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The Importance of Small Water Bodies for Carbon Capture in Northumberland

Otaigbe Philip Inegbedion

A thesis submitted in partial fulfilment of the requirements of the University of Northumbria at Newcastle for the degree of Doctor of Philosophy

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Abstract

Small water bodies (SWBs) are an important biogeochemical sub-compartment of the global carbon cycle that has been given little or no attention. They have similar capabilities to oceans, large lakes and river systems to exist in flux and could store more carbon in their sediments than the above systems. This research is aimed at determining the number and surface area of inland water bodies in Northumberland, the carbon stocks in the sediments of those water bodies and the microbial influence on the carbon stocks. These further define the Northumberland regional carbon stocks, the level of allochthonous and autochthonous carbon influence in the regional carbon stocks and the effects of surrounding vegetation, sediment wetness, dry bulk density, microbes, anoxia, pond permanence and temporariness on carbon stock variations.

The importance of SWBs is in their abundance and the ability to estimate this will aid the understanding of their actual contributions to the global carbon cycle as a net source or sink. Using Landsat-8 and World Imagery data, number and surface area of water bodies in Northumberland were identified by manual digitising of water bodies on ArcGIS 10.0. This showed variation in number and surface area of water body abundance with respect to imagery types, time and scale of analysis. The correctness of estimating water body abundance is subject to the continuous temporal change of small water body abundance. The continuous changes are associated with the nature of water bodies, regional/sub-regional landscape (hypsometry), precipitation and land use.

Carbon stock in Northumberland was determined by Total Elemental Analyser (TEA) combustion of sediment from various types and sizes of ponds collected from Druridge Bay, Northumberland. Carbon stocks varied in each ponds type and size range. These variations were influenced by the prevailing environmental/physical, biological and chemical/biochemical factors in pond sediments.

The microbial community drives carbon stock by altering the microbial community structure, allochthonous and autochthonous carbon processes and the oxygenation in the ponds. PCR pyrosequencing targeted at the 16s rRNA gene showed diversity in the microbial composition of the Northumberland pond sediments and the results showed a varying level of anoxia triggered by factors such as anoxic Proteobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria and Chlorobi dominance. These dominant phyla also influenced other phyla to develop anoxic ecological relationships and produce predominantly anoxia based processes like methanogenesis and fermentation. Anoxic pond bottoms were also triggered by high terrestrial inputs amongst other factors.

This research shows for the first time that carbon stock in a region’s SWBs varied because of numerous physical/environmental, chemical and biological factors. Also, SWBs stock carbon from the terrestrial environment and in-situ aquatic processes. Northumberland water body distribution has shown that more carbon is stocked in the small sized water body systems than larger water body system and their global abundance places them as an important carbon capture mechanism.

Contents

Declaration

I, Otaigbe Philip Inegbedion, declare that the work contained in this thesis entitled ‘The Importance of Small Water Bodies for Carbon Capture in Northumberland’ has not been submitted for any other award and that the work is my own.

I confirm that this work was done wholly while in candidature for the research degree of Doctor of Philosophy at Northumbria University at Newcastle and that it 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 University Ethics Committee on 23/02/2016.

I declare that the word count of this thesis is 39,978 words

Name: Otaigbe Philip Inegbedion

Signature:

Date: 21/06/2017

Acknowledgements

It begins with God and it ends with God

Believing is my connection

From the moment, I was born and two months after the death of Mr Odiase (my auntie's husband) who left as dying wish to my seven months' pregnant mom that "the child (me), he was not going to see, must have the best of educations". From his library of books on Physics, Geography and Literature, I got into reading at an early age.

To my mom and dad, who squeezed out daily miracles to keep my siblings and me fed, sheltered and educated.

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I remember the day my dad could not afford to pay my computer lesson fee; the days I got sent home from the different schools because we had to owe sometimes. I remember dad kept telling me to go back every morning and somehow, mom and dad worked out miracles.

I remember Handsome Preparatory School; I remember Benevolent Nursery/Primary School; I remember St. Greg's Nursery/Primary; I remember Government College Lagos (GCL); I remember Royal Star Comprehensive College; I remember Royal Kriston College; I remember the University of Benin (Uniben); I remember Newcastle University and I remember Northumbria University. I can only look back and be grateful.

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I love you, mom and dad.

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My love for you will never run out…
“making it in life is easy because I have faith, courage and enthusiasm”.
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If there is anything I learnt in this PhD is to never give up.
Faith, Courage and Believe.
And love without borders.
Thank you all and God bless you all.

Abbreviations

% - Percentage
%C – Percentage Carbon
%N – Percentage Nitrogen
: - ratio
µm - micrometre
^{0}C - Centigrade
bp – base pair
BU – Border Upland
C – carbon
C:N – Carbon – Nitrogen ratio
CA - California
CFC – Chlorofluorocarbon
CH_4 – methane
cm – centimetre
CO_2 – Carbon (IV) oxide/Carbon dioxide
CP – Coastal Plain
DBD – Dry Bulk Density
DEM – Digital Elevation Model
DNA - Deoxyribonucleic acid
DOC – Dissolved Organic Carbon
DOM - Dissolved Organic Matter
e.g. – for example
ETM+ - Enhanced Thematic Map Plus
FCB - Fibrobacteres-Chlorobi-Bacteroidetes
g – gram
g/cm^3 – gram per cubic centimetre
GC – Gas Chromatography
GHG – Green House Gas
GIS – Geographic Information Systems
GLWD – Global Lakes and Wetland Database
GLOWABO – Global Water Body database
GPP – Gross Primary Productivity
GTC – Gigaton Carbon
h – height
H_2O – Water
H_2S – Hydrogen Sulfide
HCFC – Hydrochlorofluorocarbon
HFC - Hydrofluorocarbon
IPCC - Intergovernmental Panel on Climate Change
IRT - Inhibitor Removal Technology
Kg – Kilogram
Kg C/sq. m – Kilogram Carbon per square metre
m – metre
mg - milligram
mi – miles
ml/min – millilitre per minute
mm - millimetre

MNWDI – Modification of Normalised Difference Water Index
MODIS – Moderate Resolution Imaging Spectroradiometer
MSSL – Mullard Space Science Laboratory
N – Nitrogen
N_2O – Dinitrogen monoxide or nitrous oxide
NCA – National Character Area
NDWI – Normalised Difference Water Index
NEP – Net Ecosystem Productivity
NHD - National Hydrography Dataset
NR – Nature Reserve
NWBD – Northumberland Water Body Distribution
O_2 – Oxygen
OLI - Operational Land Imager
OTU - Operational Taxonomic Units
PCR – Polymerase Chain Reaction
Pg - petagram
POC – Particulate Organic Carbon
POM - Particulate Organic Matter
ppb – part per billion
ppm – part per million
ppt – part per trillion
PVC - Planctomycetes-Verrucomicrobia-Chlamydiae
Q-PCR – Quantitative Polymerase Chain Reaction
r – radius
rRNA - ribosomal RNA
SDS - sodium dodecyl sulfate
SMTZ - sulfate methane transition
SOP – Standard Operating Procedure
sq. km – square kilometre
sq. m – square metre
SRTM – Shuttle Radar Topography Mission
SWB – Small Water Body
TCD - Thermal Conductivity Detector
TEA - Total Elemental Analyser
Tg – Terragram
TOC – Total Organic Carbon
™ - trademark
UAV – Unmanned Aerial Vehicle
UK – United Kingdom
UNEP - United Nations Environment Programme
USA – United States of America
USCCSP - United States Climate Change Science Program
WW2 - World War 2

List of Figures

List of Tables

Chapter 1 - Introduction

The increase in global temperature, melting of the polar ice caps, desert encroachment, changes in biodiversity and wildlife are recent climate change observations that have been related to elevated atmospheric CO_2 concentrations and other Green House Gases (GHGs). There is an urgent need to quantify the global carbon cycle and create a holistic carbon budget that fully comprehends the various processes and interactions across the various sub-compartments of the cycle (Michmerhuizen and Striegl, 1996 and Cole *et al.*, 2007). The identification and quantification of carbon sinks and stores have been recognised as high importance to carbon budgeting (Downing *et al.*, 2006, Downing *et al.*, 2008 and Downing, 2010). Cole *et al.* (2007) referred to a "missing carbon sink", which despite well-constrained estimates of anthropogenic CO_2 release and oceanic uptake suggest around 1.9 Pg per year of carbon remains unaccounted for. Siemens (2003) and Janssens *et al.* (2003) speculate that the imbalance in the European carbon budget is accounted for in the export of carbon to rivers and storage in sediment. Stallard (1998) and Smith *et al.* (2001) argued much of the missing sink (0.6 - 1.5Pg per year) may be largely in human-made aquatic and semi-aquatic environments such as farm ponds, stating that these environments may be quantitatively significant. There is the need for research on a regional and global scale in determining the carbon cycle flux compartments and sub-compartments, as this would help to create the much need changes in policy and human attitude towards climate change related issues.

Recent attempts to create a holistic global inventory of terrestrial water bodies estimate that 304 million water bodies cover around 3.4 million sq. km, and that this is dominated by small lakes and ponds, correcting a century-long misconception that large lakes are more significant (Downing, 2010, Downing *et al.*, 2006, Lehner and Doll, 2004 and

Tranvik *et al.,* 2009). Downing (2010) suggests a negative correlation between water body size and global frequency. If this correlation were to be applied to small water bodies (SWBs), there would undoubtedly be more globally abundant smaller water bodies than large lakes. The potential importance of smaller water bodies as carbon sinks has been highlighted further by the analysis of organic carbon burial rates in aquatic systems, suggesting a disproportionately greater intensity of carbon processing occurring within smaller aquatic systems (Downing, 2010).

SWBs have been identified as miscellaneous freshwater features such as small lake, spring, marsh, pond, watered gravel pit, drainage ditch or any other individual part of an ecosystem where water is a dominant environmental factor (Battin *et al.,* 2009, Downing, 2010, Downing *et al.,* 2006, Lehner and Doll, 2004, Seekell and Pace, 2011, Seekell *et al.,* 2013, Seekell *et al.,* 2014 and Tranvik *et al.,* 2009). For this research, small water bodies are defined as land depressions seen in map or satellite imagery that are <0.1 sq. km in size area, capable of holding water seasonally and process carbon and other bio-elements (Seekell and Pace, 2011 and Seekell *et al.,* 2013). This research also categorise water bodies into size ranges of:

1 – 10 sq. m

10 – 100 sq. m

100 – 1,000 sq. m

1,000 – 10,000 sq. m

10,000 sq. m – 0.1 sq. m

SWBs such as small lakes, ponds and temporary pools have previously been ignored in audits and all major global reports, under the assumption that systems of this size would not play any major role, and that large lakes and reservoirs dominated terrestrial limnological processing (Alonso, 2012, Downing, 2010 and UNEP, 2008). Nowhere is this more evident than the global carbon cycle, where there has been substantial research into the role of forests, wetlands and larger inland water bodies such as lakes and reservoirs as global carbon sinks but SWBs remain almost completely ignored (Boyd *et al.*, 2010, Dean and Gorham, 1998, Downing, 2010, Gilbert *et al.*, 2014, Oertli *et al.*, 2009 and Smith *et al.*, 2001). Predictions of the number and global coverage of natural water bodies in the range of 1 sq. m to 1 sq. km are restricted due to constraints in satellite imagery and limited regional data. With the recent and proven effectiveness of satellite imagery and UAVs (mapping to the centimetre scale), it is now possible for the first time to produce high-resolution maps of the spatial distributions of SWBs between 10 sq. m – 1 sq. km. This will be invaluable in addressing the ‘missing gap’ in water body distribution below 1 sq. km. Spatial distribution of water bodies can further be combined with carbon levels to develop regional or sub-regional carbon stocks in water bodies.

