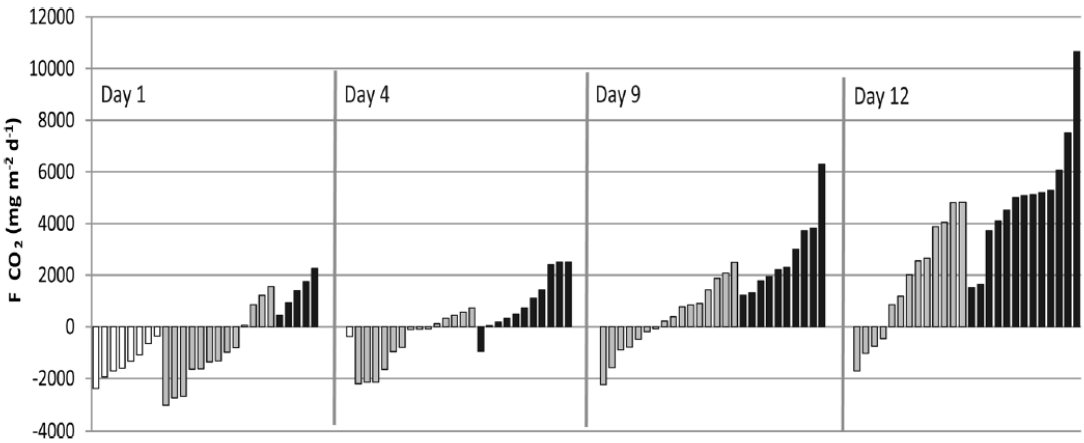


Fig. 3 CO_2 flux for each pond on the four sampling days. Ponds are grouped by hydrology and ordered by flux rates. Over the course of the 4 days more ponds dry out and become net sources of net CO_2. Positive values = net CO_2 emission, negative values = net CO_2 capture. ▭ = wet ponds, ▒ = transitional, ■ = dry ponds

Discussion

The objective of this study was to quantify the changes in the *F* CO_2 for small temporary ponds: the data provide evidence of very fine-grained spatial and temporal heterogeneity of CO_2 fluxes from these habitats.

Temporal heterogeneity

The *E*–*F* CO_2 for all the ponds during the survey period indicates that these small ponds act as a net source of *C* to the atmosphere during prolonged absence of rainfall during summer months. This behaviour has been frequently reported for similar temporary aquatic systems during drying periods i.e. ponds, river courses, wetland and tidal regions (Fromin et al., 2010; von Schiller et al., 2014; Catalán et al., 2014).

However, daily means varied significantly. Whilst the cluster of ponds acted as a net sink on Day 1, this had shifted to a net source of CO_2 only 3 days later, and reached a 9-fold increase in CO_2 efflux within 2 weeks. Consequently *F* CO_2 at the beginning and

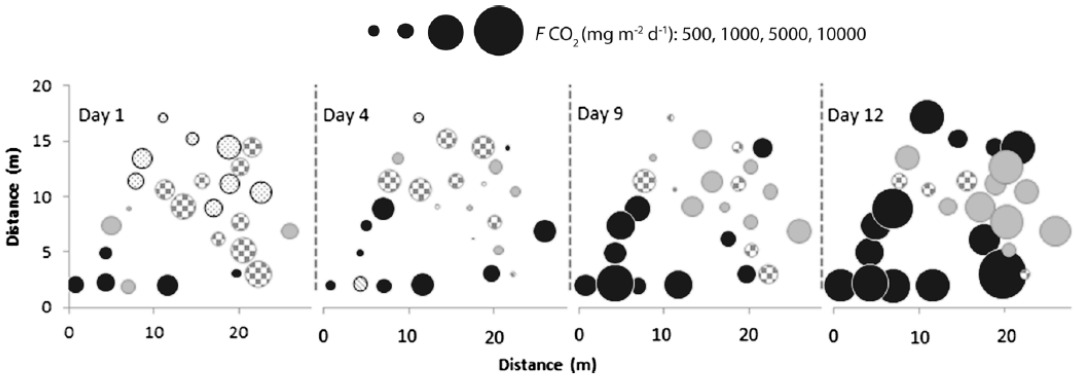


Fig. 4 CO_2 flux for each pond on the four sampling days and their spatial distribution across the site. The position of each pond on the site is shown by a *circle* and the *circle shading* indicates hydrological state and whether the pond is a net CO_2 source or sink. *Circles with fine dots* wet ponds with negative CO_2 flux, *checker board circles* transitional ponds with negative CO_2 flux, *grey circles* transitional ponds with positive CO_2 flux, *circles with diagonal lines* dry ponds with negative CO_2 flux and *black circles* dry ponds with positive CO_2 flux

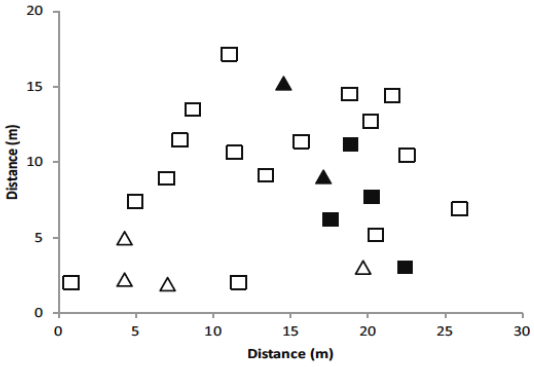


Fig. 5 The distribution of the ponds and plant community types as classified by TWINSPAN. Each marker represents the location of an individual pond spatially across the site, the *y*-axis representing the north–south orientation. The four pond plant communities are represented by *filled square* group 1 (dominated by *Glyceria fluitans* and *Carex otrubae*), *open square* group 2 (dense *Eleocharis palustris*), *open triangle* group 3 (sparser *E. palustris* with *Spirogyra* sp.) and *filled triangle* group 4 (No *E. palustris*). See text for detailed description of plant survey and analysis)

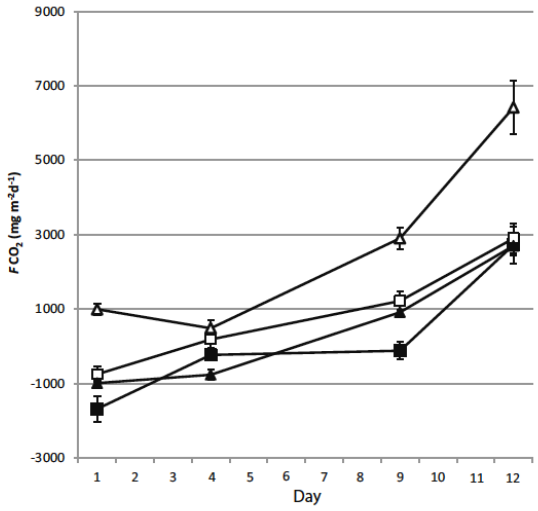


Fig. 6 CO_2 flux rates (mean ± standard deviation) grouped by vegetation types over the four sampling days. The four pond types are *filled square* group 1 (dominated by *Glyceria fluitans* and *Carex otrubae*), *open square* group 2 (dense *Eleocharis palustris*), *open triangle* group 3 (sparser *E. palustris* with *Spirogyra* sp.) and *filled triangle* group 4 (No *E. palustris*)

end of the sampling period were comparable to both the lower and upper end of effluxes previously reported for freshwater ecosystems, respectively (Raymond et al., 2013).

Similarly, during the drying phase of a temporary pond in southeastern France, Fromin et al. (2010) observed a peak in *F* CO_2 at the beginning of the drying processes with substrate-induced respiration from microbial activity peaking after 3 weeks. Beyond 3 weeks the CO_2 emissions continued to decrease throughout the drought period. Whilst it is likely that *F* CO_2 beyond our survey period may decrease when all ponds enter a stable drought phase, our study highlights the severity of *F* CO_2 emissions during the initial drying phase. For temperate ponds

that undergo several wetting and drying cycles during summer months this poses complications for management practices intended to enhance *C* capture and storage.

Another factor which might have exacerbated CO_2 emissions between Day 4 and Days 9 and 12 was the slight precipitation on Days 6 and 8. Whilst only small and not enough to fully rehydrate the ponds, rewetting of sediments or soils after dry periods is known to increase *C* lability and microbial activity resulting in rapid release of CO_2 (Fromin et al., 2010; von Schiller et al., 2014). Equally the resulting water stress on the macrophytes is likely to have reduced photosynthetic activity limiting the intake of atmospheric CO_2, which would counter balance the release of CO_2 during hydrated periods. This may be one factor contributing to the continued increase in CO_2 flux rates over the sampling period as the swards of grasses and moss overlying the bottom of the ponds continued to dry out during the switch from transitional phase to dry phase. Interestingly, the thick vegetation held in moisture, slowing the processes of sediment desiccation compared to ponds with a looser vegetation profile. Whilst no statistical relationship was found between vegetation type and CO_2 flux rates over this two-week study, during periods when rehydration occurs before complete desiccation, these mats might act as a 'buffer' preventing complete sediment desiccation and extensive CO_2 release. The sediments underneath the vegetation were conspicuously darker, damper and apparently anoxic compared to the few ponds lacking extensive vegetation cover.

The organic *C* content of sediments from other ponds in the area is high compared to adjacent non-wetland habitat indicating a net accumulation of *C* within temporal aquatic systems (Gilbert et al., 2014). Yet the data presented here highlight the interaction of *C* fluxes with the atmosphere. Taken together, the sediment storage and flux rates suggest that these ecosystems have the potential to be highly active sequesters of atmospheric *C* when hydrated during summer months as illustrated by the net intake of CO_2 on Day 1. However, this high degree of temporal variability in the *F* CO_2 over such a short period of time poses serious complications for extrapolations of measurements to seasonal averages from singular measurements alone and highlights the need for more comprehensive surveys when trying to extrapolate results.

Spatial heterogeneity

Whilst the temporal variability of the *F* CO_2 over several days poses complications for the infrequent measurement of temporal systems, the variability in *F* CO_2 amongst superficially similar ponds on the same day is equally variable (Figs. 2, 3). Flux rates amongst all ponds resulted in % RSDs of 232, 1320, 144 and 81 on Days 1, 4, 6 and 9, respectively, indicating high variability in the *F* CO_2 amongst individual ponds. This was supported by the outcome of the repeated measure ANOVA too, showing that the individual ponds behaved differently, and it matters which pond was being measured. On Day 1 there was a rough divide across the middle of the site between those ponds emitting and those taking in CO_2 (Fig. 3), which typically marks the marsh line as described in "Site description" section. However as the survey period progressed and more ponds began to enter the transitional or dry phase of their hydrological cycle, fluxes of CO_2 amongst ponds became more uniform.

No statistical variation was found in flux rates between vegetation types as characterized by the TWINSPAN analysis indicating that hydrology rather than vegetation is the dominant driver in CO_2 release. However several limitations exist. Firstly this study only represents a 2-week snap shot of the flux rates from these ponds in their life cycle. Vegetation communities in temporary systems change regularly depending on annual climate variations (Jeffries, 2008) and as such plant species that have greater impact on *F* CO_2 may have been missed in this study. Equally it may be that the previous macrophyte communities, which now form the sediment layer result in differences in the lability of organic matter, and subsequently may be more important than the growth of current plant communities.

This large degree of spatial variability poses complications for accurately quantifying the F CO_2 on a landscape scale if too few ponds or an unrepresentative group is chosen. The use of eddy covariance for monitoring terrestrial net ecosystem exchange over comparably large areas (100 m^2) provides a useful comparison to the flux from individual ponds (Abnizova et al., 2012) or for monitoring *F* CO_2 from ponds with a larger surface area (Fromin et al., 2010). However the use of eddy covariance on a landscape scale can easily overlook the influence of individual ponds, especially during wet and dry cycles. More

effort is needed to underpin the constraints of hydrology on the frequency of drying and rewetting cycles and their impact on F CO_2 amongst ponds across the landscape if accurate regional extrapolations of these small systems are to be acquired.

These results also suggest important practical outcomes, notably the potential of small ponds as CO_2 sinks, if the frequency of inundation periods is managed. Ponds and small wetlands are relatively easy to create and constructed wetlands have been widely used for the containment and treatment of a diversity of contaminated effluent (Vymazal, 2014). Typical uses include amelioration of acid mine water discharge (e.g. Shoeran & Sheoran, 2006), excess agricultural nutrients (Fink & Mitsch, 2004), road run-off (Gill et al., 2014) and nutrient-enriched river water (Tang et al., 2013). Small ponds are natural features of intensively used lowland landscapes throughout the world and can bring additional benefits to wildlife, although some studies of constructed wetlands built for controlling contamination show that the optimum designs for effluent reduction may be less suitable for maximizing benefits to wildlife (Hansson et al., 2005) or that the contamination is associated with degraded biodiversity compared to pristine wetlands (Batty et al., 2005). The potential of ponds as C sinks is clear, especially in the longer term when sequestration of CO_2 outweighs methane emissions (Mitsch et al., 2013). Creating ponds as part of our attempts to mitigate against C emissions looks to be both a practical and beneficial strategy. However, the few studies of C fluxes from exiting constructed wetlands, constructed for other purposes, shows they can also be net sources (e.g. Liikanen et al., 2006) and that the plants present can be important drivers of CO_2 emissions but precise outcomes can vary with plant species, e.g. Ström et al. (2005). More encouragingly Teiter & Mander (2005) explored using the example of domestic water treatment wetlands and estimated their C emissions would not be significant globally; a key management outcome from our study is the need to get the design of wetlands right to maximize their effectiveness in the face of natural climate variations and the threat of greater climate variation.

Without complete annual and diurnal flux measurements and C burial rates it would be inappropriate to extrapolate this study to state whether small ponds act as a net sources or net sinks to the atmosphere. Nonetheless, their flux rates are comparable with those of ecosystems with some of the highest rates of CO_2 sequestration/emissions on the planet highlighting the possibility that small seasonal aquatic systems are important cyclers of atmospheric C.

This study set out to monitor spatial and temporal changes to CO_2 flux rates in small, temporary ponds in a typical lowland European landscape during a summer drying phase. The results show striking temporal change in E–F CO_2 linked to hydrological changes, with ponds at the start of the 2-week study period being net CO_2 sinks, and as the site dried out the ponds increasingly became net sources of CO_2 to the atmosphere. A 9-fold difference in flux rates from the beginning to end of the study period resulted in F CO_2 comparable to both the lower and upper flux rates reported for aquatic ecosystems (Raymond et al., 2013). There was a broad spatial gradient in the behaviour of ponds across the site as the site dried out. In addition, the repeated measures analysis of the gas fluxes suggested that the behaviour of individual ponds varied but that the precise plant communities did not affect the CO_2 flux. Small ponds and wetlands like those in this study are found throughout the Earth's terrestrial biomes, from tropics to polar regions. Our results show that small-scale spatial and temporal changes can result in large variations in wetland CO_2 fluxes to the atmosphere suggesting that these ubiquitous habitats may be an important but overlooked component of global C dynamics.

Acknowledgments We are grateful to the Northumberland Wildlife Trust for permission to work on the Hauxley site and to the organizers of *Wetlands 2014* in Huseca and *SEFS 2015* Geneva for allowing the opportunity to present the results of this study.

References

Abnizova, A., J. Siemens, M. Langer & J. Boike, 2012. Small ponds with major impact: the relevance of ponds and lakes in permafrost landscapes to carbon dioxide emissions.

Global Biogeochemical Cycles 26: 2.
Allende, L. & G. Mataloni, 2013. Short term analysis of the phytoplankton structure and dynamics in two ponds with distinct trophic states from Cierva Point (maritime Antarctica). Polar Biology 36: 629–644.
Battin, T. J., S. Luyssaert, L. A. Kaplan, A. K. Aufdenkampe, A. Richter & L. J. Tranvik, 2009. The boundless carbon cycle. Nature Geoscience 2: 598–600.
Batty, L. C., L. Atkin & D. A. C. Manning, 2005. Assessment of the ecological potential of mine-water treatment wetlands using a baseline survey of macroinvertebrate communities. Environmental Pollution 13: 412–419.
Batzer, D. P., S. E. Dietz-Brantley, B. E. Taylor & S. E. DeBiase, 2005. Evaluating regional differences in macroinvertebrate communities from forested depressional wetlands across eastern and central North America. Journal of the North American Benthological Society 24: 403–414.
Boix, D., J. Biggs, R. Céréghino, A. P. Hull, T. Kalettka & B. Oertli, 2012. Pond research and management in Europe: "small is beautiful". Hydrobiologia 689: 1–9.
Bouchard, V., S. D. Frey, J. M. Gilbert & S. E. Reed, 2007. Effects of macrophyte functional group richness on emergent freshwater wetland functions. Ecology 88: 2903–2914.
Boven, L., R. Stoks, L. Forró & L. Brendonck, 2008. Seasonal dynamics in water quality and vegetation cover in temporary pools with variable hydroperiods in Kiskunság (Hungary). Wetlands 28: 401–410.
Catalán, N., D. von Schiller, R. Marcé, M. Koschorreck, L. Gomez-Gener & B. Obrador, 2014. Carbon dioxide efflux during the flooding phase of temporary ponds. Limnetica 33: 349–360.
Cereghino, R., D. Boix, H.-M. Cauchie, K. Marten & B. Oertli, 2014. The ecological role of ponds in a changing world. Hydrobiologia 723: 1–6.
Chanda, A., A. Akhand, S. Manna, S. Dutta, I. Das, S. Hazra, K. H. Rao & V. K. Dadhwal, 2013. Measuring daytime CO_2 fluxes from the inter-tidal mangrove soils of Indian Sundarbans. Environmental Earth Sciences 72: 417–427.
Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, C. M. Duarte, P. Kortelainen, J. A. Downing, J. J. Middelburg & J. Melack, 2007. Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems 10: 172–185.
Collinson, N. H., J. Biggs, A. Corfield, M. J. Hodson, D. Walker, M. Whitfield & P. Williams, 1995. Temporary and permanent ponds: an assessment of the effects of drying out on the conservation value of aquatic macroinvertebrate communities. Biological Conservation 74: 125–133.
Davies, B., J. Biggs, P. Williams, M. Whitfield, P. Nicolet, D. Sear, S. Bray & S. Maund, 2008. Comparative biodiversity of aquatic habitats in the European agricultural landscape. Agriculture, Ecosystems and Environment 125: 1–8.
Downing, J. A., 2010. Emerging global role of small lakes and ponds: little things mean a lot. Limnetica 29: 9–24.
Downing, J. A., Y. T. Prairie, J. J. Cole, C. M. Duarte, L. J. Tranvik, R. G. Striegl, W. H. McDowell, P. Kortelainen, N. F. Caraco & J. M. Melack, 2006. The global abundance and size distribution of lakes, ponds, and impoundments. Limnology and Oceanography 51: 2388–2397.
Ewald, N., T. Kalettka & L. Brendonck, 2012. Eyes of the landscape – value, conservation and management of European ponds. Limnologica – Ecology and Management of Inland Waters 42: 251–253.
Fink, D. F. & W. J. Mitsch, 2004. Seasonal and storm event nutrient removal by a created wetland in an agricultural watershed. Ecological Engineering 23: 313–325.
Foster, G., 1993. Pingo fens, water beetles and site evaluation. Antenna 17: 184–195.
Fowler, H. J. & C. G. Kilsby, 2002. Precipitation and the North Atlantic Oscillation: a study of climatic variability in northern England. International Journal of Climatology 22: 843–866.
Fromin, N., G. Pinay, B. Montuelle, D. Landais, J. M. Ourcival, R. Joffre & R. Lensi, 2010. Impact of seasonal sediment desiccation and rewetting on microbial processes involved in greenhouse gas emissions. Ecohydrology 3: 339–348.
Gala, T. S. & A. M. Melesse, 2012. Monitoring prairie wet area with an integrated LANDSAT ETM+, RADARSAT-1 SAR and ancillary data from LIDAR. Catena 95: 12–23.
Gallagher, A. & K. Huissteden, 2011. Increased greenhouse gas emission from thaw ponds in Siberian arctic tundra on continuous permafrost. Geophysical Research Abstracts 13: EGU2011-3830.
Gilbert, P. J., S. E. Taylor, D. A. Cooke, M. Deary, M. Cooke & M. J. Jeffries, 2014. Variations in sediment organic carbon among different types of small natural ponds along Druridge Bay, Northumberland, UK. Inland Waters 4: 57–64.
Gill, L. W., P. Ring, N. M. P. Higgins & P. M. Johnston, 2014. Accumulation of heavy metals in a constructed wetland treating road runoff. Ecological Engineering 70: 133–139.
Hansson, L.-A., C. Brönmark, P. A. Nilsson & K. Åbjörnsson, 2005. Conflicting demands on wetland ecosystem service: nutrient retention, biodiversity or both? Freshwater Biology 50: 705–714.
Jeffries, M. J., 1998. Pond macrophyte assemblages, biodisparity and spatial distribution of ponds in the Northumberland coastal plain, UK. Aquatic Conservation: Marine and Freshwater Ecosystems 8: 657–667.
Jeffries, M. J., 2008. The spatial and temporal heterogeneity of macrophyte communities in thirty small, temporary ponds over a period of 10 years. Ecography 31: 765–775.
Jeffries, M. J., 2010. The temporal dynamics of temporary pond macroinvertebrate communities over a 10-year period. Hydrobiologia 661: 391–405.
Jeffries, M. J., 2012. Ponds and the importance of their history: an audit of pond numbers, turnover and the relationship between the origins of ponds and their contemporary plant communities in south-east Northumberland, UK. Hydrobiologia 689: 11–21.
Jonai, H. & W. Takeuchi, 2014. Comparison between global rice paddy field mapping and methane flux data from GOSAT. Geoscience and Remote Sensing Symposium (IGARSS) 2014: 2098–2101.
Jones, I., 2013. The Impact of Extreme Events on Freshwater Ecosystems. British Ecological Society, London.
Keeley J. & P. Zedler 1998. Characterization and global distribution of vernal pools. In: Witham C., E. Bauder, D. Belk, Ferren W.R. Jr. & R. Ornduff (eds) Ecological Conservation and Management of Vernal Pool

Ecosystems. – Proceedings rom a 1996 Conference. California Native Plant Society. Sacramento: 1–14.

Kutschker, A. M., L. B. Epele & M. L. Miserendino, 2014. Aquatic plant composition and environmental relationships in grazed Northwest Patagonian wetlands, Argentina. Ecological Engineering 64: 37–48.

Liikanen, A., J. T. Huttunen, S. M. Karjalainen, K. Heikkinen, T. S. Väisänen, H. Nykanen & P. J. Martikainen, 2006. Temporal and seasonal changes in greenhouse gas emissions from a constructed wetland purifying peat mining runoff water. Ecological Engineering 26: 241–251.

Lunn, A., 2004. Northumberland. Harper Collins, London.

Mitsch, W. J., N. Bernal, A. M. Nahlik, Ü. Mander, L. Zhang, C. J. Anderson, S. E. Jøgensen & H. Brix, 2013. Wetlands, carbon and climate change. Landscape Ecology 28: 583–597.

Mozley, A., 1937. The ponds, lakes and streams of the Kirghiz Steppe. The Scottish Geographical Magazine 53: 1–10.

Mo, Y., Z.-H. Deng, J.-Q. Gao, Y.-X. Gua & F.-H. Yu, 2015. Does richness of emergent plants affect CO_2 and CH_4 emissions in experimental wetlands? Freshwater Biology 60: 1537–1544.

Raymond, P. A., J. Hartmann, R. Lauerwald, S. Sobek, C. McDonald, M. Hoover, D. Butman, R. Striegl, E. Mayorga, C. Humborg, P. Kortelainen, H. Dürr, M. Meybeck, P. Ciais & P. Guth, 2013. Global carbon dioxide emissions from inland waters. Nature 503: 355–359.

Seekell, D. A. & M. L. Pace, 2011. Does the pareto distribution adequately describe the size-distribution of lake? Limnology and Oceanography 56: 350–356.

Shoeran, A. S. & V. Sheoran, 2006. Heavy metal removal mechanism of acid mine drainage in wetlands: a critical review. Minerals Engineering 19: 105–116.

Ström, L., M. Mastepanov & T. R. Christensen, 2005. Species-specific effects of vascular plants on carbon turnover and methane emissions from wetlands. Biogeochemistry 75: 65–82.

Tang, W., W. Zhang, Y. Zhao, Y. Wang & B. Shan, 2013. Nitrogen removal from polluted river water in a novel ditch-wetland-pond system. Ecological Engineering 60: 135–139.

Teiter, S. & U. Mander, 2005. Emission of N_2O, N_2, CH_4 and CO_2 from constructed wetlands for wastewater treatment and from riparian buffer zones. Ecological Engineering 25: 528–541.

Torgersen, T. & B. Branco, 2008. Carbon and oxygen fluxes from a small pond to the atmosphere: temporal variability and the CO_2 /O_2 imbalance. Water Resource Research 44: WO2417. doi:10.1029/2006WR005634.

Tranvik, L., J. A. Downing & J. Cotner, 2009. Lakes and reservoirs as regulators of carbon cycling and climate. Limnology and Oceanography 54: 52–68.

Turetsky, M. R., A. Kotowska, J. Bubier, N. B. Dise, P. Crill, E. R. C. Hornibrook, K. Minkkinene, T. R. Moore, I. H. Myers-Smith, H. Nykänen, D. Olefeldt, J. Rinne, S. Saarnio, N. Shurpali, E.-S. Tuitila, J. M. Waddington, J. R. White, K. P. Wickland & M. Wilmking, 2014. A synthesis of methane emissions from 71 northern, temperature and subtropical wetlands. Global Change Biology 20: 2183–2197.