1.1 Research content

In determining the importance of SWBs to carbon capture in Northumberland, this research uncovers the total number and surface area of water bodies, the carbon stocks in water bodies' sediments, their carbon source and some factors that control carbon stocks dynamics in water body sediments. The whole of Northumberland served as the site for determining water body distribution and then selected ponds in Druridge Bay, South East Northumberland were analysed for carbon stocks and microbial contents.

1.1.1 Chapter Structure

Chapter 1 sets the scene for the thesis, by introducing carbon capture, SWBs and their potential carbon storage, and states the research aims

Chapter 2 review the literature on carbon capture and climate change, the dynamics of various carbon cycle compartments, small water bodies' carbon stocks and their omission from the global carbon budget. The literature review also explores factors controlling carbon stock and the importance of microbes in SWBs.

This chapter also reviews the geological, landscape, vegetation and anthropogenic activities of Northumberland region and Druridge Bay, South East Northumberland.

Chapter 3 presents methods for estimating Northumberland inland water body distribution (NWBD) with a focus on lentic systems. It uses high and low-resolution imagery for estimating number and surface area of water bodies in Northumberland.

Chapter 4 explores the carbon stocks in ponds and carbon stock variations with respect to pond size and pond type. It also identifies the influence of sediment wetness and dry bulk density on carbon stock.

Chapter 5 explores microbial communities within the pond sediments analysed for carbon stock. It also explores allochthonous and autochthonous carbon processing and oxygenation in the ponds. Potential metabolic processes associated with the microbial community structures in different ponds are discussed.

Chapter 6 combines the carbon stocks identified in Chapter 4 NWBD in Chapter 3 to estimate carbon stock in Northumberland water bodies. In this chapter, an estimate of the allochthonous and autochthonous carbon inputs was also determined from the microbes that utilise and produce carbon in pond sediments.

This chapter also discusses the importance of SWBs in carbon capture, identifies the research limitations and recommends potential future small water body research.

Finally, it summarises findings on the importance of SWBs for carbon capture in Northumberland.

1.2 Research Aims

The research aim is to determine the number and surface area of inland water bodies in Northumberland, the carbon stocks in the sediments of those water bodies and the microbial influence on the carbon stocks. These further define Northumberland carbon stocks, the level of allochthonous and autochthonous carbon influence in Northumberland water bodies and the effects of surrounding vegetation, sediment wetness, dry bulk density, microbes, anoxia, pond permanence and temporariness on carbon stocks variations.

The research aim is achieved by:

> Chapter 3:
>
> estimating the number and surface area of water bodies in Northumberland and investigating the size distribution
>
> Chapter 4:
>
> quantifying the carbon in SWBs, and its variation according to size and type of pond and down the sediment profile
>
> Chapter 5:
>
> identifying key microbial processes occurring within pond sediments and how they drive carbon stock
>
> Chapter 6:
>
> estimating the importance of SWBs in carbon capture by using Northumberland water body distribution (NWBD) to establish a regional carbon stock and carbon stock proportions from allochthonous and autochthonous sources.

Chapter 3, 4 and 5 present research questions to help address the research aims.

Chapter 2 - Literature Review and Background

2.1 Inland water bodies distributions

Before research in the hydrological contributions of inland water bodies to climate change, lakes and other water bodies were mainly considered important as sources for water and agricultural land (Lehner and Doll, 2004). Inland water bodies were also defined based on their ability to be seen in imagery, and these included natural and manmade lakes and impoundments (Downing *et al.,* 2006, Downing, 2010 and Lehner and Doll, 2004), with the exception of lotic systems such as streams, rivers and their tributaries which connect to oceans and seas. SWBs were usually overlooked or seen as part of the terrestrial ecosystem (Battin *et al.,* 2009). It has been suggested that there are around 8 million water bodies, with sizes >0.01 sq. km (Meybeck, 1995). Recent research has shown that the number of lakes and other inland water bodies is a lot more than previously thought (Verpoorter *et al.,* 2014). This is associated with the distribution and identification of more SWBs of different polygonal shapes, sizes and depths on a global scale with improving imageries (Downing *et al.,* 2006).

The Caspian Sea (~438,000 sq. km) is the world's largest lake based on surface area and the second largest is Lake Superior. Other large lakes include Lake Victoria, the Aral Sea, Lake Huron, Lake Michigan and Lake Tanganyika. They account for 10 - 12% of the total global surface area of inland water bodies (Liu, 2013 and Scheffers and Kelletat, 2016). Inland water bodies differ by numerous factors such as depth (Lake Baikal with depths >1700 m and Lake Patos, a Brazilian floodplain), elevation (Lake Titicaca with 3,810 m above sea level (Juengst *et al.,* 2017)), ice/ice-free state, salinity, temperature and watershed area (Fluet-Chouinard *et al.,* 2016 and Winslow *et al.,* 2015). Inland water bodies can exist because of the change in natural tectonics and geology or anthropogenic land usage. The distribution of water bodies below 10 sq. km are poorly understood because of diverse geologic and anthropogenic factors that control the abundance of water bodies <10 sq. km compared to water bodies >10 sq. km, which are more geologically influenced (Downing *et al.,* 2006, Lehner and Doll, 2004 and Scheffers and Kelletat, 2016).

2.1.1 Historical assessments of water body abundance and size

The earliest inventories of global lakes in modern records were published by Halbfaß (1922) and Thienemann (1925). Halbfaß (1922) identified lakes in Germany and Thienemann (1925) expanded the Halbfaß (1922) database to include European lakes. Thienemann (1925) showed that 1.8% of the land surface is made up of inland lakes and ponds. In that database, the Caspian Sea accounted for about 15% and Lake Superior accounted for 3.3% of earth's inland water bodies. The database suggested around 2.5 million sq. km as the total surface area of lakes and ponds. The databases also showed high levels of accuracy for water bodies that were seen on the available global maps at that time but poorly identified details for lakes and other water bodies <2,000 sq. km (Downing and Duarte, 2009 and Downing, 2010). Also, Schuiling (1977) first presented the concept of a relationship between numbers of lakes and surface area. Schuiling (1977) characterised the distribution of lentic water systems which improved Halbfaß (1922) database by 800 planimetered lakes.

Wetzel (1990) showed the earliest interest in the importance of SWBs abundance stating that there are more small lakes globally than large lakes. The Wetzel perception was regarded as a conceptual analysis rather than quantitative (Downing *et al.,* 2006). He postulated, "small lakes dominate the area of inland surfaces covered by water and the earth contains so many small, shallow lakes with small lacustrine ecosystems that cover more land than large lakes”. This postulation opened research into determining the actual abundance of SWBs, which were previously unacknowledged in almost 100 years of research in limnology since Halbfaß (1914).

2.1.2 Global distribution of water bodies

Several authors have used mathematical extrapolations to estimate the number of water bodies that cannot be identified on classical maps. Meybeck (1995) adopted an orthogonal regression system that calculates the number of lakes in many geographical regions that showed a consistent decrease in the number of water bodies with increasing size range. Meybeck (1995) sampled selected lakes in different sub-regions globally to define his orthogonal regression database. The database suggested few large lakes with smaller lakes being numerically abundant. The database is limited because mathematical extrapolation techniques do not consider changes across regional hypsometry and other factors that control small water body abundance (Seekell and Pace, 2011, Seekell *et al.*, 2013 and Tranvik *et al.*, 2009). Thus, Meybeck (1995) and other mathematical extrapolations of water body abundance are potentially flawed in their representation of water bodies <0.1 sq. km (Seekell and Pace, 2011 and Seekell *et al.*, 2013). Lehner and Doll (2004) inventoried lakes and other water body sizes >0.1 sq. km using satellite imagery and suggested a Pareto distribution pattern could estimate the abundance of water bodies with surface areas down to 0.001 sq. km (Downing *et al.*, 2006). The Lehner and Doll (2004) global lake and wetland distribution (GLWD) database contained 17,357 natural lakes >10 sq. km, around 250,000 wetlands, floodplains, lakes, rivers, and reservoirs (See Appendix A.1).

Estimates of the abundance of large water bodies identified from mathematical extrapolations and classical maps have good accuracy due to the availability of multiple but similar data sources (Lehner and Doll, 2004). However, there are wide variations in the SWBs abundance obtained from mathematical extrapolations because of temporal change of SWBs and varying data sources used in estimating SWBs. Also, classical maps are limited by imagery quality and resolutions used in identifying SWBs. The need to improve accuracy for estimating small water body abundance are further intensified by global biogeochemical cycling importance of SWBs (Battin *et al.*, 2009 and Gilbert *et al.,* 2014).

2.1.3 Small water bodies (SWBs) distribution

The suggestions of a Pareto distribution of inland water bodies to estimate the rest of the global inland water bodies distribution (<0.1 sq. km) has been discussed during the last 18 years to address the shortfall of most databases and wide variations in estimating water bodies <0.1 sq. km (Downing *et al.,* 2006, Lehner and Dolls, 2004, Seekell and Pace, 2011 and Verpoorter *et al.,* 2014). Downing *et al.* (2006) used mathematical extrapolations to suggest that the GLWD database (Lehner and Doll, 2004) can be drawn even further to estimate SWBs <0.1 sq. km. Given a paretian increase in lake abundance as lake sizes decrease between the Caspian Sea and lakes with sizes > 10 sq. km. This approach gave high levels of goodness-to-fit for a log-abundance (size range)-log-size area regression of water bodies as seen in Figure 2.1, without consideration of factors that influence SWBs abundance (Seekell and Pace, 2011). The database was used to estimate around 304 million water bodies that cover around 4.2 million sq. km of earth surface with sizes from the Caspian Sea (378,119 sq. km) to 0.0001 sq. km (Lehner and Doll, 2004). The Pareto pattern was also favoured by the existing notion of a relationship between the increase in the number of lakes and decrease in the surface area as suggested by Schuiling (1977) and Wetzel (1990). Although, Wetzel idea was purely conceptual and was not scientifically proven.

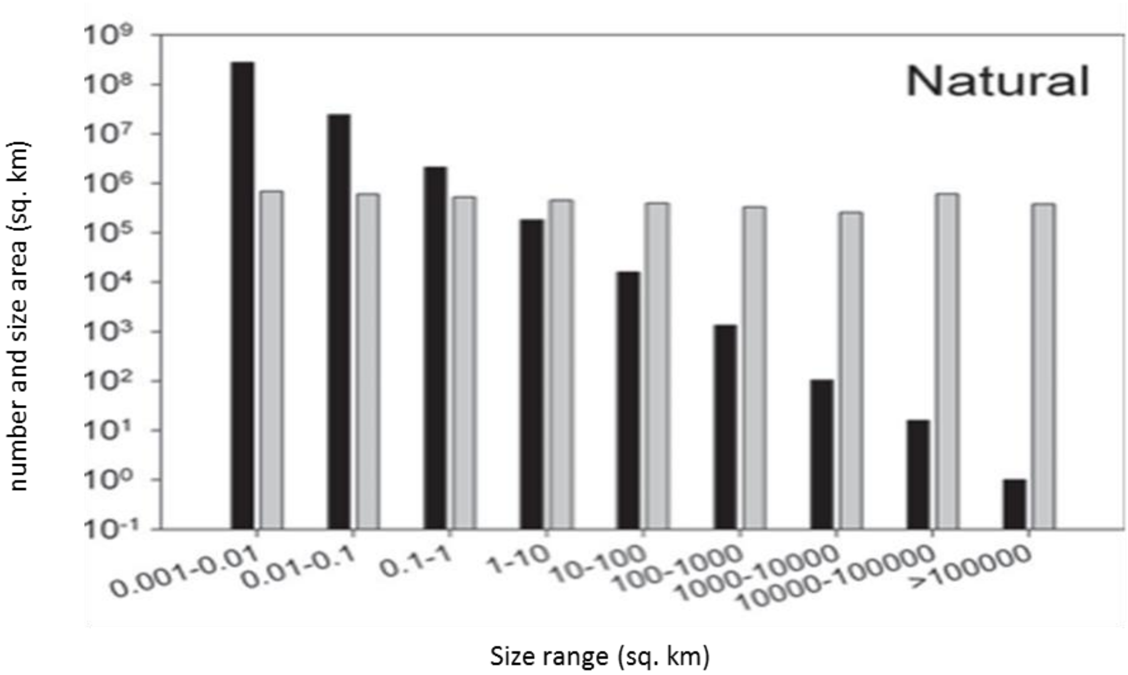

Figure 2.1: Pareto distribution of natural water bodies globally by Downing *et al*. (2006) and Downing (2010) (where the black bars represent the number of water bodies and the grey bars are the size area of water bodies)

Seekell and Pace (2011) showed that regional water body distribution did not necessarily follow the Pareto distribution and so advocated for a more regional estimate approach. Regional deviation from the Pareto distribution was seen in the analyses of water abundance in Adirondack Mountains of New York and the Northern Highland Lake District of Wisconsin, USA. Seekell and Pace (2011) analysis suggested a lognormal regional water body distribution would be one of many better approaches to estimating global water body abundance instead of the Pareto distribution. In the analyses of ponds in lowland Northumberland, Jeffries (2016) also showed that water body size distributions do not follow a Pareto distribution.

Water body abundance follows patterns defined by regional environmental, physical, and climatic factors and an extrapolation model should consider regional influences as constraints. Otherwise, there will be inaccuracies and the potential to overestimate the global abundance of water bodies <0.1 sq. km (Seekell and Pace, 2011 and Jeffries, 2016). Therefore, deriving theoretical databases for SWBs' size-distribution (Pareto, lognormal or otherwise) comes with levels of uncertainty as seen in Downing *et al.* (2006) and Seekell and Pace (2011). Thus, recalculation of the Global Lakes and Wetland Database (GLWD) (Lehner and Doll, 2004) and any other databases has shown the need for more regional field data to improve mathematical extrapolations.

Other recent methods for SWB abundance estimations include GLOWABO (Verpoorter *et al.*, 2014) and a sub-regional/regional database by Jeffries (2016) and McDonald *et al.* (2012) respectively. What is clear is that limnology research into water body abundance has come a long way with improving methodologies since Halbfaß (1922). However, limnology research should focus on developing a more accurate database based on actual field data of SWBs <0.1 sq. km with awareness of constraints of land use, hypsometry and other environmental variations. This would be best achieved through aerial imagery, remote sensing and ground survey for water bodies in fine detail. It should also be noted that the calculation of global small water body distributions will also be complicated by regional weather and climate variations (Downing *et al.*, 2006). Thus, SWBs abundances are continuously changing due to precipitation (Jeffries, 2016).

2.2.1 Climate change and carbon cycle

It was well established in the 20th century that climate change is the biggest threat to humanity in the modern times (Crowley, 2000, Fritz, 1996 Lamb, 2002, IPCC 1995). The effects of climate change such as the increase in sea levels, loss of wildlife diversity, melting of the ice caps and extreme weather conditions are believed to be more frequent and disastrous than previously expected (Butman and Raymond, 2011, Crate, 2011, Fankhauser, 2013 and Watkiss *et al.,* 2005). These effects are associated with increased carbon emission from land and water bodies into the atmosphere and have led ecosystems and biomes to adapt and adjust to changes affecting biological – environmental interactions (IPCC 1995, IPCC, 2001 and IPCC, 2014).

There has been no complete compilation of carbon stock from all sub-compartments of the global carbon cycle. Le Queré *et al.* (2015) and Cole *et al.* (2007) identified an uneven distribution of carbon amongst the atmosphere, aquatic and land environments, with land and aquatic environments being mainly reservoirs in the carbon cycle while the atmosphere serves as gaseous exchange points. There are uncertainties associated with the land's carbon sinks as their location and magnitude are largely unknown. These carbon sources and sinks continually vary as they exist in a flux state (Battin *et al.,* 2009, Canadell *et al.,* 2007, Cole *et al.,* 2007, Downing *et al.,* 2006, Lal, 2004, Le Queré *et al.,* 2015, Raymond *et al.,* 2013 and Verpoorter *et al.*, 2014), with atmospheric CO_2 being on the continuous increase from ~280 ppm during the pre-industrial revolution to levels over 384 ppm in 2008 (Battin *et al.,* 2009) and has risen around 400 ppm between 2008 to 2015 (NOAA/ESRL, 2016). Half of this CO_2 emission was from anthropogenic sources while the rest is sourced from oceans and land (Canadell *et al.,* 2007).

Le Queré *et al.* (2015) and Sabine *et al.* (2004) showed the different sub-compartments of the carbon cycle between 2004 – 2013 and the 1990s respectively and their interactions. The general overview was that more atmospheric carbon was being deposited because of anthropogenic carbon processes or land use. Land use and anthropogenic activities such as agriculture, fossil fuel combustion and cement production increased the atmospheric carbon cycle by 165 GTC per year, with fossil fuel contributing 6.4 GTC per year in the 1990s (Sabine *et al.*, 2004) and fossil fuel and cement production accounting for 8.9 GTC per year between 2004 – 2013 (Le Queré *et al.*, 2015) (Figure 2.2 & 2.3). Land use and anthropogenic processes make the terrestrial or land compartment of the carbon cycle become more of a net carbon source as it loses more carbon than it sequesters annually (Figure 2.3). These land carbon excesses end up in the atmosphere and water bodies, and atmospheric carbon levels are known to affect the biosphere (IPCC 1995, IPCC, 2001, IPCC, 2014)

Le Queré *et al.* (2015) and Sabine *et al.* (2004) (Figure 2.2 & 2.3) also identified compartments of the global carbon cycle and their net source, sink and reservoir value. But carbon in the various compartments does not equate to a net carbon source or sink. Thus, there is the need for detailed carbon estimations, projections and identification of knowledge gaps in calculating the carbon budget of compartments and sub-compartments of the carbon cycle (IPCC, 2014).

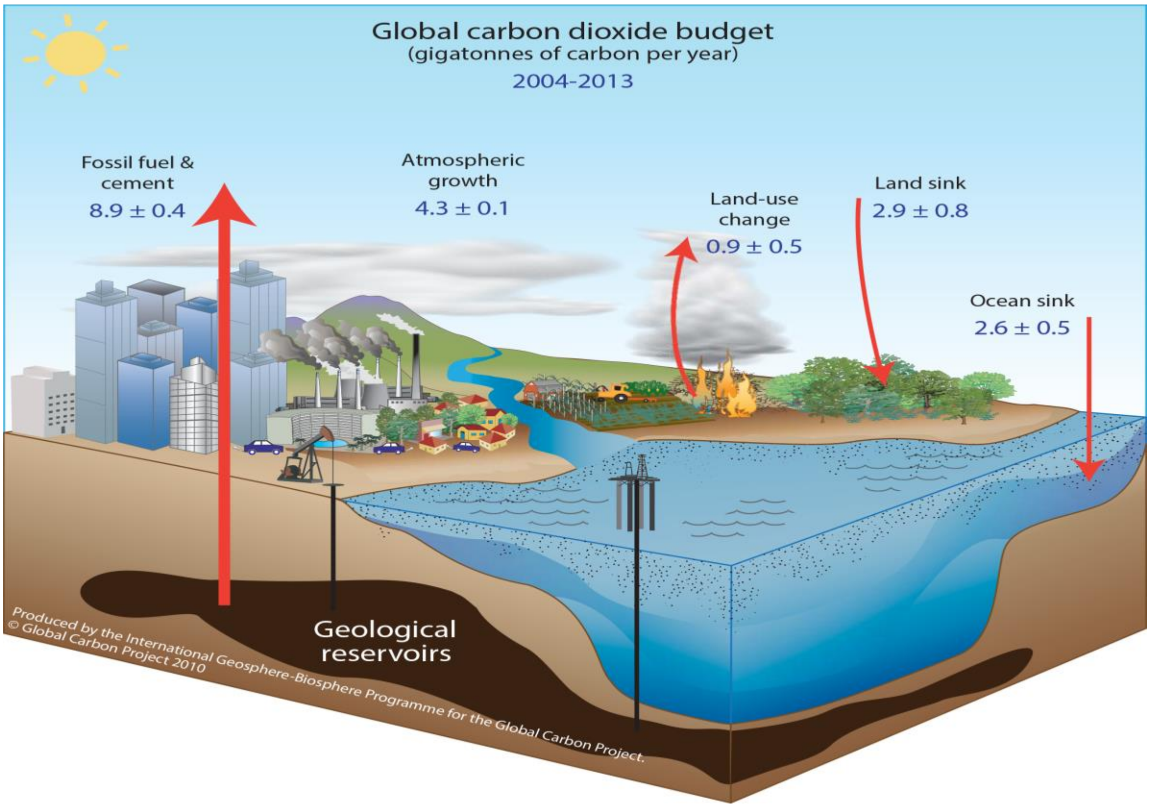

Figure 2.2: The global carbon cycle in the 2004 – 2013 showing the main annual fluxes in GTC per year (Le Queré et al., 2015)

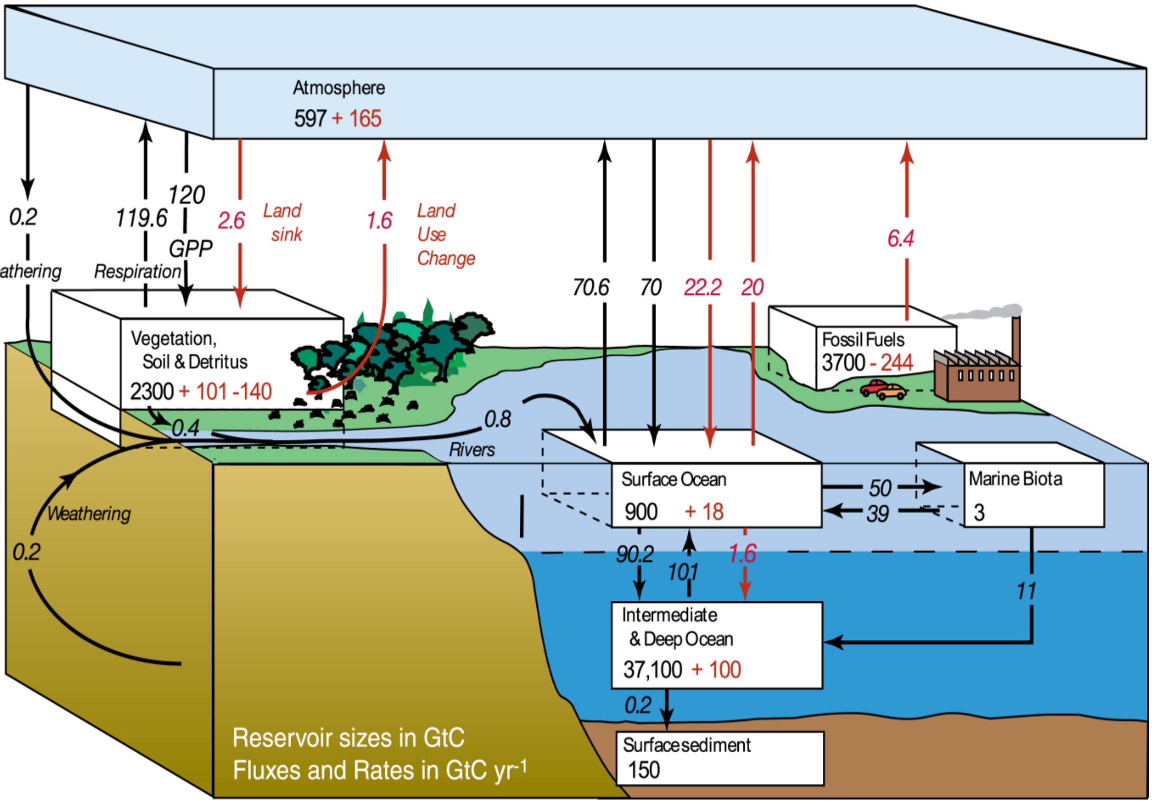

Figure 2.3: The global carbon cycle in the 1990s, showing the main annual fluxes in GTC per year: natural sources (black) and anthropogenic sources (red), (Sabine et al., 2004)