Verpoorter, C., T. Kutser, D. A. Seekell & L. J. Tranvik, 2014. A global inventory of lakes based on high-resolution satellite imagery. Geophysical Research Letters 41: 6396–6402.

von Schiller, D., R. Marcé, B. Obrador, L. Gómez-Gener, J. Casas-Ruiz, V. Acuña & M. Koschorreck, 2014. Carbon dioxide emissions from dry watercourses. Inland Waters 4: 377–382.

Vymazal, J., 2014. Constructed wetlands for treatment of industrial wastewaters: a review. Ecological Engineering 73: 724–751.

Williams, P., J. Biggs, G. Fox, P. Nicolet & M. Whitfield, 2001. History origins and importance of temporary ponds. Freshwater Forum 17: 7–15.

Williams, P., M. Whitfield, J. Biggs, S. Bray, G. Fox, P. Nicolet & D. Sear, 2004. Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in southern England. Biological Conservation 115: 329–341.

Appendix B: Gilbert et al. (2014)

Article

Variations in sediment organic carbon among different types of small natural ponds along Druridge Bay, Northumberland, UK

Peter J. Gilbert[1*], Scott Taylor[1], David A. Cooke[1], Michael Deary[1], Martin Cooke[2], and Michael J. Jeffries[1]

[1] *Northumbria University, Ellison Building, NE1 8ST, Newcastle upon Tyne, UK*
[2] *Newcastle University, Drummond Building, NE1 7RU, Newcastle upon Tyne, UK*
* *Corresponding author email: peter2.gilbert@northumbria.ac.uk*

Received 4 April 2013; accepted 13 September 2013; published 10 January 2014

Abstract

Small natural ponds from Druridge Bay, Northumberland (UK), were sampled to investigate the variations in sediment organic carbon (OC) content among pond types. Sediment OC was highest in uncompacted sediments from permanent ponds with extensive natural vegetation (means ranged between 7.68 and 12.86% OC) but lower in compacted sediments (mean 3.72% OC) or from ponds in arable or pasture fields (mean 3.44% OC) and from adjacent soil controls (means of 3.13–3.38% OC). The extent of 4 distinct pond types (permanent naturally vegetated, arable field, grass pasture field, and dune slack) varied across years. This study highlights ecological variations among pond types that can result in large variations in sediment OC content and, combined with annual variations to the extent of ponds, poses significant implications for upscaling carbon burial rates based solely on combined surface area.

Key words: Northumberland, pond ecology, sediment organic carbon

Introduction

To develop a comprehensive understanding of the factors controlling climate change, there is significant need to fully quantify the processes and intricate interactions within sub-compartments of the global carbon cycle (Cole et al. 2007, Stets and Striegl 2012). While substantial research has recently been conducted into the role of inland seas, large lakes, reservoirs, and aquaculture impoundments as sinks for carbon sequestration (Dean and Gorham 1998, Lehner and Döll 2004, Boyd et al. 2010, Downing 2010), smaller waterbodies have been almost wholly neglected in audits of the global carbon cycle (USCCSP 2003, Downing 2010, Alonso et al. 2012). Several developments over recent years have highlighted the potential significance of ponds in global cycles. Recent attempts to create holistic global inventories of terrestrial waterbodies estimate that around 304 million features cover ~4.2 million km^2, dominated by small lakes and ponds (Lehner and Döll 2004, Downing et al. 2006, Tranvik et al. 2009). Constraints in satellite imagery and limited regional data limit our ability to quantify ponds smaller than 100–1000 m^2 (Downing et al. 2012), yet if the negative correlation between size and global frequency were to apply, they would undoubtedly have significant global coverage.

Analysis of organic carbon (OC) burial rates in small aquatic systems has highlighted their potential as major contributors in the carbon cycle. Agriculturally eutrophic impoundments may bury carbon at an average rate of 2122 g m^{-2} yr^{-1}, far exceeding those found in any other global ecosystem (Mulholland and Elwood 1982, Dean and Gorham 1998, Downing 2010). This high accumulation can be attributed to high nutrient concentrations driving primary productivity, comparably high levels of soil particulate transfer from adjacent land due to the high ratio of pond edge to area, and high rates of preservation due to nearly continuous sediment anoxia (Munsiri et al. 1995, Verstraeten and Poesen 2002, Downing et al. 2008). Because global coverage of ponds has been drastically underestimated, and rates of carbon sequestration in small lakes and ponds are nearly double that of larger waterbodies, inland waters are now believed to process about 1 petagram yr^{-1} more C than previously thought (Downing 2010) and may dominate terrestrial global carbon processing (Meyers and Ishiwatari 1993, Cole et al. 2007, Boyd et al. 2010, Downing 2010).

DOI: 10.5268/IW-4.1.618

Inland Waters (2014) **4**, pp. 57-64

Substantial gaps in our understanding of small ponds in the global carbon cycle still remain, however. Most studies focus specifically on aquaculture ponds (Xinglong and Boyd 2006, Ntengwe and Edema 2008, Boyd et al. 2010), and of those studies that do focus on small natural waterbodies, most overlook the diversity of pond types and the heterogeneity of pond wildlife that can occur across landscapes (Williams 2004).

Given that pond biodiversity is characteristically heterogeneous at the landscape scale, we hypothesise that the carbon capture potential of different pond types will be equally variable (e.g., with pond permanence or adjacent land-use). By grouping ponds together we may be overlooking some of the key components that dictate a pond's efficiency for carbon capture and limiting the application of results. This study quantifies organic carbon from small ponds with different vegetation, substrate, seasonal extent, and adjacent land-use in North East England.

Study sites

We sampled ponds from Druridge Bay (Northumberland, UK). The Northumberland coastal plain has a relatively cool, dry, temperate climate (summer mean maximum temperatures are seldom >20 °C, annual rainfall usually <800 mm). The Druridge Bay area is densely populated with wetlands, primarily large lakes, dating from the 1950s onward, created for nature conservation (~10% of the area) or by land subsidence from underlying abandoned coal mines (Jeffries 2012). Smaller ponds and dune slacks occupy ~2% of the landscape; the majority are <400 m^2, and many of these are pools and flashes of <10 m^2. Two sites were selected to best represent the ponds in the region.

Six ponds were studied around Blakemoor Farm (59°40′56″N; 42°84′39″E) at the southern end of Druridge Bay (ponds B1–B6), sampled May–June 2011. Ponds were located within both pasture fields (ponds B2 and B3) and arable fields (ponds B5 and B6) subject to crop spraying, while 2 naturally vegetated ponds were located adjacent to fields (ponds B1 and B4). Pond water recharge is predominantly from precipitation, with pond winter maximum areas ranging from 10 to 12,000 m^2, and all but 2 (ponds B1 and B4) drying out during prolonged periods of no rainfall. Some ponds displayed evidence of subsurface upwelling with elevated conductivity, indicating seawater intrusion via mine tunnels, although the pond environments were wholly freshwater in character.

Ten ponds were studied at Hauxley Nature Reserve (60°27′61″N; 42°85′48″E) at the northern end of Druridge Bay (ponds H1–H10), sampled February 2012. All 10 ponds are 1 m^2 and were constructed in 1994 to monitor plant and invertebrate community assembly (Jeffries 2002). Located on a backfilled opencast coal mine site topped with a layer of clay and ~50 cm topsoil, the field has been left as a nature reserve and has not been subjected to direct agricultural inputs.

Methods

Sediment samples: Two sampling methods were adopted. For compacted sediments typical of the ponds at Hauxley and the peripheries of the larger ponds at Blakemoor, a 4 cm internal diameter "pole corer" was manually driven into the sediment as far as possible to ensure penetration of the original compacted bottom soil. For the uncompacted sediments at Blakemoor Farm with high moisture content, a "freeze corer" was constructed for *in situ* sampling, simplified from Shapiro (1958). Both of these techniques allowed the removal of an intact sediment profile, allowing the analysis of the percent organic carbon (%OC) over depth.

One sediment core per pond was collected from the 10 ponds at Hauxley, and between 1 and 5 sediment cores were collected from the 6 ponds at Blakemoor Farm depending on the waterbody size, forming a transect through the centre. While the 5 cores from pond B3 at Blakemoor Farm resulted in a precision of 31% (calculated from the inter-core mean %OC values), ponds with <5 cores greatly varied in precision (22–81%). Pittman et al. (2013) suggested in an analysis of impoundments ~100,000 m^2 that 10 cores was the minimum required to gain a 25% precision, with only marginal gains beyond 10, and significant reduction with fewer. The ponds in this study are ~2–3 orders of magnitude smaller and may be perceived to be more homogeneous. Although this study was designed as exploratory research, the large seasonal variations in area and subsequent complexities in substrate constituency of small ponds makes it difficult to gain a high level of precision.

In addition to sediment cores, soil cores were also collected to provide an indication of the %OC in the underlying and surrounding soils. Two samples were taken at Hauxley and one from within 2 m above the high winter inundation line of each pond at Blakemoor Farm. All sediment and soil cores were extruded and wrapped in aluminium foil on site and stored at 4 °C prior to preparation for analysis.

Sample preparation: Samples were air dried for >4 d before dissection. The samples from Hauxley were separated into 3 layers based on visible laminations, consisting of the top layer of accumulated sediment, an underlying transitional layer, and the original undisturbed pond bottom, as described by Munsiri et al. (1995). Sediment cores from Blakemoor Farm were dissected into

 DOI: 10.5268/IW-4.1.618

~1.5 cm separations. Once dissected, all samples were ground using a pestle and mortar.

Sediment analysis: Sediment OC in the Hauxley cores was determined using the Walkley-Black titration method (Nelson and Sommers 1982). Sediment OC in the Blakemoor Farm samples was pretreated for inorganic carbonates and determined by dry combustion (Schumacher 2002). All samples were blank corrected and the limit of quantification calculated to be 0.08% (lowest OC sampled was 1.29%). Duplicates and triplicates (included every 5th and 10th sample) comprised a full range of depths, with all except 2 relative standard deviations (RSDs) <6.56%, and most <5%RSD. While dry combustion is currently the standard technique for OC analysis, Walkley-Black has often been the standard method for comparison and yields comparable results to dry combustion (Nelson and Sommers 1982, Soon and Abboud 1991, Schumacher 2002).

Sediment characteristics: One core was also collected from each of the ponds at Blakemoor Farm (taken from the area most representative of the ponds vegetation) to provide a detailed quantification of the dry bulk density (DBD) and moisture content variations throughout the sediment profile. Cores were dissected every ~1 cm, noting the exact length to calculate volume, and weighed immediately upon return to Northumbria University. Cores were then air dried >4 d before obtaining the dry weight to calculate moisture content and DBD.

Extent of pond types at Blakemoor: In November 2010, 2011, and 2012 the site was walked (overall search area ~1 km^2) to map the approximate areas of ponds onto aerial images, which were then measured using Edina Digimap (2012). Ground walking allows observation of small, ephemeral pools such as tyre ruts and gateway puddles to be located, while the autumn date allows refilling after summer dry phases but before extensive winter inundation obscures differences. Vegetation in many of the ponds has been recorded as part of long term studies (Zealand and Jeffries 2009). Our purpose here is not to explore the detailed variations of plant communities, but we identified 4 distinct pond types with gross variations in plants and adjacent land-use that may affect carbon sequestration (Table 1). The small ponds at Hauxley have been monitored intensively (Jeffries 2002), and the vegetation is similar to the naturally vegetated ponds at Blakemoor, although the Hauxley ponds dry most years.

Results

Sediment OC ranged from 1 to 19% (n = 141) but with marked differences between ponds, sediment types, and core depth. The highest %OC was found in the upper layers (to a depth of ~4 cm) of the uncompacted sediment from naturally vegetated ponds at Blakemoor (mean ± 1SD, 12.86% ± 3.97) in comparison to the compacted samples (3.72% ± 2.13; Fig. 1). In addition, the upper

Table 1. Broad characterisation of 4 pond types at Blakemoor Farm.