2.2.2 Aquatic and atmospheric compartments of the carbon cycle

Carbon mainly enters the biosphere through photosynthesis by phototrophic assimilation of CO_2 (Lorenz and Lal, 2010). Other bio-elements enter the biosphere through less effective chemotrophic mechanisms and biological assimilation (Wang *et al.*, 2016). The assimilated CO_2 via photosynthesis serves as gross primary productivity (GPP) of the global carbon budget. Other GPP processes involve oxidation or non-oxidation such as respiration, combustion and pyrolysis, and these processes are referred to as mineralisation. The un-mineralised GPP accounts for the net ecosystem production (NEP) which is stored in the ecological niche to form carbon reservoirs or exported by fluvial processes (Falkowski *et al.*, 2000, Larouche, 2015 and Roley, 2013). Also, abiotic CO_2 have been found to be stored in the aquatic environment directly from the atmosphere by a partial pressure increase of CO_2 to go into solution (Falkowski *et al.*, 2000). The rates of accumulation or sequestration of carbon are dependent on the nature of the depositional environment (Cole *et al.*, 2007).

Cole *et al.* (2007) discussed the dynamics of the carbon cycle compartments as continuously exchanging carbon through oxidation, storage and transport of terrestrial carbon in streams, lakes, wetlands, rivers and estuaries. It is estimated that 1.9 Pg per year of carbon are transported from land with only 0.9 Pg per year entering into oceans as organic and inorganic carbon. During the transport, ~0.2 Pg per year are buried in inland aquatic sediments and ~0.8 Pg per year enter into the atmosphere as gas exchange. Rivers are also important sources of CO_2 into the atmosphere via biological gaseous exchange. Rivers also connect with groundwater systems to increase CO_2 availability. Groundwater is both an anthropogenic and a natural source of CO_2 and water can physically weather carbonate rocks during fluvial transportation to release inorganic CO_2 into the atmosphere. Groundwater has also been known to discharge inorganic carbon directly into the oceans.

Lal *et al.* (2004) estimated global carbon emission at over 400 Pg because of increased fossil fuel, deforestation and biomass burning. Also, over 78 Pg of soil organic carbon depletion has been lost to atmospheric CO_2 causing an increase by 31%. These losses can easily be associated with poorly managed land usage between the 1750s and the present day (IPCC, 2001) with an increase in global atmospheric CO_2 averaging 1.76 ppm per year between 1979 – 2015 and CH_4 averaging 11.5 ppb per year between 2014 – 2015. Trace atmospheric gases or GHGs of CO_2, CH_4 and N_2O are on the steady rise globally while CFC, HFC and HCFC are still in low abundance globally (NOAA/ESRL, 2016) (Figure 2.4). These GHGs could remain in the atmosphere for decades causing the rapid increase in radiative forcing and global surface temperature by 0.6 ^{0}C. The warming rate is currently estimated at ~0.17 ^{0}C/decade (IPCC, 2001, IPCC, 2013 and NOAA/ESRL, 2016). And the increase in atmospheric emissions and temperature pose indelible socio-economic and health risks (IPCC 2013).

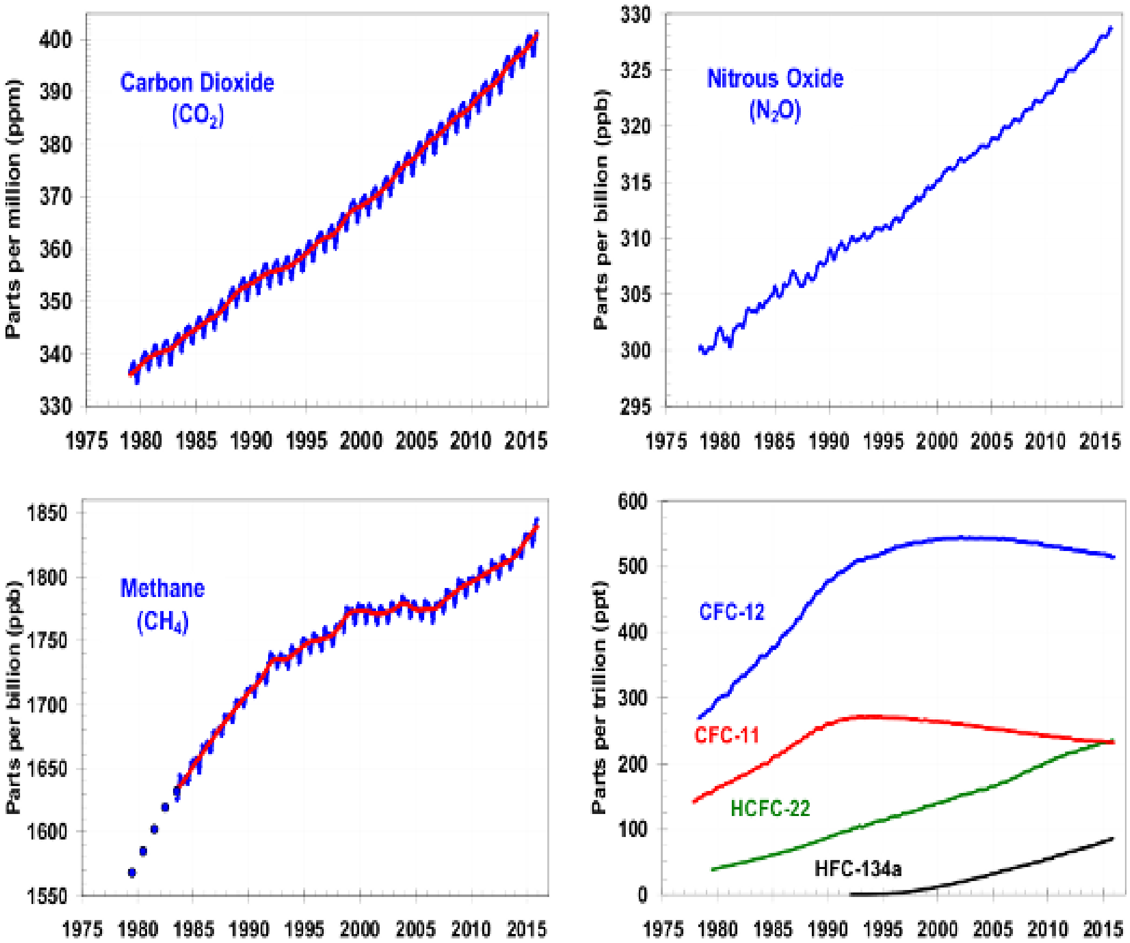

Figure 2.4: Changes in the trace atmospheric gases, NOAA/ESRL, 2016

2.2.3 Terrestrial or land compartments of the carbon cycle

2.2.3.1 Peatland and Forest

Global soil organic carbon was estimated at 4,000 Tg with 33% buried in peatlands (Premke *et al.*, 2016). Peatland occupies 3% of the earth land area (mostly located in the Northern hemisphere) and it is characterised by carbon-rich wetlands. Forest land accounts for ~30% of earth's land area and provides an effective carbon sink that absorbs 30% of the CO_2 from fossil fuel and deforestation emissions (Canadell *et al.*, 2007). The forest holds its carbon reserves in both trees and soils and interacts with the atmosphere through photosynthesis and surrounding small ponds, lakes and soil through deposition of detritus (Premke *et al.*, 2016 and Couwenberg *et al.*, 2011).

2.2.3.2 Cropland, Grassland and Large Lakes

Cropland and grassland account for 34% of the earth's non-glaciated landscape and are mainly used for agricultural purposes (Ramankutty *et al.,* 2008 and Smith, 2008). Croplands are classified as carbon neutral because the level of CO_2 emission and soil organic carbon stock are relatively constant (Sommer and Bossio, 2014). Grasslands can be carbon neutral or carbon sinks and their change in net carbon flux is dependent on variation in rainfall and temperature. High rainfall and low temperature lead to more carbon storage and low rainfall and high temperature balances out the net source and sinks (Leifeld and Fuhrer, 2009 and Smith, 2014).

Large lakes are CO_2 rich and are net heterotrophic systems with carbon loss into the atmosphere. Factors that contribute to increased heterotrophy are the excess dissolved organic carbon (DOC) and water pH, lake surface vegetation, drought rewetting, lake stratification, DOC colour change and increased temperature (Cole *et al.,* 1994. Evan *et al.,* 2005 and Premke *et al.,* 2016).

2.2.3.3 Small water bodies

It has been shown that rivers deliver terrestrial carbon into oceans and large lakes. River systems are also able to process carbon derived from terrestrial and aquatic sources. Little significance is given to SWBs. They are usually considered as 'carrier pipes' (Battin *et al.,* 2009 and Cole *et al.,* 2007), or transport intermediates into lakes, rivers and ocean (Downing *et al.,* 2006), or a part of the terrestrial ecosystem (Battin *et al.,* 2009 and Tranvik *et al.,* 2009). Tranvik *et al.* (2009) showed that land export of organic carbon into inland lakes equates to ~1.4Pg per year. During transport, ~0.6Pg per year enter inland water bodies for storage and oxidation (outgassing) while only 0.9Pg per year of organic carbon originating from land end up in the oceans.

SWBs are involved in more efficient phototrophic and chemotrophic carbon processing compared to larger water bodies due to high nutrients and bio-elements dilution in the small sizes of the water body feature (Downing, 2010, Kortelainen *et al.,* 2004 and Shirokova *et al.,* 2013). SWBs' autochthonous carbon production by microbes has not been estimated in literature. Also, Battin *et al.* (2009) approximately estimated 20% of carbon buried in the terrestrial ecosystem (trees and soils) is adjacent to inland water bodies. If this is equated as 0.6 Pg per year of carbon buried in inland water bodies (Tranvik *et al.,* 2009), SWBs (a compartment of inland water bodies) would have more importance than previously thought because of their proximity to access terrestrial carbon and ability to produce autochthonous carbon. Some SWBs have prevalent anoxia which aid efficient carbon burial (Battin *et al.,* 2009, Briée *et al.,* 2007 and Gilbert *et al.,* 2014). In stable sedimentary basins and over thousands of years, carbons can be permanently stored in the lithosphere as reservoir carbon. Thus, SWBs can serve as short and long term carbon sinks amongst other earthly features of the lithosphere (Battin *et al.,* 2009).

2.3 Carbon stores, sinks and the missing carbon budget

Like Le Queré *et al.* (2015) and Sabine *et al.* (2004) (Figure 2.2 & 2.3), Lal *et al.* (2004) discussed dynamics of the carbon cycle reserves, source and sinks. Lal *et al.* (2004) showed that land usage (1.6 Pg), fossil fuel combustion and cement production (6.3 Pg) were the major carbon sources into the environment. Their report also identifies that the atmosphere and oceanic environment were only able to re-capture 4.9 Pg (as sinks). This leaves a deficit of 3.0 Pg of a 7.9 Pg carbon cycling system unaccounted for in the 1990s (Table 2.1). The report suggests that the unaccounted carbon budgets are recaptured in the terrestrial environment. The general assumptions are that the major chunk of these carbon budgets exist as soil organic/inorganic carbon (Lal *et al.*, 2004), forest plants (Luyssaert *et al.*, 2008), buried in streams and rivers, buried in the world's large lakes, glaciers, ground water, deltas, bays and estuaries. But it is also possible that SWBs are "a missing link" or the "missing link" that store the unaccounted carbon in Table 2.1.

Table 2.1: Sources, sinks and missing links in the calculation of carbon budget (Lal et al., 2004)

Source/Sink	*1980s (Billion ton)*	*1990s (Billion ton)*
Source		
1.Fossil fuel utilisation	5.0	6.3
2.Land use change	1.7	1.6
Total	6.7	7.9
Known sinks		
Atmosphere	3.3	3.2
Oceans	1.9	1.7
Total	5.2	4.9
Missing sinks (probably in terrestrial sinks)	1.5	3.0

2.4.1 Productivity of small water bodies

The net ecosystem production (NEP) of water bodies is the sum of the carbon budget cycling in the ecosystem from aquatic primary productivity and allochthonous entry with the deduction of carbon loss to atmospheric emissions. NEP varies in different environments (Crooks *et al.,* 2014, Falkowski *et al.,* 2000, Larouche, 2015, Matthews, 2013 and Roley, 2013). Lentic lake systems have better carbon retention capacity over lotic (river-like) ecosystems due to their confined nature, a high concentration of nutrients, rewetting, autochthonous and allochthonous carbon entry, reduced perturbation and anoxia amongst other features (Cole *et al.,* 2007). Downing (2010) suggested that biogeochemical processes are 115 times greater in lake systems compared to the oceanic environment and biogeochemical processes are 33 times more in lake systems compared to the terrestrial environment (soil). Also, carbon and bio-element storage commonly increase with increasing lake productivity and decreasing lake size (Kortelainen *et al.,* 2004, Mulholland and Elwood, 1982 and Shirokova *et al.,* 2013).

2.4.2 Carbon storage in small water bodies

Shirokova *et al.* (2013) showed that there were significant eutrophic and oligotrophic bacterial activities occurring across different SWBs studied in Western Siberia. There was high gross primary productivity (GPP) in all shallow SWBs studied with 75 - 92% oxygen consumption by bacterioplankton which connotes high organic matter mineralisation, respiration and water column activities. The storage of organic carbon was identified as particulate organic carbon (POC) in sedimentary bottoms of lakes but carbon storage did not show direct correlation with varying levels of gross primary productivity in individual sample areas. This was because high oxygenation of water bodies reduced carbon storage (Shirokova *et al.,* 2013) but storage improves with the increase in anoxia (Gilbert *et al.,* 2014).

Paleo-environmental study of the world's large lakes showed a general trend of carbon burial rate increasing with decreasing surface area (Einsele *et al.,* 2001). Inland water bodies found in the terrestrial environment are numerically more abundant in nature and can store carbon over a long period through drainage from the surrounding terrestrial environments (Downing *et al.,* 2006, Shirakova *et al.,* 2013, Sobek *et al.,* 2005 and Sobek *et al.,* 2011). Alongside fluvial transport, SWBs can store carbon from in-situ biological processes. Generally, inland water bodies have high terrestrial inclusion and high primary productivity, even with little or no transportation and this is because of in-situ vegetation growth, microbial phototroph and heterotroph activities with nutrient supply. SWBs have been estimated to store as much as 30 – 60% more organic carbon than oceans, despite, world oceans covering over 70% of the planet while SWBs account for ~3% (Cole *et al.,* 2007, Downing, 2010 and Downing *et al.,* 2006).

2.4.3 Carbon flux in small water bodies

Carbon fluxes in inland water bodies relate to the nature of physical, chemical and biological processes that transform terrestrial and aquatic carbon into source or sink. Carbon entry is either used up as a nutrient and/or buried in sediment via aquatic transformation by microorganisms (Aufdenkampe *et al.,* 2011 and Scharnweber *et al.,* 2014), POC sedimentation, or photosynthetic plant burial (Boyd *et al.,* 2010 and Raymond *et al.,* 2013). In agricultural land and densely vegetated areas, entry of terrestrial carbon has direct impact on carbon stock in the receiving aquatic system and its allochthonous transport is largely aided by water or aeolian transport (Frielinghaus *et al.,* 1998) into the geomorphic depressions and water bodies. Also like oceans, inland water bodies source inorganic carbon as products of rock weathering and ground water (Cole *et al.,* 2007 and Marce *et al.,* 2015), and some of these are loss to the atmosphere. Atmospheric CO_2 contributions from SWBs pose a new question on the heterogeneity of carbon sink/source, that is, which of terrestrial and/or aquatic carbon in SWBs are the net source or sink?

Premke *et al.* (2016) discussed variability in the landscape, landscape composition and extents of landscape elements as factors that constantly vary globally and they alter carbon flux estimates. These coupled with other factors such as nature of organic carbon, microbial loads, nature of water bodies sediment, regional climate, water depth, oxic and anoxic conditions influence physical transport and sedimentation into/within SWBs and activities of natural and adaptive microorganisms in SWBs, and consequently carbon flux (Cole *et al.,* 2007, Emerson and Hedges, 2003, Gilbert *et al.*, 2014, Grabowski *et al.*, 2011, Shirokova *et al.,* 2013 and Sims *et al.,* 2013).

2.5 Microbes and microbial interaction in water bodies

Microbial diversities widely vary in water bodies and the connections between trophic levels are usually complex in the ecosystem. The main microbial components of an inland water body’s food web generally include autotrophs, omnivores, heterotrophs, decomposers and phagotrophs (Maiti, 2013, Misra, 2009 and Sharma, 2009). They provide pathways for entry of soluble organic and inorganic nutrients into the biotic ecosystems (Maiti, 2013 and Teoh *et al.,* 2016). Cell density estimates indicate not just diversity but ubiquity in marine and freshwater ranging from $10^4 – 10^7$ cells per ml of water. Freshwater lakes, saline lakes and rivers contain 10 x 10^5 cells per ml of water and open ocean and continental shelf contains 5 x 10^5 cells per ml of water (Whitman *et al.,* 1998).

Various microbes exhibit a combination of hetrotrophism, decomposition, autotrophism and phagotrophism strictly or obligatorily based on their taxonomic classification, trophic relationship with other microbes and environmental influence (Maiti, 2012 and Maiti, 2013). Producers utilise sunlight, inorganic and organic matter in the environment for energy. They usually replicate rapidly and form the primary biomass for other trophic levels’ growth and development. These replications tie to nutrient supply and favourable environmental conditions (Pearce, 2016, Ramaraj *et al.,* 2015 and Teoh *et al.,* 2016). Consumers are feeders of algal and bacterial picoplankton, larger algae, protista and metazoans (Bärlocher and Boddy, 2016 and Crump *et al.,* 2013). Mixotrophs also exist in the inland water body ecosystem with abilities to create nutrients as producers and prey on other organisms as consumers. Decomposers scavenge dead small and large organisms and break them down to release nutrients (Bärlocher and Boddy, 2016 and Falkowski and Raven, 2013).

In SWBs, most naturally occurring bacteria are heterotrophs and they are ubiquitous in the natural environments. Heterotrophic bacterioplankton with range in size from ranges 0.2 - 1.0 µm. Coccoid cyanobacteria and small green algae with size range 0.5 – 2.0 µm are mainly autotrophic. Protista are mainly flagellate, amoeba and ciliates. Their size ranges from 2 – 200 µm and taxa vary as mainly autotrophs and heterotrophs. Pigmented ciliates and flagellates are known to be primary producers and consumers of organic matter. Rotifers range from 50 – 200 µm and are mainly heterotrophic particulate feeders of algae, phytoplankton and picoplankton. They are also known to predate other rotifers and ciliates (Bärlocher and Boddy, 2016, Falkowski and Raven, 2013 and Trebilco *et al.,* 2013).

Figure 2.5 typifies the transfer of energy in the food web, and the fate of DOC and POC in water bodies. The energy transfers within a typical inland water body system start off with small organisms and then energy moves through the food web by larger organism’s consumption. Energy transfer ends at decomposition which leads to mineralisation or carbon storage or re-entry into the food web (Dray, 2014, Grant, 2013 and Nanda *et al.,* 2016). Overall, movement of energy is not 100% effective with the loss of energy through respiration and other cell metabolic processes. As energy gets transferred to higher trophic levels from primary production, energy supplied to more complex structured organisms reduces with varying efficiency. These processes create atmospheric carbon sources and settling of dead microbes and organic carbon from allochthonous sources in lake bottoms, that is, carbon sinks (Aufdenkampe *et al.,* 2011, Falkowski and Raven, 2013, Frielinghaus *et al.,* 1998, Grossart and Rojas-Jimenez, 2016, and Scharnweber *et al.,* 2014).

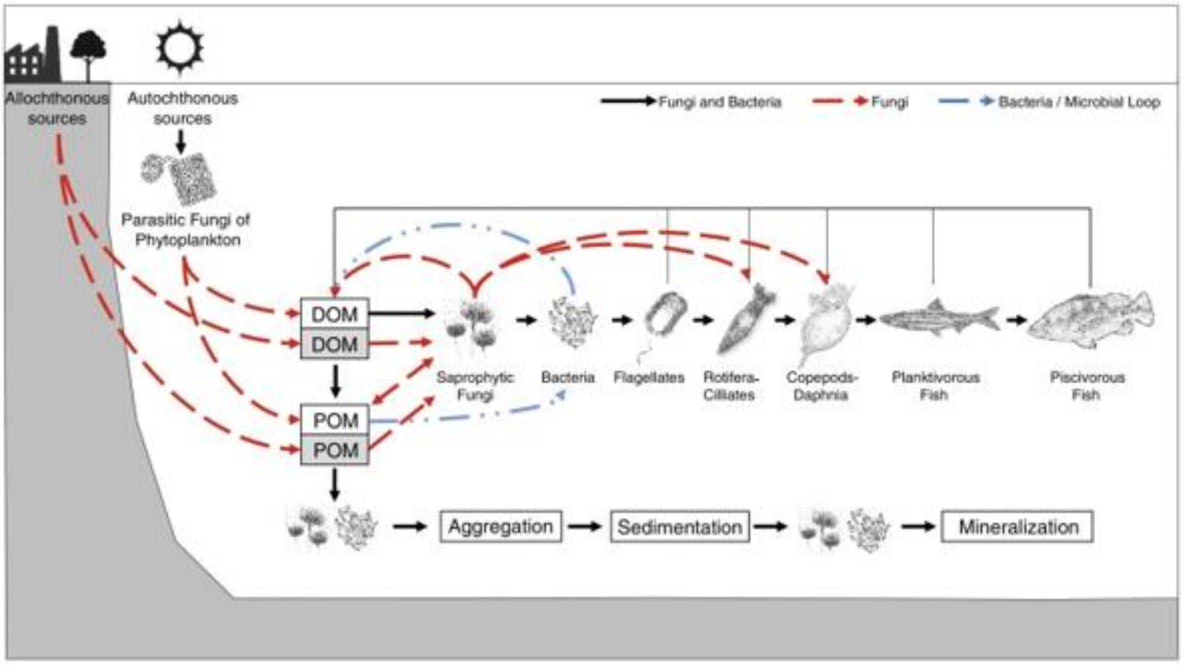

Figure 2.5: Energy transfer and fate of allochthonous and autochthonous carbon source in an aquatic system (Grossart and Rojas-Jimenez, 2016)

2.6 Other factors controlling processes in SWBs

Some notable other factors that control carbon stock in SWBs are outlined below.