Pond type	Characteristics. Conductivity (µS cm^{-1}), as mean (min, max)
Arable field ponds	Shallow (<30 cm), usually temporary ponds in arable cereal and oil seed rape fields. Bare ploughed soil substrate, with no or limited (<5%) vegetation cover, primarily ubiquitous weeds of disturbed ground (e.g., common knotgrass [*Polygonum aviculare*] and wild chamomile [*Chamomilla suaveolens*], OV18 community; Rodwell 2000). Mean Cond. = 383 µS cm^{-1} (89–1200)
Grazing pasture ponds	Shallow (<30 cm) permanent and temporary ponds in sheep or cattle pasture fields. Soil substrate, extensive vegetation (>90%), mostly seeded fodder (e.g., perennial ryegrass [*Lolium perenne*]) or amphibious grasses (e.g., flote grass [*Glyceria fluitans*]). Mean Cond. = 581 µS cm^{-1} (234–1151)
Dune slack ponds	Shallow (<30 cm), temporary, on landward side of dunes. Organic rich mud substrate over sandy soil. Occasional brackish water inundation. Extensive natural vegetation (>90%) characteristic of such sites (e.g., common silverweed [*Potentilla anserine*]). Mean Cond. = 728 µS cm^{-1} (244–1392)
Deeper permanent ponds	Permanent with extensive deep zones (>0.5 m) as well as shallow swamp zones. Typically with organic rich, anoxic, uncompacted substrate. Extensive but patchily vegetated (20–60%) with characteristic pond emergents (e.g., branched bur-reed, [*Sparganium erectum*]) and submerged species (e.g., water-starworts [*Callitriche* spp.]). Mean Cond. = 817 µS cm^{-1} (555–1436)

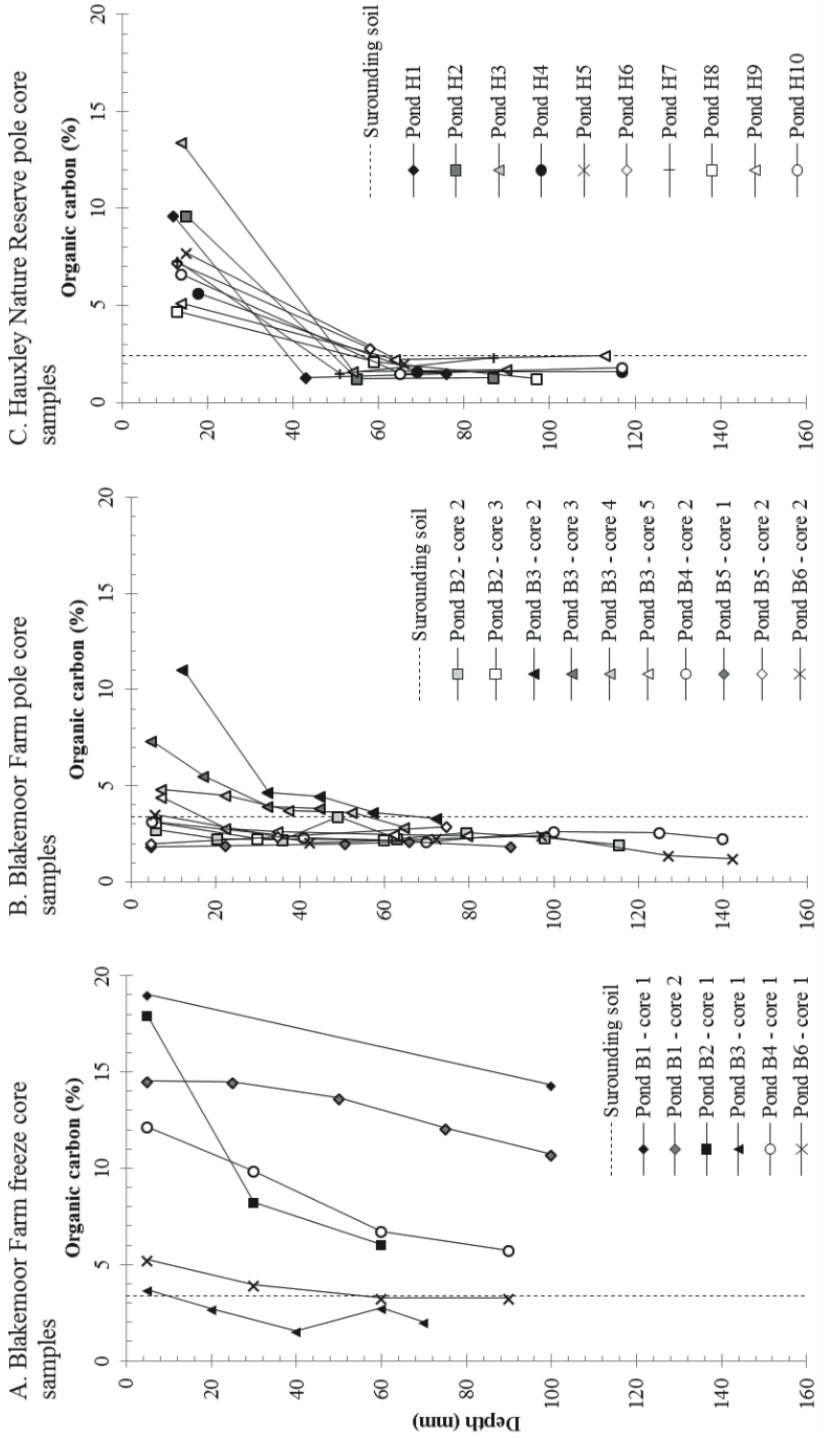


Fig. 1. Sediment organic carbon concentrations in successive core segments taken from ponds at Druridge Bay, Northumberland, UK. (A) Blakemoor Farm freeze core samples represent high moisture content uncompacted sediments. (B) Blakemoor Farm pole cores represent low moisture content compacted sediments. (C) Hauxley Nature Reserve cores obtained using pole core method, collecting one core per pond. Number of cores collected was dependent on size of pond; all cores collected are shown. Lines are provided for guidance only and do not represent trend lines.

 DOI: 10.5268/IW-4.1.618

layers of the limited number of uncompacted sediment samples from arable and pasture ponds at Blakemoor Farm (ponds B3 and B6; Fig. 1) contained much lower %OC (3.44% ± 1.43). The %OC in the accumulated sediment of cores from the Hauxley ponds was 7.68% ± 2.61. Results for OC in the surrounding soils were lower at all sites: 3.38% ± 1.41 at Blakemoor and 3.13% ± 0.14 at Hauxley (Fig. 1). The %OC from uncompacted sediments at Blakemoor was significantly higher than from all other sediments, and the %OC from Hauxley ponds was significantly higher compared to the controls or Blakemoor compacted sediments (ANOVA, $F = 14.4$, df 4, 30, $P < 0.001$; data arcsine transformed, post-hoc comparisons used Tukey's test, and where more than one core was taken within a pond, the mean values were used to avoid pseudoreplication). There were no significant differences between %OC from the controls and compacted sediment.

Cores ranged in length from 4.5 to 15 cm, averaging 8.9 cm (n = 31). All pole core samples both from Blakemoor and Hauxley contained >5% OC in the top layers and displayed a gradual decrease in OC with increase in depth (Fig. 1) to a depth of ~4 cm, where OC content became indistinguishable from the surrounding soils. The distinct separation in the Hauxley cores above and below ~4 cm depth represents a clearly visible boundary between organic-rich sediment accumulated since their creation and the underlying original clay-based soil. Coupled with our knowledge of the exact age of the ponds at Hauxley, we estimated that the ponds are accumulating on average 149 g m^{-2} yr^{-1} OC (108–173). OC also decreased with an increase in depth in the uncompacted sediments of vegetated ponds from Blakemoor; however, the increase was at a much lower rate than in the compacted sediments, with %OC remaining distinctly higher than background soil levels for the full extent of the cores. Overall, the freeze core samples contained significantly higher %OC concentrations than those collected using the pole corer, averaging 9 and 3% OC respectively.

DBD averaged 0.54 g cm^{-3} (0.13–0.88) in the uppermost layer of sediment (Fig. 2), increasing gradually with increasing depth until reaching a mean of 1.43 g cm^{-3} (1.59–1.33) in the 5–6 cm core segment. DBD in the remaining core segments below 6 cm fluctuated on either side of the 1.33 g cm^{-3} mean but showed no overall increase or decrease with depth.

Sediment moisture content averaged 53.3% (83.2–33.8) in the surface layer, gradually decreasing to 26.7% in the 6–7 cm core segment. For the remainder of the cores sediment moisture content fluctuated by <10% of the 26.7% mean and showed no overall increase or decreasing trend.

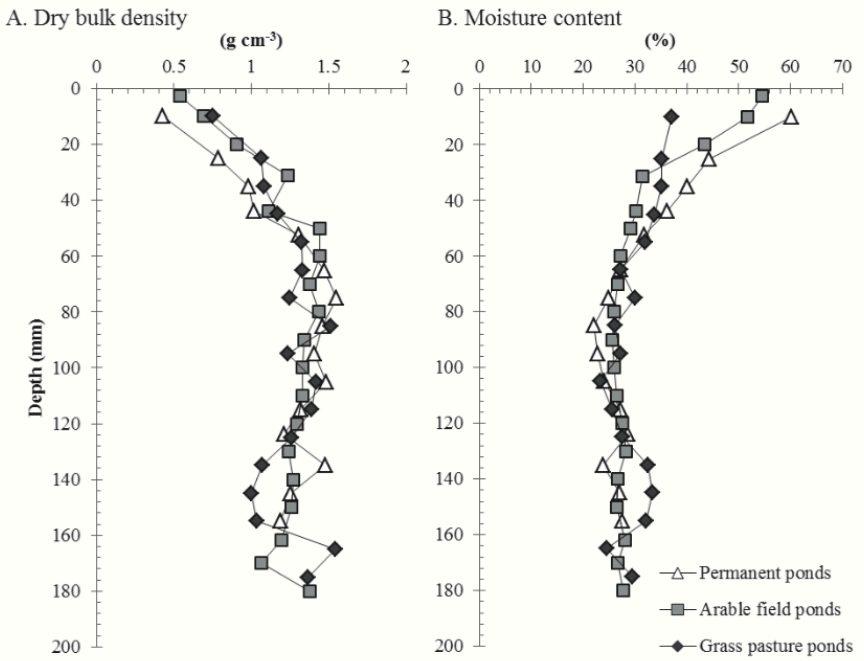


Fig. 2. Depth profiles for sediment (A) dry bulk density and (B) moisture content in successive core segments from ponds at Blakemoor Farm, Northumberland, UK. Data represent the mean of 2 ponds from each pond variety, plotted at the vertical midpoint of each section.

The 3-year survey of the ponds at Blakemoor showed marked variation in total aerial extent (range 5–12% of total survey area), particularly in the coverage of arable field and grass pasture ponds (Fig. 3). The UK experienced remarkably wet weather in 2012, resulting in enlarged ponds and the inundation of parts of fields not normally flooded, while in 2011 most arable and pasture ponds dried in summer and did not refill in autumn. The extent of the vegetated ponds was continually higher than all 3 other types combined and was much less vulnerable to annual variation.

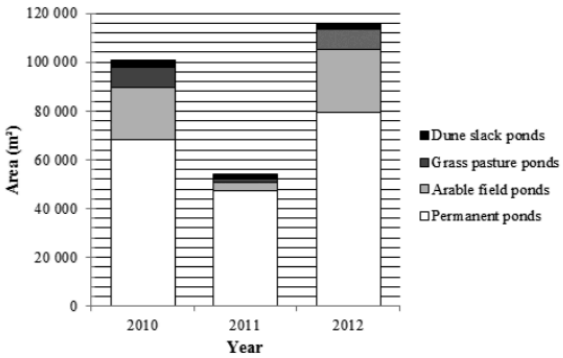


Fig. 3. Total area covered by 4 pond types at Blakemoor Farm, monitored November 2010, 2011, and 2012, which represented 10.1, 5.5, and 11.7% of the survey area, respectively.

Discussion

The overall range of %OC observed in this study is comparable to those reported in similar studies (Dean and Gorham 1998, Steeby et al. 2004, Downing et al. 2008, Boyd et al. 2010), supporting the limited evidence that ponds are powerful carbon sinks. The results also show that pond and substrate type have an important influence on their effectiveness at carbon sequestration.

The highest %OC concentrations were found in uncompacted, permanently submerged sediments of ponds at Blakemoor. The Hauxley ponds also had high levels in sediment that had accumulated since the ponds were dug. Although these ponds are temporary, they have developed a thick sward of moss that keeps sediment damp and anoxic even when they dry out (Jeffries 1998). The high %OC in the Blakemoor and Hauxley sediments is typical of the near-surface layers characterised by Munsiri (1995), with low bulk density and high moisture content. Such conditions possibly slow rates of organic matter degradation, preserving higher OC concentrations to a greater depth. While little variation in sediment DBD and moisture content was observed between the varying habitat types (Fig. 2), the permanent, naturally vegetated ponds accumulated the highest %OC and were the least affected by annual variations in rainfall when their combined extent is examined over the landscape.

The OC burial rates calculated for Hauxley are among some of the highest reported in literature, and while typically lower than those reported for artificial impoundments, they are higher than any other natural aquatic habitat (Mulholland and Elwood 1982, Schlesinger 1997, Duarte et al. 2004, Downing et al. 2008).

Bulk density of sediment typically increases with depth (Dadey et al. 1992, Munsiri et al. 1995), and while the %OC recorded in this study decreases with increase in depth, OC content (in g cm^{-3} OC) would inevitably not decrease at such a high rate, if at all, when the bulk density is factored in.