2.6.1 Food web

The structure, composition and access to terrestrial deposition in inland water body systems allow for high biotic richness and gross primary productivity. In combination with prevailing physical and chemical factors, the abundance of microbes (algae, phytoplankton, zooplanktons and macrophytes) are the baseline for the creation of a widely-diversified food web and feeding mechanisms of higher life forms (Maiti, 2013, Misra, 2009, Sharma, 2009 and Vadeboncoeur *et al.*, 2011). Thereafter, higher life forms (birds, amphibians, fish and invertebrates) serve as a source for organic matter supply in bottom sediments. The ecological abundance of higher life forms is strongly controlled by the abundance of the prey in the predator-prey relationship and energy transfer decreases with increasing trophic level (Baum and Worm, 2009, Chiang *et al.*, 2014 Grossart and Rojas-Jimenez, 2016 and Hairston Jr and Hairston Sr, 1993).

2.6.2 Material transport, nature of organic carbon and pond depths

Material transport, organic matter size and gravity determine the material resident time and transport within the water column. Shirokova *et al.* (2013) described low weight organic matter with particle size <0.45 µm as DOC. This matter is prevalent in all water columns because of their low resident time and this makes them bio-available for microbes' utilisation. There is the tendency for DOC to form colloidal DOC which enhances gravitational pull to lake bottoms and this is also influenced by pond depths and currents. The resulting colloidal DOC in bottom sediment can be remobilized or stored. Shirokova *et al.* (2013) also confirmed that the percentage of colloidal DOC ranges from

30 – 90% in small short columned SWBs and is lowest in large lakes with longer water columns. These further stress the importance of water depth in carbon capture. Aquatic sediments have been found to contain more POC than DOC. This can be related to the shorter resident time required for larger sized particulates to travel down the water column. Therefore, there is a direct relationship between total organic carbon (TOC) and stored POC in aquatic sediment in SWBs, and POC supply could either be aquatic and/or terrestrial sources (Dunalska *et al.,* 2003 and Scharnweber *et al*., 2014).

2.6.3 Light penetration and temperature

Processes of photosynthesis, other energy transfer mechanisms utilised microbes and environmental conditions provide the baseline for determining for nature and source of carbon in SWBs (Bärlocher and Boddy, 2016 and Briée *et al.,* 2007). But sunlight through photosynthesis is the main source for energy entry into the food web (Chyba and Hand 2001 and Dimijian, 2000). Phytoplankton and phototrophic bacteria play a major role as the primary producers in an aquatic system through photosynthesis. Therefore, light penetration and turbidity are important in the development of biodiversity and trophic relationships in an aquatic ecosystem, as high light penetration begets high productivity of phototrophs which will be utilised by heterotrophs. Although, these dynamics can vary depending on other factors/influences in the water body environment (Bidle and Flakowski, 2004, Field *et al.,* 1998 and Tissot and Welte, 2013).

SWBs' temperature determines the adaptive survival of existing organisms. At constant temperature, existing microbes are more effective in carbon processing than in temperature extremes or flux. In lower trophic levels, temperature has a direct effect on the productivity of heterotrophic and autotrophic picoplankton while microplankton are less affected by temperature change. In higher trophic levels, temperature strongly regulates biochemical activities within and between cells/organisms (Agawin *et al.,* 2000 and Porter *et al.,* 1988).

2.6.4 Water body stratification

Water body stratification is related to oxygenation and temperature. Oxygenation decreases with water column depth, with the top pond or part closest to the pond surface, known as the epilimnion zone, being oxygen rich. Epilimnion zones are highly productive environments with planktonic communities and predating larger organisms. In summer, stratification and oxygenation of water bodies favours sedimentation and sequestering of POC and DOC burial. The thermocline reverses in lower temperature conditions. Productivity is based on in-situ oxygenation and microbes' ability to survive temperature change (Figure 2.6). Oxygen depleted lake bottoms reduce activities of highly oxygenic microbes and encourage burial of organic carbon (Dunalska *et al.,* 2003 and Shirokova *et al.*, 2013). Similarly, the epilimnion is filled with DOC that is readily utilised by the microbial communities in oxic conditions and this results in high CO_2 excursion (Isidorova *et al.*, 2016).

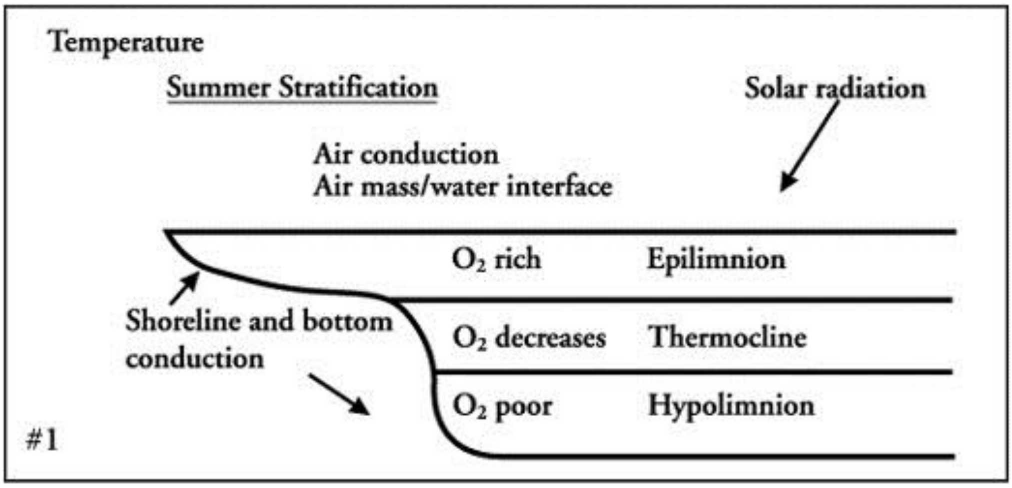

Figure 2.6: Pond Stratification and oxygenation in water bodies

In winter, stratification occurs due to the expansion of the oxygen rich zone and lower temperature. Microbial activities are generally slowed but autotrophic productivity increases for well-adapted microbes. Irrespective of seasons, POC settles to the bottom but winter is associated with slowed microbial utilisation. DOC is least utilised in the winter and settles into water body bottoms with reduced microbial perturbation (Dunalska *et al.*, 2003 and Shirokova *et al.*, 2013). The fate of DOC resets partially in springtime with the increase in temperature, erosion and increased microbial productivity. Situations can exist where excess autotrophic summer productivity and terrestrial carbon entry into the water body sustain stratification through the winter and into the new season. It involves the combined adaptability of microbial primary producers to respond to physical environmental changes and relatively insignificant changes in water body column depths (Stockner, 1988), amongst other prevailing environmental factors and this is consistent with eutrophic lakes.

2.6.5 Bio-elements

There are variations in the bio-elemental concentration of all aquatic systems. These variations are based on the nature and source of bio-elements, material transportation (aeolian and fluvial), burial, utilisation by microorganisms and prevailing environmental conditions. The combination of these factors directly affects carbon productivity and storage. Bio-elements activities in SWBs are best identified by interactions with carbon productivity. This can be achieved by measuring organic and inorganic biogeochemical activities such as trace elements, organic and inorganic carbon activities, nitrogen, sulphur, iron, potassium and phosphorus activities (Baker *et al.*, 2015, Briée *et al.*, 2007, Chapelle, 2001, Coby *et al.*, 2011, Kluber and Conrad, 1998, Shirokova *et al.*, 2013 and Weber *et al.*, 2006) and biochemical interactions amongst microbes. Within water bodies that experience stratification, bio-element distribution can vary in concentration and this creates variation in microbial processes within ponds (Briée *et al.,* 2007, Isidorova *et al.,* 2016 and Shirokova *et al.,* 2013).

Agricultural activities have also been associated with increased availability of bio-elements through run-off into SWBs and this has been associated with microbial blooms in SWBs (Cai *et al.,* 2014 and Xiao *et al.,* 2017). A high dilution factor of nutrients in large lakes and marine systems are responsible for the low productivity of such ecosystems. However, shorelines possess high gross primary productivity (GPP) given a higher concentration of nutrients from land before they are diluted into the open ocean.

In summary, it has been established that storage, gaseous exchange and recycling of carbon in water bodies are in constant interaction and flux with each other and they are controlled by several physical/environmental, biological and chemical/biochemical factors (Aufdenkampe *et al.,* 2011, Bärlocher and Boddy, 2016, Falkowski and Raven, 2013 Grabowski *et al.,* 2011, Jeffries, 2016, Premke *et al.,* 2016 and Sobek *et al.,* 2005). Other factors include vegetation entry, precipitation, sediment type and aggregation, hypsometry, and land use. The level of influence of an individual factor is interdependent on others in determining carbon source or sink and the way an ecosystem responds to carbon or bio-elemental cycling processes. Small water body systems have banks or pond edges and shorter water columns which access an active supply of allochthonous materials, minerals, and microbes and this creates high nutrient concentrations and biological processing compared to large lakes and oceans (Cole *et al.,* 2007).

2.7.1 Northumberland

Northumberland is in the North East of England, bordering Scotland to the North and West and the North Sea to the East. It has a diversity of physical geographies with flat land near the North Sea (Elliott, 2011, Jeffries, and 2008, Lunn, 2004) and increasingly upland elevation toward the West. It elevation ranges from sea level in the East to ~800 m in the North West (Figure 2.7a).

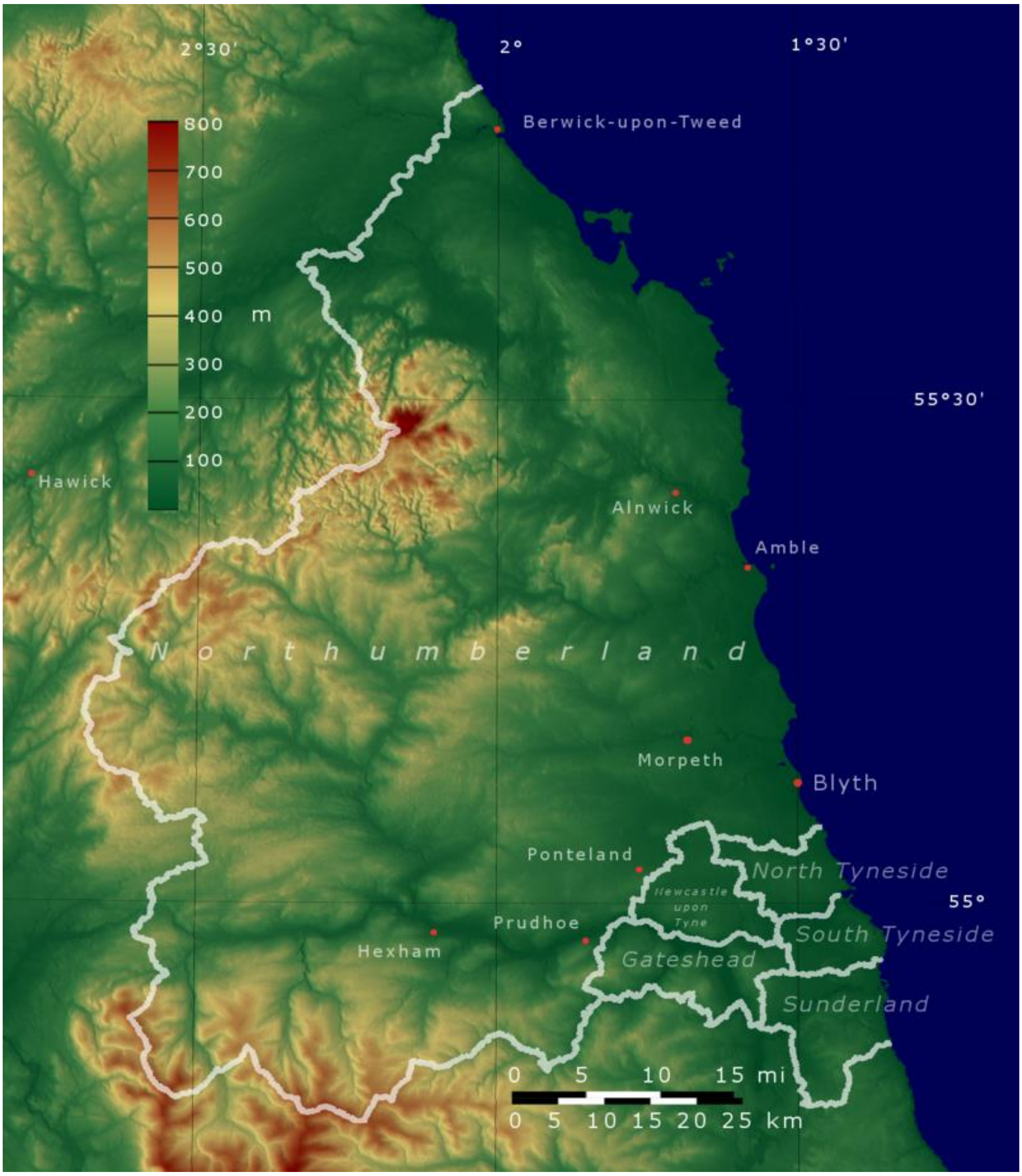

Figure 2.7a: Map of Northumberland (map created with SRTM)