Cores containing the lowest %OC were from the temporary ponds in arable fields at Blakemoor (ponds 5 and 6). Typically these are shallow (<30 cm when full), dry out to expose bare mud, have little vegetation, and are disturbed by ploughing. Although these ponds are regular features of the landscape and cover large areas during the winter months, they seem relatively inactive in terms of trapping OC. The permanent, vegetated ponds with the highest %OC have significant variation. The 10 ponds studied at Hauxley ranged from 5 to 13% OC, despite having the same age and size, the same underlying clay-based soil, a location within 30 m of each other, and the same rainfall and nutrient inputs. The hydrology of the site does differ slightly among the 3 ponds, with sediments containing the highest %OC located in the region that is first to flood and dry out. This slight variance has created differences in the plant communities, with the 3 ponds containing the highest %OC dominated by thick moss swards and aquatic grasses since the late 1990s. Ponds that retained a more open flora of algae such as the stonewort (*Chara vulgaris*) or water buttercup (*Ranunculus aquatilis*) do not create a thick blanket over the substrate when the ponds dry.

Although little research has examined the influence of dominant vegetation type on subsequent sediment %OC concentrations, these results indicate that the individual ecology of a waterbody plays a significant role in determining %OC sequestration rates. Over the 3-year survey of aerial extent at Blakemoor, waterbodies of a similar size to those at Hauxley (<2 m^2) comprised only 0–0.0014% of the 1 km^2 study region; however, they did account for 0–17% of the waterbody stock. Pools of this size, often simply well-established tyre track ruts, can greatly contribute to the aquatic species richness of a region, supporting a distinct fauna not found in large, permanent ponds (Armitage et al. 2012).

The results from this study provide evidence from small ponds that confirms their potential significance alongside larger ponds and small lakes for carbon capture.

 DOI: 10.5268/IW-4.1.618

The substantial variation in %OC between substrates from varying pond types, and also within individual ponds, coupled with their variable productivity depending on pond permanence, nutrient inputs, plant ecology, and trophic status, poses significant implications when trying to upscale findings for carbon budgets. The results show that the heterogeneous nature of pond ecological communities is important when characterising differences in the carbon capture function of different ponds.

Acknowledgements

We thank Blakemoor Farm, Rio Tinto Alcan, and Hauxley Nature Reserve for access to land and in-depth site history information.

References

Alonso I, Weston K, Gregg R, Morecroft M. 2012. Carbon storage by habitat: review of the evidence of the impacts of management decisions and condition of carbon stores and sources. Natural England Research Report NERR043.

Armitage PD, Hawczak A, Blackburn JH. 2012. Tyre track pools and puddles – anthropogenic contributions to aquatic biodiversity. Limnologica. 42:254–263.

Boyd CE, Wood CW, Chaney PL, Queiroz JF. 2010. Role of aquaculture pond sediments in sequestration of annual global carbon emissions. Environ Pollut. 158:2537–2540.

Cole JJ, Prairie YT, Caraco NF, McDowell WH, Tranvik LJ, Striegl RG, Duarte CM, Kortelainen P, Downing JA, Middelburg JJ, et al. 2007. Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. Ecosystems. 10:172–185.

Dadey KA, Janecek T, Klaus A. 1992. Dry bulk density: its uses and determination. Proceedings of the Ocean Drilling Program, Scientific Results. 126:551–554.

Dean W, Gorham E. 1998. Magnitude and significance of carbon burial in lakes reservoirs and peatlands. Geology. 26:535–538.

Downing JA. 2010. Emerging global role of small lakes and ponds: little things mean a lot. Limnetica. 29:9–24.

Downing JA, Prairie YT, Cole JJ, Duarte CM, Tranvik LJ, Striegl RG, McDowell WH, Kortelainen P, Caraco NF, Melack JM. 2006. The global abundance and size distribution of lakes, ponds, and impoundments. Limnol Oceanogr. 51:2388–2397.

Downing JA, Cole JJ, Middelburg JJ, Striegl RG, Duarte CM, Kortelainen P, Prairie YT, Laube KA. 2008. Sediment organic carbon burial in agriculturally eutrophic impoundments over the last century. Global Biogeochem Cy. 22:1–10.

Downing JA, Cole JJ, Duarte CM, Middelburg JJ, Melack JM, Prairie YT, Kortelainen P, Striegl RG, McDowell WH, Tranvik LJ. 2012. Inland Waters. 2:229–236.

Duarte CM, Middelburg JJ, Caraco N. 2004. Major role of marine vegetation on the oceanic carbon cycle. Biogeosci Disc. 1:659–679.

Edina Digimap. 2012. Digimap roam [cited 10 March 2013]. Available from: http://digimap.edina.ac.uk/roam/os

Jeffries MJ. 1998. Pond macrophyte assemblages, biodisparity and spatial distribution of ponds in the Northumberland coastal plain, UK. Aquat Conserv. 8:657–667.

Jeffries MJ. 2002. Evidence for individualistic species assembly creating convergent ratios amongst pond invertebrate communities. J Anim Ecol. 71:177–187.

Jeffries MJ. 2012. Ponds and the importance of their history: an audit of pond numbers, turnover and the relationship between the origins of ponds and their contemporary plant communities in south-east Northumberland, UK. Hydrobiologia. 689:11–21.

Lehner B, Döll P. 2004. Development and validation of a global database of lakes, reservoirs and wetlands. J Hydrol. 296:1–22.

Meyers PA, Ishiwatari R. 1993. Lacustrine organic geochemistry: an overview of indicators of organic matter sources and diagenesis in lake sediments. Org Geochem. 20:867–900.

Mulholland PJ, Elwood JW. 1982. The role of lake reservoir sediments as sinks in the perturbed global carbon cycle. Tellus. 34:490–499.

Munsiri P, Boyd C, Hajek B. 1995. Physical and chemical characteristics of bottom soil profiles in ponds at Auburn, Alabama, USA and a proposed system for describing pond soil horizons. J World Aquacult Soc. 26:346–377.

Nelson DW, Sommers LE. 1982. Total carbon, organic carbon, and organic matter. In: Page AL, Miller RH, Keeney DR, editors. Methods of Soil Analysis. Part 2. Chemical and microbiological properties. 2nd ed. Madison (WI): American Society of Agronomy and Soil Science of America. p. 539–579.

Ntengwe FW, Edema MO. 2008. Physico-chemical and microbiological characteristics of water for fish production using small ponds. Phys Chem Earth. 33:701–707.

Pittman B, Jones JR, Millspaugh JJ, Kremer RJ, Downing JA. 2013. Sediment organic carbon distribution in 4 small northern Missouri impoundments: implications for sampling and carbon sequestration. Inland Waters. 3:39–46.

Rodwell JS. 2000. British plant communities: Volume 5; Maritime communities and vegetation of open habitats. Cambridge (UK): Cambridge University Press.

Schlesinger W. 1997. An analysis of global change. Biogeochemistry. Waltham (MA): Academic Press 2:588–589.

Schumacher B. 2002. Methods for the determination of total organic carbon (TOC) in soils and sediments. US Environmental Protection Agency.

Shapiro J. 1958. The core-freezer: a new sampler for lake sediments. Ecology. 39:758.

Soon Y, Abboud S. 1991. A comparison of some methods for soil organic carbon determination. Commun Soil Sci Plan. 22:943–954.

Steeby JA, Hargreaves JA, Tucker CS, Kingsbury S. 2004. Accumulation, organic carbon and dry matter concentration of sediment in commercial channel catfish ponds. Aquacult Eng. 30:115–126.

Stets EG, Striegl RG. 2012. Carbon export by rivers draining the conterminous United States. Inland Waters. 2:177–184.

Tranvik L, Downing JA, Cotner J. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. Limnol Oceanogr. 54:52–68.

[USCCSP] US Climate Change Science Program. 2003. Vision for the program and highlights of the scientific strategic plan.

Verstaeten G, Poesen J. 2002. Using sediment in small ponds to quantify sediment yield from small catchments: Possibilities and limitations. Earth Surf Proc Land. 27:1425–1439.

Williams P. 2004. Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. Biol Conserv. 115:329–341.

Xinglong J, Boyd CE. 2006. Relationship between organic carbon concentration and potential pond bottom soil respiration. Aquacult Eng. 35:147–151.

Zealand AM, Jeffries MJ. 2009. The distribution of pond snail communities across a landscape: separating out the influence of spatial position from local habitat quality for ponds in south-east Northumberland, UK. Hydrobiologia. 632:177–187.

 DOI: 10.5268/IW-4.1.618

Appendix C: Gilbert et al. (2015)

Environ Geochem Health (2015) 37:757–766
DOI 10.1007/s10653-015-9696-x

ORIGINAL PAPER

Arsenic hazard in Cambodian rice from a market-based survey with a case study of Preak Russey village, Kandal Province

Peter J. Gilbert · David A. Polya · David A. Cooke

Received: 31 October 2014 / Accepted: 27 March 2015 / Published online: 17 April 2015

Abstract This study comprises a market-based survey to assess the arsenic (As) hazard of Cambodian rice, encompassing rice from seven Cambodian provinces, comparisons with rice imported from China, Vietnam and Thailand, and assessments of 15 rice varieties. Rice samples ($n = 157$) were collected from four large markets in Kandal Province and analysed for As using inductively coupled mass spectrometry. The mean As concentration for Cambodian rice (0.185 μg g^{-1}, range 0.047–0.771 μg g^{-1}) was higher than that for imported rice from Vietnam and Thailand (0.162 and 0.157 μg g^{-1}, respectively) with mean As concentrations highest in rice from Prey Veng Province resulting in a daily dose of 1.77 μg kg^{-1} b.w. (body weight) d^{-1}. Between unmilled rice varieties, Cambodian-grown White Sticky Rice had the highest mean As concentration (0.234 μg g^{-1}), whilst White Sticky Rice produced in Thailand had the lowest (0.125 μg g^{-1}), suggesting that localised conditions have greater bearing over rice As concentrations than differences in As uptake between individual varieties themselves. A rice and water consumption survey for 15 respondents in the village of Preak Russey revealed mean consumption rates of 522 g d^{-1} of rice and 1.9 L d^{-1} of water. At water As concentrations >1000 μg L^{-1}, the relative contribution to the daily dose from rice is low. When water As concentrations are lowered to 50 μg L^{-1}, daily doses from rice and water are both generally below the 3.0 μg kg^{-1} b.w. d^{-1} benchmark daily limit for a 0.5 % increase in lung cancer, yet when combined they exceeded this value in all but three respondents.

Keywords Arsenic · Rice · Rice consumption · Water consumption · Dietary As intake · Cambodia

P. J. Gilbert (✉) · D. A. Cooke
Faculty of Engineering and Environment, Northumbria University, Ellison Building,
Newcastle upon Tyne NE1 8ST, UK
e-mail: p.gilbert@northumbria.ac.uk

D. A. Polya
School of Earth, Atmospheric and Environmental Sciences, The University of Manchester, Williamson Building-G27, Manchester M13 9PL, UK

Introduction

It is estimated that around 110 million people in South and South-east Asia are exposed to drinking water supplies which are polluted with naturally occurring arsenic (As) (Brammer and Ravenscroft 2009). Whilst high levels of As contamination in areas such as India and Bangladesh have been well documented over the past 20 years (Smedley and Kinniburgh 2002; Patel et al. 2005; Williams et al. 2006; Mondal and Polya 2008; Brammer and Ravenscroft 2009; Sohel et al. 2009), As within groundwaters of the Cambodian Mekong floodplain was only identified in the late 1990s (Japan International Cooperation Agency 1999

unpublished) with the first publication in the public domain scientific literature not until 2003 (Polya et al. 2003). While the As hazard in aquifers along the Mekong Delta is less substantive than in comparable shallow aquifers with similar geochemistry (typically circumneutral characterised by low Eh and high iron and manganese concentrations) such as the Ganges, Brahmaputra and Meghna (Polya et al. 2004; Buschmann et al. 2007, 2008), localised hotspots still pose a serious threat to regional populations.

Due to the long dry season (November to May) and the lack of water treatment and pathogen-free surface water in rural areas, drilling of tube wells in the mid-1990s meant that shallow groundwater quickly became the main source of drinking water for 9 months of the year in many parts of rural Cambodia (Phan et al. 2010). Approximately 51 % of groundwater wells contain As concentrations greater than the WHO Maximum Contaminant Level (MCL) of 10 μg L^{-1}, 41 % of wells greater than the Cambodian MCL of 50 μg L^{-1}, and 29 % of wells greater than 500 μg L^{-1} (Lado et al. 2008; Sampson et al. 2008). Several studies have attempted to model groundwater arsenic hazard across Cambodia (Polya et al. 2005; Berg et al. 2007; Buschmann et al. 2008; Lado et al. 2008; Sovann and Polya 2014), and it is estimated that approximately 15 % of Cambodians (2.25 million people) are living in As risk zones (UNICEF 2009). While As concentrations have been reported as high as 2500 μg L^{-1} in Bangladesh, 3200 μg L^{-1} in West Bengal and 3050 μg L^{-1} in Vietnam (Smedley and Kinniburgh 2002), tube well As concentrations in Kandal Province, Cambodia, have been reported up to 3500 μg L^{-1} (Sampson et al. 2008).