The North to South Northumberland hard geology dates from the Palaeozoic era from ~440 million to ~250 million years ago. It features igneous andesite and granite of the Cheviots Hills in the North and Carboniferous dolerite intrusion in the Hadrian's Wall area in South Northumberland (Figure 2.7b). In the Quaternary era, between 25,000 and 12,000 years ago, the landscape of Northumberland and North of England were glacially shaped. Coastal Northumberland near the Farne Islands has dolerite outcrops (Bird, 2016, Lunn, 2004 and Oswald *et al.,* 2013). The south-east Northumberland area are characterised by coal fields.

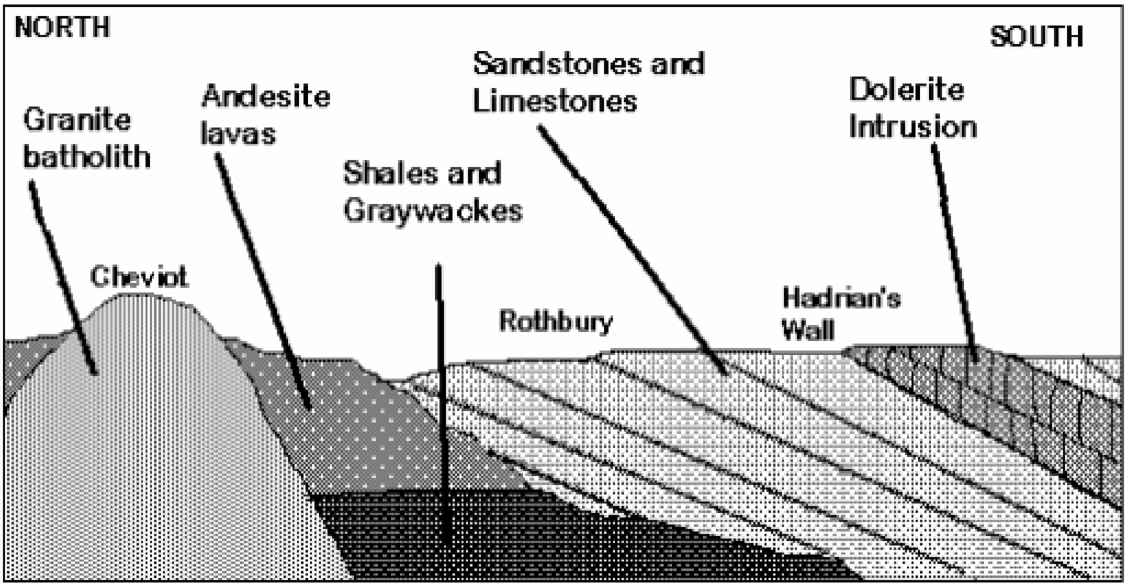

Figure 2.7b: North to South geological sections of Northumberland (Sourced from Northumberland National Park)

Northumberland lies wholly or partially in the following National Character Areas (NCA) (Natural England, 2012):

- North Northumberland Coastal Plain
- South East Northumberland Coastal Plain
- Mid NorthumberlandBorder Moors and Forests
- Cheviots

- Cheviot Fringe
- Northumberland Sandstone Hills
- Tyne Gap and Hadrian's Wall

The North Northumberland Coastal Plain consists mainly of arable and pasture land with sparse woodland confined to a river valley. It is a narrow and windswept strip located on the England –Scotland border. The coastline also contains sandy beaches, dunes and Whin Sill outcrops. The South-East Northumberland Coastal Plain lies south of the North Northumberland Coastal Plain. It is a low-lying strip with large fields, restored and active open cast coal mines, coastal rocky headlands and farmlands. Like the North, it has sand dunes as well as coastal flood plains and the rivers Blyth, Wansbeck, Coquet, Pont and Seaton Burn. Mid Northumberland features include a gently undulating plateau with farmlands, ridges and river valleys. Border Moors and Forests are an extensive and sparsely populated upland plateau dissected by the rivers North Tyne, Rede, Lyne and Irthing. Border Moors and Forests have an underlying geology of carboniferous deposits and subsequent glacial and fluvial depositions that formed peat. Cheviots NCA is part of an upland chain of Northumberland moors. The uplands rise steeply above the lowland belts of the Cheviots Fringe. Its features include some hill tops, rocky outcrops and scree slopes of igneous geology. Cheviot Fringe are undulating landscapes formed by glacial processes and weathering of underlying bedrocks deposited into river valleys. It also consists of arable farmland, pasture and meadow. Northumberland Sandstone Hills is located in the middle of Northumberland, with distinctive flat-topped ridges. Its ridgetop and grass moorland are covered by conifers. Tyne Gap and Hadrian’s Wall are underlain by sedimentary carboniferous rocks, limestone, sandstones, shales and igneous rock dolerites. It is covered in arable and pasture land, conifers and wooded valleys as well as being drained by the River Tyne (Natural England, 2012 and Natural England, No date).

The Northumberland coast has a temperate climate with a summer mean maximum temperature of ~20 ^{0}C and rainfall of ~800 mm per year (Gilbert *et al.*, 2014 and Lunn, 2004). Coastal Northumberland is characterised by salt marsh and extensive dunes. There are also rocky reefs and sea caves which house marine communities and seabirds (Gilbert *et al.,* 2014, Jeffries, 2008, Jeffries, 2012, Jeffries 2016 and Lunn, 2004). Mainland Northumberland is characterised by grasslands, and woodlands found in the steep-sided valleys of the Rivers Coquet, Aln and Wansbeck. The rivers are also home to a wide variety of trout, crayfish, salmon and other river dwellers. Subsidence wetland post-mining in South East Northumberland are home for plants, invertebrates, amphibians and birds. The upland of Northumberland is characterised by heath, blanket bog and hay meadows with wader, raptor, pignut and globeflower populations. Some of the main land uses in Northumberland are arable and pasture agricultural land, and urban development (Abraham, 2016, Bird, 2016, Elliott, 2011 and Groom *et al.,* 2012).

2.7.2 Druridge Bay

Druridge Bay is an area of coastal Northumberland situated between the towns of Amble and High Hauxley to the north and Cresswell and Lynemouth to the south (Figure 2.8). Geological development of Druridge Bay and the whole of Northumberland can be dated back to the early Carboniferous (350 million years ago) which is responsible for the coal deposition in the entire region. In the 20th century, open cast mining was replaced with deep mining processes with massive excavation (and then subsidence) which changed the landscape. The shoreline areas were exploited for their sands which led to erosion and accretion (Bird, 2016). However, site restoration is currently in advanced stages from the previous anthropogenic induced subsidence (Elliott, 2011, Jeffries, 2008, Lunn, 2004 and Waddington, 2010). The deep mining restorations created opportunities for land conservation and tourism including wetland conservation at Hauxley and East Chevington and Druridge Bay Country Park. Druridge Bay land features include sandy beaches, intertidal rock platforms, narrow dune ridge, dune grassland and flat coastal plain farmland (Jeffries, 2008, Jeffries, 2011 and Lunn 2004). Ponds within this area have been predominantly associated with changes in dominant species with changing seasons (Jeffries, 2008). The ponds are dried out in summer and increase in surface area during the winter, thus, have more surface area for carbon sequestration and material draining. Figure 2.8 shows the area where the cores were collected in Druridge Bay, Northumberland for carbon and microbial content analyses.

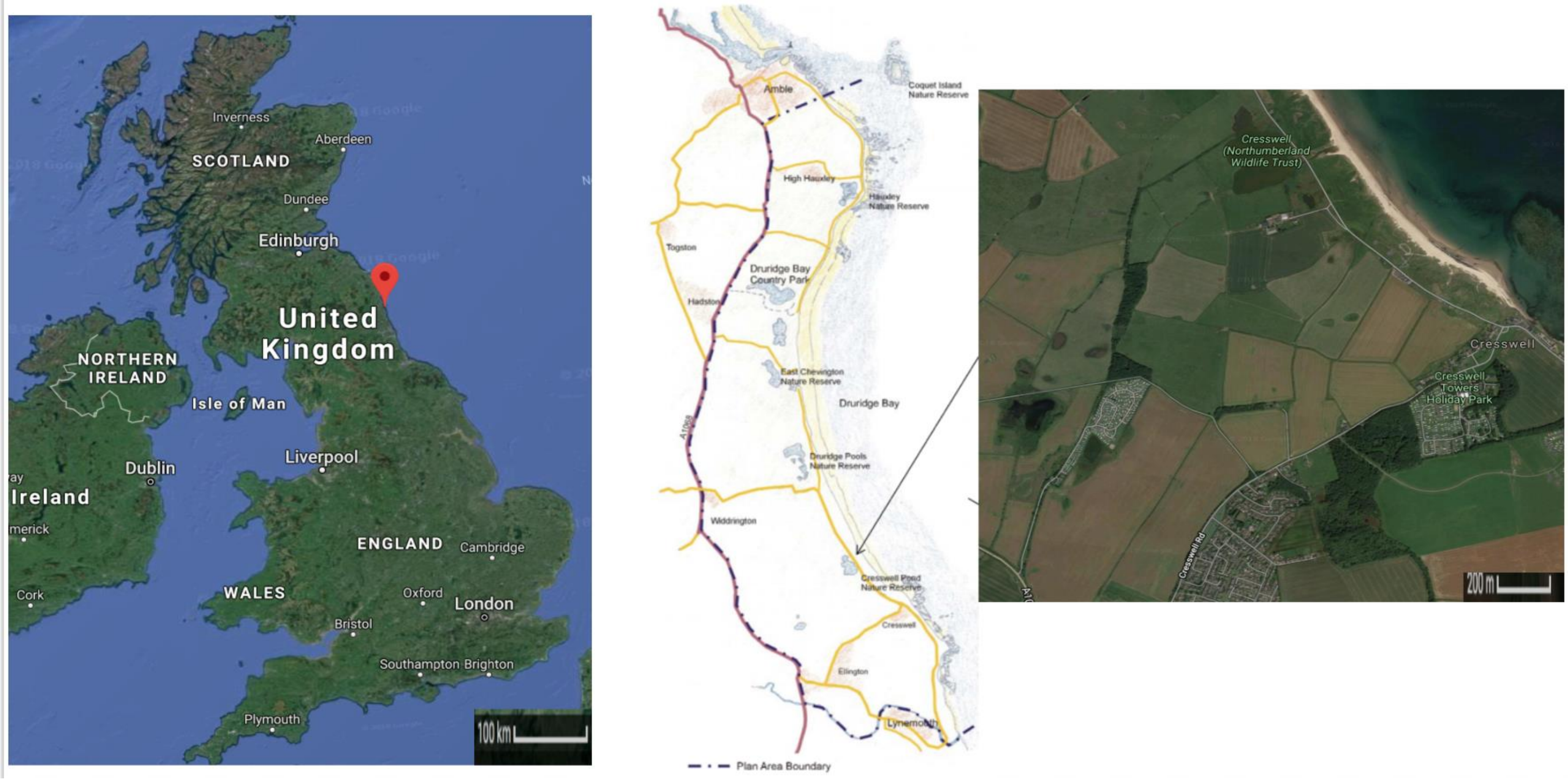

Figure 2.8: Research site location for carbon and microbial analysis at Druridge Bay, Northumberland (on Google ™ Maps