Approximately 1 million people in Kandal Province stopped using surface water as drinking water due to water-borne pathogen diseases which were responsible, among other factors, for infant mortality rates of 71 deaths per 1000 live births (Sthiannopkao et al. 2008). An estimated 98.65 % of residents in Kampong Kong commune, Kandal Province, are confronted with As toxicity through the groundwater drinking pathway with cancer risks estimated to average 5 per 1000 exposed (Phan et al. 2010). The first documented cases of the visible manifestations of arsenicosis in Cambodia were discovered in the village of Preak Russey (Kampong Kong commune, Kandal) in 2006 and have since been repeatedly documented with increasing frequency (Samnang 2006 *unpublished report* cited by Sampson et al. 2008; Mazumder et al. 2009; Agusa et al. 2010; Hashim et al. 2013; Huang et al. 2014). Whilst the prevalence of arsenicosis in Kampong Cham and Kratie Provinces is relatively low compared to other As-affected regions of Asia (0.00 and 1.30 %, respectively, compared to a range of 1.4–18.1 %), prevalence in Kandal Province reached 35.47 % (all P values $\leq$0.001; Hashim et al. 2013). Of great concern, one study reported that 37 % of children (<16 years) in Preak Russey had skin lesions of arsenicosis (Mazumder et al. 2009), whilst another reported that children aged 1–9 years had the highest hair As concentrations of all age groups (>55 μg g^{-1}; $P \leq 0.000$; Phan et al. 2011), suggesting a high daily dosage for children. As the time for visual manifestations to appear is typically 10–15 years, it can be expected that the frequency of arsenicosis cases will increase over the coming decade due to the relatively recent introduction of contaminated water sources. This said, cases of arsenicosis in the village of Lvea Em (Kandal Province) have been documented following exposure times as brief as 3 years (Kol 2007 *unpublished report* cited by Sampson et al. 2008).

Paddy rice is efficient at accumulating As and is increasingly recognised as a major exposure route for the dietary intake of As, demonstrated by strong associations between rice consumption and urinary As concentrations (Cascio et al. 2011; Banerjee et al. 2013; Zhao et al. 2013; Sharma et al. 2014). Recently, several studies have reported elevated concentrations of As within Cambodian rice (O'Neill et al. 2013; Wang et al. 2013; Phan et al. 2013, 2014; Seyfferth et al. 2014) with the mean rice As concentration in these studies (0.181 μg g^{-1}, range 0.029–0.314 μg g^{-1}) being comparable to means reviewed by Sahoo and Kim (2013) for neighbouring Thailand and Vietnam (0.15 and 0.12–0.20 μg g^{-1}, respectively) and European market rice (0.13–0.32 μg g^{-1}). While the speciation of As in rice grain varies between countries, dependent on regional environmental factors, it is widely reported that approximately 80 % of total As (*T*As) in Asian rice is found to be inorganic As (*i*As), compared to 64 and 42 % in European and US rice, respectively (Williams et al. 2005; Mondal and Polya 2008; Hanh et al. 2011; Zhao et al. 2013). To our knowledge, no work has been published on the speciation of As in Cambodian rice, so until such study is conducted the Asian average of 80 % *i*As is a reasonable assumption. Cambodian rice

production has soared dramatically in the past decade with 90 % of arable land now used for rice production, along with rice exports increasing from 12,613 T in 2009 to 378,856 T in 2013 (ARPEC 2014). Moreover, rice accounts for 70 % of the daily calorific intake for rural Cambodians, typically eaten three times per day (O'Neill et al. 2013), and as such has potential to form a large exposure route for As ingestion, alongside contaminated drinking water, by the Cambodian population.

Whilst at the time of project inception no studies into As contamination in Cambodian-grown rice had been reported, this study adds to the data published since (Phan et al. 2013, 2014; Hunag et al. 2014). The market-based survey approach adopted in this study allowed for the analysis of rice grown from a wider geographical range providing samples from three previously unreported provinces (Takeo, Kampong Speu and Kampot) as well as rice imported from China, Vietnam and Thailand. Equally, comparison of As concentrations between rice varieties as well as of the same variety produced in neighbouring countries provides further insight into the concentrations of As within Cambodian rice. The objectives of this study were to (1) identify which regions of Cambodia produce rice with the highest As concentration; (2) determine which varieties of rice contain the highest As concentration; (3) determine rice and water consumption rates for the village of Preak Russey; and (4) assess the relative contributions of rice and water to daily intake of As in Preak Russey.

Methodology

Study region

This study encompasses rice samples from several countries and provinces of Cambodia collected from markets across Kandal Province in June 2009. June typically marks the beginning of the rainy season where temperatures typically range between 24 and 32 °C. Over the same period, a rice and water consumption survey was also conducted for 15 respondents in the rural village of Preak Russey (Kampong Kong commune, Kampong Thom district, Kandal Province) located downstream of Phnom Penh between the Mekong River and Bassac River. The village of Preak Russey is comprised of 383 households and has been the focus of several previous studies, representing an area with high arsenic contamination (Hashim et al. 2013; Mazumber et al. 2009; Phan et al. 2010, 2013; See Sampson et al. 2008, Figure 3, for a map of groundwater As concentrations throughout Kandal Province and the specific location of Preak Russey village).

Rice survey

Collectively, 157 rice samples were analysed. In total, 137 rice samples were obtained from 21 market stalls across Kandal Province, noting the rice variety and province of origin (or country of origin for imports), and further 20 samples directly from the households included in the consumption survey, which were all grown locally around the village of Preak Russey. As the study aimed to assess the risk of As exposure to the population of Kandal Province (rather than purely assessing As concentrations in all Cambodian rice), the market-based sampling approach was adopted to assure that all rice sampled was intended directly for human consumption and to be consumed locally (as opposed to sourcing rice directly from the field where it may be exported or used as livestock feed). As the sample size is relatively small with a specific locational focus, this should not be viewed as a comprehensive national survey, but as an indication of worst-case scenarios. Samples were transported to Northumbria University, England, for analysis. Samples were washed with deionised water and oven-dried at 60 °C before they were ground to powder using a ball mill. Approximately 0.2 g of sample was accurately weighed and predigested in 2 mL of Analar nitric acid overnight prior to heating on a hot plate at 100 °C for 15–20 min to evaporate the samples to dryness. The samples were rehydrated with 10 mL of deionised water, sonicated and then stored at 4 °C prior to analysis by inductively coupled mass spectrometry (ICP–MS). Blanks were run alongside every tenth sample (mean As concentration ≤ 0.000 μg g^{-1}) and every tenth sample analysed in triplicate (n triplicates = 32), with the precision of the analyses of these samples averaging 7.5 % (range 2.2–12.5 %). Standard reference material was run alongside every 15th sample with all final calculations corrected for analytical recovery.

Consumption survey

Water and rice consumption rates were monitored for 20 adult respondents in the village of Preak Russey;

however, due to a religious event over the survey period, complete rice consumption rates were unobtainable for five of the individuals and as such have been excluded from the study, resulting in 15 respondents (males = 5, females = 10). Personal observations indicated that socio-economic factors were consistent across the village, with the majority of households being subsistence farmers. All respondents were from separate households and as such represent 4 % of the 383 households in Preak Russey village.

Water consumption rates were monitored by providing a premeasured bottle to be used as their drinking vessel over the 24-h period, and where the respondents preferred to drink tea, this was also used as their drinking vessel. Each household's rice consumption was measured at three meal times throughout the day by weighing the amount of rice prior to cooking. This was then divided by the number of household members (taking children as ½) to achieve a mean adult rice consumption rate for each respondent. While the brief sampling period may be seen as limiting, due to time constraints, it was the only feasible to conduct a 24-h survey. Should a similar survey be repeated it would be recommended to conduct a longer more detailed study.

Estimates of daily arsenic intake

Estimates of daily *i*As intake were calculated assuming that the inorganic content of total As is 80 % for rice (Sanz et al. 2005; Williams et al. 2005; Mondal and Polya 2008; Zhao et al. 2013) and 100 % for water (Smedley and Kinniburgh 2002; Patel et al. 2005). Daily *i*As intake was calculated by multiplying the concentration by the mean rice consumption rate reported in this study (522 g d^{-1}), and for the individuals in Preak Russey by using the reported rice and water consumption rates for each respondent. Daily dose was calculated from daily intake based on mean body weights for males and females (51.1 and 47.4 kg, respectively) living in Kandal Province as reported by Phan et al. (2010) and was averaged (49.25 kg) for nongender-specific daily dose calculations reported in the rice survey.

Statistical analysis

All statistical analyses of data were performed using IBM SPSS Statistics 21. Concentrations between country and province of origin, as well as differences in concentration among individual rice varieties were subject to one-way ANOVA where data were arcsine-transformed and a post hoc comparison of Tukey's test applied. Differences between male and female water consumption rates, as well as relative contributions of rice and water to daily intake of As were analysed using independent-samples *t* test.

Results and discussion

Cambodian-grown rice was found to contain elevated levels of As (Table 1) ranging from 0.047 to 0.771 μg g^{-1}, with a mean concentration of 0.185 ± 0.103 μg g^{-1} ($n = 131$, median = 0.185 μg g^{-1}). Based on the calculated daily intake of *i*As, the mean daily dose of Cambodian-grown rice [1.57 μg d^{-1} kg^{-1} b.w.; 95 % CI (1.43, 1.74)] was less than the lower limits on the benchmark dose for a 0.5 % ($BMDL_{0.5}$) increase in lung cancer (3.0 μg d^{-1} kg^{-1} b.w.) stated by the WHO (2011). The levels reported in this study are in line with those reported in other studies on Cambodian-grown rice (mean = 0.190 μg g^{-1}, range 0.008–0.649 μg g^{-1}) (O'Neill et al. 2013; Phan et al. 2013, 2014; Wang et al. 2013; Seyfferth et al. 2014). Mean As concentrations are also comparable to other As-affected Asian countries such as Bangladesh (mean = 0.13 μg g^{-1}; Meharg et al. 2009; mean = 0.14 μg g^{-1}; Rahman et al. 2009), India (mean = 0.07 μg g^{-1}; Meharg et al. 2009; mean = 0.14 μg g^{-1}; Patel et al. 2005), China (mean = 0.14 μg g^{-1}; Meharg et al. 2009), Thailand (mean = 0.15 μg g^{-1}; Adomako et al. 2011; mean = 0.11 μg g^{-1}; Williams et al. 2005), Japan (mean = 0.19 μg g^{-1}; Meharg et al. 2009) and Vietnam (mean = 0.20 μg g^{-1}; Schoof et al. 1998; mean = 0.225 μg g^{-1}; Hanh et al. 2011). Mean As concentrations in Cambodian-grown rice were higher than rice imported from Vietnam ($n = 4$, mean = 0.162 μg g^{-1}) and Thailand ($n = 21$, mean = 0.157 μg g^{-1}) yet not significantly (ANOVA, $F = 0.807$, $P \geq 0.05$). Many factors determine rice As concentrations, and they frequently differ between countries. Groundwater As concentrations, speciation and mobilisation vary depending on the type of fluvial deposits and localised environmental conditions. Socio-economic and educational factors influence farming practices and the use of contaminated water for

Table 1 Summary of As concentrations in rice (μg g^{-1}) grown in different regions and their subsequent *i*As concentrations, daily intake and daily dose

Country	Province	*n*	[*T*] As			[*i*] As[b]		
			Mean ± SD	Median	Range	Mean	Daily intake[c] (μg d^{-1})	Daily dose[d] (μg kg^{-1} b.w. d^{-1})
Cambodia	Prey Veng	8	0.220 ± 0.093	0.231	0.047–0.364	0.176	92.3	1.87
	Takeo	13	0.197 ± 0.173	0.151	0.074–0.771	0.158	82.7	1.67
	Kampot	3	0.197 ± 0.042	0.197	0.146–0.249	0.158	82.6	1.67
	Battambang	85	0.181 ± 0.102	0.169	0.070–0.766	0.145	75.9	1.54
	Kandal[a]	20	0.180 ± 0.044	0.177	0.097–0.289	0.144	75.2	1.52
	Kompong Thom	1	0.177	–	–	0.142	74.1	1.50
	Kompong Speu	1	0.158	–	–	0.126	66.1	1.34
	Mean	131	0.185 ± 0.103	0.185	0.047–0.771	0.148	77.5	1.57
China		1	0.205	–	–	0.164	85.8	1.74
Vietnam		4	0.162 ± 0.076	0.135	0.089–0.290	0.129	67.8	1.37
Thailand		21	0.157 ± 0.055	0.159	0.064–0.298	0.125	65.7	1.33

SD standard deviation

[a] Samples from Kandal are those collected from households in Preak Russey; all were grown locally

[b] *i*As calculated assuming 80 % of total As is inorganic

[c] daily intake based on a daily rice consumption rate of 522 g

[d] daily dose based on body weight of 49.25 kg

irrigation, or even the use of illegal pesticides containing As (i.e. methyl parathion). Subsequently, it is beyond the scope of this study to cover all these factors for each of the aforementioned countries. However, the broadly comparable rice As concentrations between the Cambodian rice reported here and those of other As-affected regions of the world further adds concern to the relatively understudied hazard posed by rice As consumption in Cambodia.

Cambodian rice As concentrations varied between regions and were highest in rice from Prey Veng whilst lowest from Kompong Speu. However, in comparison with other studies (Fig. 1), the degree of variability between the means reported in this study is relatively low. Mean rice As concentrations for Prey Veng, Kandal and Battambang were in line with those reported in previous studies (O'Neill et al. 2013; Phan et al. 2013, 2014, Seyfferth et al. 2014; Wang et al. 2013) and were only considerably lower than those reported for Kampong Thom (Seyfferth et al. 2014). Interestingly, the variability in mean As concentration between provinces across all previous studies and this does not relate to groundwater As contamination levels. The highest groundwater As concentrations are frequently reported in Kandal Province, specifically around the area of Preak Russey where the 20 rice samples from Kandal Province in this study were grown (Sampson et al. 2008). Yet the mean rice As concentration reported here for Kandal was the fifth lowest of all provinces surveyed. Equally, it is considerably lower than those reported by Phan et al. (2013), Seyfferth et al. (2014) and Wang et al. (2013). Contrastingly, provinces with considerably lower groundwater As concentrations such as Battambang and Takeo had relatively similar rice As concentrations to those provinces with high groundwater contamination. This variation may be due to: farming practices, where numerous As educational programmes in high-risk areas, such as Kandal and Prey Veng, have altered irrigation practices, with safe water now being sourced and resulting in lower rice As concentrations; localised hotspots, which have not been fully quantified by the relatively few rice studies conducted in Cambodia, in comparison with the vast quantities of rice produced in Cambodia; and differences in rice variety, with As uptake being variable between individual species.

The variety of rice (Table 2) found to have the highest As concentration was that of unmilled Black Sticky Rice which was significantly higher than those

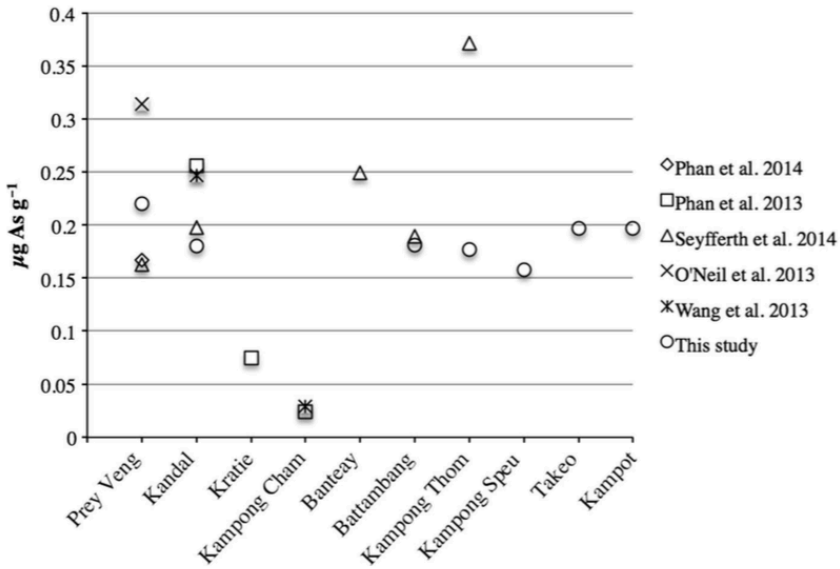


Fig. 1 Mean rice As concentrations for Cambodian provinces as reported in this study and previous studies

of all other rice varieties sampled (ANOVA, $F(15, 137) = 12.1$, $P \leq 0.000$), which was the only variety with a mean *i*As concentration greater than the 0.2 μg g^{-1} recommended by the FAO (2014) and the only variety to breach the $BMDL_{0.5}$ [6.51 μg d^{-1} kg^{-1} b.w.; 95 % CI (6.26, 6.76)]. However, due to the low availability of Black Sticky during the market survey (only two samples were available), the statistical difference between unmilled and milled varieties should be treated with caution. Despite this, the concentrations reported in this study are in line with unmilled rice from Bangladesh (Meharg et al. 2008) and the upper end from India (Halder et al. 2012). Of the milled rice varieties, White Sticky Rice from Cambodia had the highest As concentration, which was significantly higher than Ginger Flower, Neang Menh and Thai White Sticky Rice (ANOVA, $F(15, 137) = 12.1$, $P = 0.041$, 0.016, and 0.031, respectively). While the calculated daily dose from Cambodian White Sticky Rice is 1.98 μg kg^{-1} b.w. d^{-1} [95 % CI (1.54, 2.43)], it should be noted that this variety is typically used for desserts and as such the consumption rate of 522 g d^{-1} and calculated daily dose is probably rarely reached. While Samali Rice from Cambodia and Thailand was of similar concentrations, concentrations of White Sticky Rice from Cambodia were higher than those of samples of the same variety grown in China, Vietnam and Thailand, with Thai White Sticky Rice having the lowest mean As concentration of all varieties sampled. This large variation in As concentrations in rice of the same variety suggests that perhaps localised conditions and farming practices have a greater bearing over rice As concentrations than differences in As uptake of individual varieties themselves. Bilok Chong Rice had the lowest mean As concentration of rice varieties grown in Cambodia, and with the exception of Black and White Sticky Rice from Cambodia, none of the other rice varieties were found to be significantly different from one another (ANOVA, $F(15, 137) = 12.1$, $P \geq 0.05$). Excluding Black Sticky Rice, the mean As concentrations for each rice variety in this study all equate to a daily dose lower than the $BMDL_{0.5}$, suggesting that rice consumption alone in Cambodia does not pose an independent threat. This said, consuming 2 L of water per day at the WHO recommended 10 μg As L^{-1} equates to a daily intake of 20 μg d^{-1}. To equal this for *i*As, intake from rice whilst consuming 522 g d^{-1} the As concentration need only be 0.047 μg g^{-1} which is equal to the lowest value of all samples reported in this study.

Water consumption rates for the village of Preak Russey (Table 3) were significantly different between males and females (means = 2.6 and 1.5 L d^{-1}, respectively, $t(18) = -2.041$, $P = 0.013$) and ranged between 0.9 and 6.0 L d^{-1} which is in line with other water consumption rates used in South-east Asia (Watanabe et al. 2004, 2006; Phan et al. 2014). Rice

Table 2 Summary of As concentrations in differing varieties of rice (μg g^{-1}) and their subsequent *i*As concentrations, daily intake and daily dose

Rice variety	Country of production	*n*	[*T*] As			[*i*] As[b]		
			Mean ± SD	Median	Range	Mean	Daily intake[c] (μg d^{-1})	Daily dose[d] (μg kg^{-1} d^{-1})
Black Sticky	Cambodia	2	0.768 ± 0.002	0.769	0.766–0.771	0.615	321.1	6.51
White Sticky	Cambodia	16	0.234 ± 0.095	0.242	0.047–0.402	0.187	98.0	1.98
White Sticky	China	1	0.205	0.205	–	0.164	85.8	1.74
White Sticky	Vietnam	4	0.162 ± 0.076	0.135	0.089–0.290	0.129	67.8	1.37
White Sticky	Thailand	8	0.125 ± 0.046	0.126	0.064–0.195	0.100	52.5	1.06
Er Rice	Cambodia	4	0.219 ± 0.016	0.221	0.199–0.237	0.175	91.7	1.86
Jasmin Rice	Cambodia	14	0.220 ± 0.131	0.200	0.083–0.660	0.176	92.0	1.86
Unknown[a]	Cambodia	20	0.180 ± 0.044	0.177	0.097–0.289	0.144	75.2	1.52
Samali	Cambodia	4	0.179 ± 0.049	0.196	0.097–0.228	0.143	75.0	1.52
Samali	Thailand	12	0.178 ± 0.053	0.169	0.100–0.298	0.142	74.5	1.51
Brola Pdai	Cambodia	11	0.171 ± 0.058	0.178	0.072–0.286	0.136	71.5	1.45
Menh Sre	Cambodia	1	0.170	0.170	–	0.136	71.3	1.44
Bilok Knhey	Cambodia	2	0.164 ± 0.006	0.164	0.157–0.170	0.131	68.5	1.39
Thai Kon	Thailand	1	0.159	0.159	–	0.127	66.6	1.35
Red Rice	Cambodia	1	0.158	0.158	–	0.126	66.1	1.34
Neang Kon	Cambodia	14	0.149 ± 0.036	0.139	0.094–0.218	0.119	62.4	1.26
Ginger Flower	Cambodia	17	0.149 ± 0.047	0.138	0.070–0.263	0.119	62.3	1.26
Neang Menh	Cambodia	19	0.144 ± 0.038	0.139	0.082–0.257	0.115	60.4	1.22
Cat Prum	Cambodia	2	0.138 ± 0.044	0.139	0.094–0.183	0.111	58.0	1.17
Bilok Chong	Cambodia	4	0.136 ± 0.051	0.137	0.075–0.197	0.109	57.1	1.15

SD standard deviation

[a] Unknown varieties are those samples collected from households in Preak Russey

[b] *i*As calculated assuming 80 % of total As is inorganic

[c] daily intake based on a daily rice consumption rate of 522 g

[d] daily dose based on body weight of 49.25 kg

consumption rates were at the upper end of values used in previous reports from other South and South-east Asian countries (0.2–0.5 kg d^{-1}; Zhu et al. 2008a, b; Williams et al. 2005; Zavala and Duxbury 2008; Seyfferth et al. 2014) and were higher than rates reported for Cambodia by Phan et al. (2014) (mean = 0.44 kg d^{-1}), and Phan et al. (2013) (mean = 0.45 kg d^{-1}).

It should be noted that the village of Preak Russey is amongst the poorest and most isolated villages in Kandal Province and while the majority of households surveyed consumed rice three times a day, this is unlikely to be the case for all rural communities in Cambodia, where rice is often substituted for noodles in wealthier households. Equally, while June typically marks the beginning of the rainy season, no days over the survey received rainfall, with high temperatures likely elevating water consumption rates. While these factors may make this survey unrepresentative of the whole of Cambodia, they are representative of poor, isolated communities who consume the most rice and have the least access to safe drinking water supplies. Coupled with living in areas of the highest As contamination, it is these communities, which are at greatest risk.

The mean daily dose of *i*As from rice for the 15 respondents was 1.87 μg kg^{-1} b.w. d^{-1} [95 % CI (1.54, 2.21)], and with a range of 0.62–2.95 μg kg^{-1} b.w. d^{-1}, none of the individuals exceeded the $BMDL_{0.5}$ of 3 μg kg^{-1} b.w. d^{-1} from rice consumption alone. However, the daily dose of *i*As from water, based on the range of concentrations previously

Table 3 Rice and water consumption rates for 15 respondents surveyed in Preak Russey and their subsequent daily dose of *i*As

Individual	Sex	Rice[a]			Water[b]		
		Consumption ($g\ d^{-1}$)	[*i*] As ($\mu g\ g^{-1}$)	Daily dose[c] ($\mu g\ kg^{-1}\ d^{-1}$)	Consumption ($L\ d^{-1}$)	Daily dose[c] ($\mu g\ kg^{-1}\ d^{-1}$) at:	
						1000–3500[d] ($\mu g\ L^{-1}$)	50[e] ($\mu g\ L^{-1}$)
1	f	617	0.227	2.95	1.1	23–81	1.16
2	f	600	0.178	2.25	1.2	25–88	1.27
3	f	800	0.103	1.73	1.1	23–81	1.16
4	f	400	0.149	1.26	1.4	29–103	1.48
5	f	567	0.175	2.09	1.5	31–110	1.58
6	m	566	0.212	2.35	1.1	21–75	1.08
7	m	694	0.166	2.26	0.9	17–61	0.88
8	m	450	0.212	1.86	2.0	39–137	1.96
9	f	567	0.203	2.43	1.5	31–110	1.58
10	f	516	0.173	1.89	3.0	63–221	3.16
11	m	312	0.101	0.62	6.0	117–411	5.87
12	f	343	0.139	1.00	1.5	31–110	1.58
13	f	333	0.203	1.42	1.9	40–140	2.00
14	m	309	0.290	1.75	3.0	58–205	2.94
15	f	750	0.143	2.26	1.1	23–81	1.16
	Mean	522	0.178	1.87	1.9	38–134	1.92
	m	–	–	–	2.6	50–178	2.54
	f	–	–	–	1.5	32–113	1.61

[a] Rice calculations assume 80 % of total As is inorganic

[b] Water calculations assume 100 % As is inorganic

[c] Daily dose based on body weights of 47.4 and 51.1 kg for females and males, respectively, based on mean body weights reported by Phan et al. (2010) for Kandal Province

[d] Range of tube well As concentrations in Preak Russey recorded by Sampson et al. (2008)

[e] Cambodian maximum contaminant level

reported for Preak Russey (1000–3500 $\mu g\ L^{-1}$; Sampson et al. 2008), was significantly higher by an order of magnitude, meaning that at such high water As concentrations the contribution from rice to the daily dose of As is relatively low. The majority of Preak Russey village now sources water below the Cambodian MCL of 50 $\mu g\ L^{-1}$ at which point the daily dose from rice and water is no longer significantly different ($t(38) = -0.55$, $P \geq 0.05$) with the average actually higher from rice than from water for the female respondents in this survey [1.93 and 1.61 $\mu g\ kg^{-1}$ b.w. d^{-1}, respectively; 95 % CI (1.51, 2.35) and (1.18, 2.05) respectively]. When the daily doses from rice and water are combined for each respondent, all except three individuals exceed the $BMDL_{0.5}$. The mean combined daily doses for males and females are slightly lower than those for other studies conducted in Cambodia; however, it should be noted that alternative sources of As such as vegetables, fish, meat and cooking rice were not included in this study and will only contribute to the intake of As.

Conclusions

Variations in rice As concentrations of the same varieties between provinces and countries indicate that regional groundwater As levels and agricultural practices have greater bearing on grain As concentrations than the differences in As uptake between individual rice varieties. The levels of As in rice reported here are in line with those from previous studies conducted in Cambodia and are below the recommended FAO (2014) limit of 0.2 μg *i*As g^{-1}, with consumption of

rice alone resulting in a daily dose lower than the $BMDL_{0.5}$ value of 3.0 μg kg^{-1} b.w. d^{-1}. That said, when the combined daily dose from rice and water at the Cambodian MCL of 50 μg L^{-1} was calculated for 15 respondents in the village of Preak Russey, the $BMDL_{0.5}$ was breached by all but three individuals. Equally, all of the rice As concentrations reported in this study, consumed at the reported consumption rate, would equate to an intake of As greater than drinking 2 L of water at the WHO recommended level of 10 μg L^{-1}.

Acknowledgments The authors would like to thank all the staff at RDIC (Resource Development International Cambodia) for providing accommodation, assistance with transportation and translation during sample collection and interviews.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard All procedures performed in studies involving human participants were in accordance with the ethical standards of Northumbria University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

Adomako, E. E., Williams, P. N., Deacon, C., & Meharg, A. A. (2011). Inorganic arsenic and trace elements in Ghanaian grain staples. *Environmental Pollution, 159*, 2435–2442.

Agusa, T., Kunito, T., Kubota, R., et al. (2010). Exposure, metabolism, and health effects of arsenic in residents from arsenic-contaminated groundwater areas of Vietnam and Cambodia: A review. *Reviews on Environmental Health, 25*, 193–220.

ARPEC (Alliance of Rice Producers and Exporters Of Cambodia). (2014). Quantity of Cambodian rice exported. http://cambodiariceexporters.org/en/article/quantity-of-cambodian-rice-exported-from-year-2009-to-2013-(mt).html. Accessed 16 Oct 2014.

Banerjee, M., Banerjee, N., Bhattacharjee, P., et al. (2013). High arsenic in rice is associated with elevated genotoxic effects in humans. *Scientific Reports, 3*, 2195.

Berg, M., Stengel, C., Pham, T. K. T., et al. (2007). Magnitude of arsenic pollution in the Mekong and Red River Deltas-Cambodia and Vietnam. *Science of the Total Environment, 372*, 413–425.

Brammer, H., & Ravenscroft, P. (2009). Arsenic in groundwater: A threat to sustainable agriculture in South and South-east Asia. *Environment International, 35*, 647–654.

Buschmann, J., Berg, M., Stengel, C., & Sampson, M. L. (2007). Arsenic and manganese contamination of drinking water resources in Cambodia: Coincidence of risk areas with low relief topography. *Environmental Science and Technology, 41*, 2146–2152.

Buschmann, J., Berg, M., Stengel, C., et al. (2008). Contamination of drinking water resources in the Mekong delta floodplains: Arsenic and other trace metals pose serious health risks to population. *Environment International, 34*, 756–764.

Cascio, C., Raab, A., Jenkins, R. O., et al. (2011). The impact of a rice based diet on urinary arsenic. *Journal of Environmental Monitoring, 13*, 257–265.

FAO (Food and Agricultural Organisation). (2014). Joint FAO/WHO food standards programme, Codex Alimentarius commission, Geneva. Report of the eighth session of the codex committee on contaminants in foods (pp. 14–18).

Halder, D., Bhowmick, S., Biswas, A., et al. (2012). Consumption of brown rice: A potential pathway for arsenic exposure in rural Bengal. *Environmental Science and Technology, 46*, 4142–4148.

Hanh, H. T., Kim, K. W., Bang, S., & Hoa, N. M. (2011). Community exposure to arsenic in the Mekong river delta, Southern Vietnam. *Journal of Environmental Monitoring, 13*, 2025–2032.

Hashim, J. H., Radzi, R. S. M., Aljunid, S. M., et al. (2013). Hair arsenic levels and prevalence of arsenicosis in three Cambodian provinces. *Science of the Total Environment, 463–464*, 1210–1216.

Huang, J. W., Cheng, Y. Y., Sung, T. C., et al. (2014). Association between arsenic exposure and diabetes mellitus in Cambodia. *BioMed Research International, 2014*, 683124.

Lado, L. R., Polya, D., Winkel, L., et al. (2008). Modelling arsenic hazard in Cambodia: A geostatistical approach using ancillary data. *Applied Geochemistry, 23*, 3010–3018.

Mazumder, D. N. G., Majumdar, K. K., Santra, S. C., et al. (2009). Occurrence of arsenicosis in a rural village of Cambodia. *Journal of Environmental Science and Health Part A: Toxic/Hazardous Substances and Environmental Engineering, 44*, 480–487.

Meharg, A. A., Lombi, E., Williams, P. N., et al. (2008). Speciation and localization of arsenic in white and brown rice grains. *Environmental Science and Technology, 42*, 1051–1057.

Meharg, A. A., Williams, P. N., Adomako, E., et al. (2009). Geographical variation in total and inorganic arsenic content of polished (white) rice. *Environmental Science and Technology, 43*, 1612–1617.

Mondal, D., & Polya, D. A. (2008). Rice is a major exposure route for arsenic in Chakdaha block, Nadia district, West Bengal, India: A probabilistic risk assessment. *Applied Geochemistry, 23*, 2987–2998.

O'Neill, A., Phillips, D. H., Kok, S., et al. (2013). Arsenic in groundwater and its influence on exposure risks through traditionally cooked rice in Prey Vêng Province, Cambodia. *Journal of Hazardous Materials, 262*, 1072–1079.

Patel, K. S., Shrivas, K., Brandt, R., et al. (2005). Arsenic contamination in water, soil, sediment and rice of central India. *Environmental Geochemistry and Health, 27*, 131–145.

Phan, K., Phan, S., Heng, S., et al. (2014). Assessing arsenic intake from groundwater and rice by residents in Prey Veng

province, Cambodia. *Environmental Pollution, 185*, 84–89.

Phan, K., Sthiannopkao, S., Heng, S., et al. (2013). Arsenic contamination in the food chain and its risk assessment of populations residing in the Mekong River basin of Cambodia. *Journal of Hazardous Materials, 262*, 1064–1071.

Phan, K., Sthiannopkao, S., & Kim, K. W. (2011). Surveillance on chronic arsenic exposure in the Mekong River basin of Cambodia using different biomarkers. *International Journal of Hygiene and Environmental Health, 215*, 51–58. doi:10.1016/j.ijheh.2011.07.002.

Phan, K., Sthiannopkao, S., Kim, K. W., et al. (2010). Health risk assessment of inorganic arsenic intake of Cambodia residents through groundwater drinking pathway. *Water Research, 44*, 5777–5788.

Polya, D. A., Gault, A. G., Bourne, N. J., et al. (2003). Coupled HPLC-ICP-Ms analysis indicates highly hazardous concentrations of dissolved arsenic species in Cambodian groundwaters. In J. G. Holland & S. D. Tanners (Eds.), *Plasma source mass spectrometry: Applications and emerging technologies* (pp. 127–140). Cambridge: The Royal Society of Chemistry Special Publication.

Polya, D. A., Gault, A. G., Diebe, N., et al. (2005). Arsenic hazard in shallow Cambodian groundwaters. *Mineralogical Magazine, 69*, 807–823.

Polya, D. A., Rowland, H. A. L., Gault, A. G., et al. (2004). Geochemistry of arsenic-rich shallow groundwaters in Cambodia. *Geochimica et Cosmochimica Acta, 68*, 2004.

Rahman, M. M., Owens, G., & Naidu, R. (2009). Arsenic levels in rice grain and assessment of daily dietary intake of arsenic from rice in arsenic-contaminated regions of Bangladesh–implications to groundwater irrigation. *Environmental Geochemistry and Health, 31*, 179–187.

Sahoo, P. K., & Kim, K. (2013). A review of the arsenic concentration in paddy rice from the perspective of geoscience. *Geosciences Journal, 17*, 107–122.

Sampson, M. L., Bostick, B., Chiew, H., et al. (2008). Arsenicosis in Cambodia: Case studies and policy response. *Applied Geochemistry, 23*, 2977–2986.

Sanz, E., Muñoz-Olivas, R., & Cámara, C. (2005). A rapid and novel alternative to conventional sample treatment for arsenic speciation in rice using enzymatic ultrasonic probe. *Analytica Chimica Acta, 535*, 227–235.

Schoof, R. A., Yost, L. J., Crecelius, E., et al. (1998). Dietary arsenic intake in Taiwanese districts with elevated arsenic in drinking water. *Human and Ecological Risk Assessment: An International Journal, 4*, 117–135.

Seyfferth, A. L., McCurdy, S., Schaefer, M. V., & Fendorf, S. (2014). Arsenic concentrations in paddy soil and rice and health implications for major rice-growing regions of Cambodia. *Environmental Science and Technology, 48*, 4699–4706.

Sharma, A. K., Tjell, J. C., Sloth, J. J., & Holm, P. E. (2014). Review of arsenic contamination, exposure through water and food and low cost mitigation options for rural areas. *Applied Geochemistry, 41*, 11–33.

Smedley, P., & Kinniburgh, D. (2002). A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry, 17*, 517–568.

Sohel, N., Persson, L. A., Rahman, M., et al. (2009). Arsenic in drinking water and adult mortality: A population-based cohort study in rural Bangladesh. *Epidemiology, 20*, 824–830.

Sovann, C., & Polya, D. A. (2014). Improved groundwater geogenic arsenic hazard map for Cambodia. *Environmental Chemistry, 11*, 595–607.

Sthiannopkao, S., Kim, K. W., Sotham, S., & Choup, S. (2008). Arsenic and manganese in tube well waters of Prey Veng and Kandal Provinces, Cambodia. *Applied Geochemistry, 23*, 1086–1093.

UNICEF. (2009). Arsenic in Cambodia. Resource document. http://www.unicef.org/cambodia/As_Mitigation_in_Cambodia_2009.pdf. Accessed 01 Oct 2014.

Wang, H. S., Sthiannopkao, S., Chen, Z. J., et al. (2013). Arsenic concentration in rice, fish, meat and vegetables in Cambodia: A preliminary risk assessment. *Environmental Geochemistry and Health, 35*, 745–755.

Watanabe, C., Kawata, A., Sudo, N., et al. (2004). Water intake in an Asian population living in arsenic-contaminated area. *Toxicology and Applied Pharmacology, 198*, 272–282.

WHO. (2011). *Evaluation of certain contaminants in food: Seventy-second report of the Joint FAO/WHO Expert Committe on Food Additives* (Vol. 959, p. 36). World Health Organization.

Williams, P. N., Islam, M. R., Adomako, E. E., et al. (2006). Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. *Environmental Science and Technology, 40*, 4903–4908.

Williams, P. N., Price, A. H., Raab, A., et al. (2005). Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. *Environmental Science and Technology, 39*, 5531–5540.

Zavala, Y. J., & Duxbury, J. M. (2008). Arsenic in rice: I. Estimating normal levels of total arsenic in rice grain. *Environmental Science and Technology, 42*, 3856–3860.

Zhao, F. J., Zhu, Y. G., & Meharg, A. (2013). Methylated arsenic species in rice: Geographical variation, origin, and uptake mechanisms. *Environmental Science and Technology, 47*, 3957–3966.

Zhu, Y. G., Sun, G. X., Lei, M., et al. (2008a). High percentage inorganic arsenic content of mining impacted and nonimpacted Chinese rice. *Environmental Science and Technology, 42*, 5008–5013.

Zhu, Y. G., Williams, P. N., & Meharg, A. (2008b). Exposure to inorganic arsenic from rice: A global health issue? *Environmental Pollution, 154*, 169–171.