2.8 Why Druridge Bay was chosen as the research site

Druridge Bay was selected for researching the carbon and microbial contents of ponds because of the various pond types present including arable, pasture, sand dune and natural ponds (Figure 2.9). These are the main types of water body features that are found in Northumberland aside lotic systems and wetlands (Gilbert *et al.,* 2014, Groom *et al.,* 2012, Jeffries, 2011 and Lunn, 2004). Although water bodies associated with peatland are dominant in the Cheviots, the general distribution of inland waters are arable or pasture ponds associated with agricultural processes, dune formed ponds or water channels of the Northumberland coastline, and natural ponds formed from coal mine subsidence or geologic depressions (Abraham, 2016, Bird, 2016, Elliott, 2011 and Groom *et al.,* 2012). And these systems are found in Druridge Bay. These water bodies also are of different shapes and sizes (Gilbert *et al.,* 2014). From physical observation of the ponds, arable ponds are found on arable farms with land depressions or subsidence of predominantly clay sediments. The arable ponds have fine sediment that limits water penetration. Pasture ponds are found on livestock farms and usually surrounded by green pasture vegetation; the pond sediments are usually dark and organic rich with little sandy sediment. Natural ponds have even darker rich organic sediments but are formed in natural depressions or subsidence. Sand dune ponds are ever-changing features of the Northumberland coastline. They are made of highly porous sandy sediments which continuously change sand dune pond’s physical structure. These four pond types typify the majority of ponds and adjacent environments found in all NCAs of Northumberland. Other significant water body features found in Northumberland are flood plains and river environments (Bird, 2016, Lunn, 2004 and Oswald *et al.,* 2013), but these systems are lotic in nature.

Druridge Bay was also selected so that the research was in continuation of work done by Gilbert *et al.* (2014), Gilbert (2016), Jeffries (2008), Jeffries (2011), Jeffries (2012), Jeffries (2016) and Taylor (2017 *unpublished*) in developing sub-regional carbon cycle activities in SWBs, and the processes that influence carbon stock in water bodies. These authors also sampled ponds similar to those used in this research. This research also embarks on classifying carbon stocks in ponds based on water body size ranges (10 – 100 sq. m, 100 – 1,000 sq. m and 1,000 – 10,000 sq. m) with each sampled pond falling into one of the sethree size categories (Figure 2.9). The work on regional water body abundance studied all lotic systems in Northumberland with surface area ranging from of 1 sq. m to ~11 sq. km (Kielder Dam). Therefore, understanding carbon cycle and water bodies abundance at sub-regional level will enhance the development of a regional carbon stock associated with inland water bodies, in line with Seekell and Pace (2011), Seekell *et al.* (2014) and Verpoorter *et al.* (2014). Alongside the extensive knowledge of the carbon activities in Druridge Bay by Gilbert *et al.* (2014), Gilbert (2016) and Taylor (2017 *unpublished*), this research site also provided a good baseline for researching the microbial influence of carbon activities in the three water body size ranges of 10 – 100 sq. m, 100 – 1,000 sq. m and 1,000 – 10,000 sq. m (Figure 2.9).

Finally, site access to Druridge Bay water bodies was easier to obtain compared to other area in Northumberland.

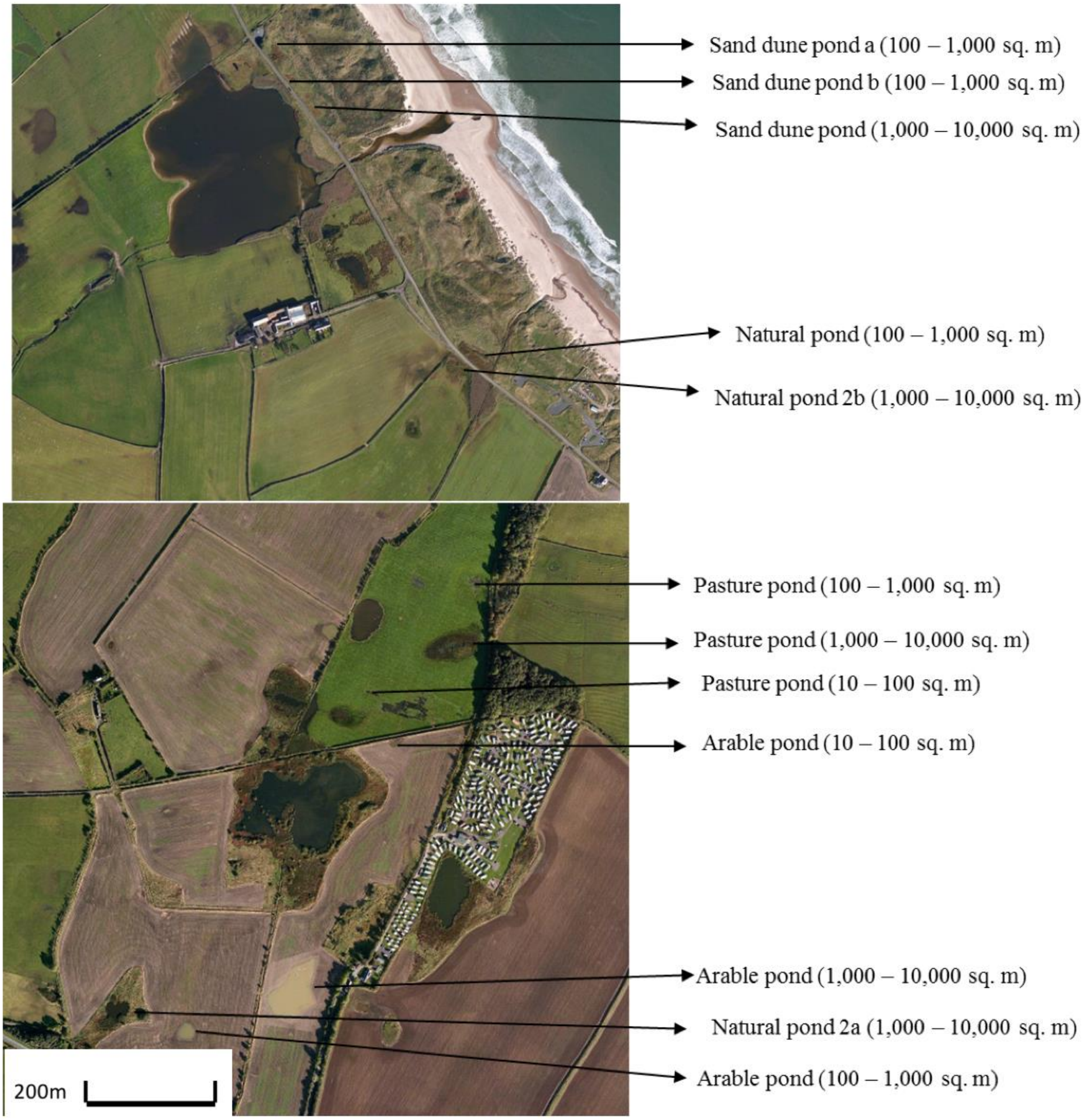

Figure 2.9: Water bodies selected to analyse carbon stock and microbial influence on capture in Northumberland (World Imagery on ArcGIS)

2.9 Chapter Summary

Inland water bodies are diverse in size and they are difficult to globally quantify below 10 sq. km. Numerous models have been developed to quantify SWBs <10 sq. km but they are limited by influence of localised or regional land use, hypsometry and climate. It is important to quantify number and size of SWBs because this further improves knowledge of their contribution to carbon cycle estimates. To combat the negative effects of climate change, it is important to identify all the contributors to atmospheric GHGs and reduce anthropogenic GHG emission sources.

The land carbon cycle sub-compartments widely vary compared to the atmosphere. Amongst the sub-compartments, SWBs are the least understood or researched. They are capable of processing allochthonous and autochthonous carbon but are influenced by physical/environmental, biological and chemical/biochemical factors. These factors determine the source or sink potentials of SWBs. Microbes in SWBs are one of the most important influencers of carbon stocks in ponds Their abundance and diversity in ponds are influenced by similar physical/environmental, biological and chemical/biochemical factors that drive carbon processing. In small ponds, microbial abundances are fostered by the high concentration of bio-elements and they can determine autotrophism, heterotrophism and decomposition levels in water bodies amongst other processes. These and other factors make SWBs more efficient at carbon processing than large lakes and oceans.

Northumberland is characterised by numerous landforms, features and vegetation. Some ponds in coastal Northumberland were developed from mining-related land subsidence and agricultural processes while others exist as a result of landscape. Druridge Bay, Northumberland was selected as the research site for carbon and microbial analysis because of the presence most of the pond types found in Northumberland, previous research done in the area and site access permission.

Chapter 3 - Northumberland Water Body Distribution (NWBD)

3.1 Background

Research into inland water bodies has shown that carbon capture processes are heterogeneous and SWBs have wider importance than being water reservoirs for agriculture, human and animal use (Battin *et al.*, 2009, Cole *et al.*, 2007, Gilbert *et al.*, 2014 and Jeffries, 2012). This research focuses on determining water body abundance and distribution for carbon capture from a regional perspective. The methodology involves combining existing knowledge on water body distribution, digitising water bodies on high resolution satellite imageries at varying scales and comparing them with known databases to estimate Northumberland regional water body distribution.

3.1.1 Mapping water bodies

Section 2.1 covered the global abundance of water bodies, the inconsistency in identifying and estimating SWBs abundance. The section also highlighted the global and regional perspective of estimating water body abundance and identified that inland water bodies are of different shapes and sizes (Downing *et al.*, 2006 and Lehner and Doll, 2004). Northumberland is similar to other regions of the world in that its SWBs are not seen on classical maps or identified accurately using extrapolation techniques. And the factors that influence SWBs abundance vary (Downing *et al.*, 2006 and Seekell and Pace, 2011). Methodologies such as Pareto and regional/sub-regional water body counts have their strengths and weaknesses in estimating water body abundance (as reviewed in Section 2.1) but the reality is water body abundance are in continuous flux due to precipitation and changes in anthropogenic land use (Jeffries, 2016). And these needs to be accounted for.

3.1.2 Identifying Northumberland water body abundance and distribution

Identifying water bodies in Northumberland can either be done through manual or automated algorithms/techniques to retrieve water bodies from remote sensing data (Ryu *et al.*, 2002) or ground survey of water bodies (Jeffries, 2016).

3.1.2.1 Computer-based techniques/approaches

The computer-based approaches include digitising water bodies through visual investigation, edge detection using single or multispectral imageries, water morphological segmentation, spectral transformation and texture analysis (Bagli *et al.*, 2004, Ryu *et al.*, 2002, Verpoorter *et al.*, 2012 and Verpoorter *et al.*, 2014). And the perimeter of earthly features such as water bodies is manually digitised by drawing polygons or identified using algorithms. Some computer-based approaches have algorithms that are supposed to distinguish other earth features from water bodies (Frazier and Page, 2000 and Carroll *et al.*, 2009). But this has been proven to be problematic because optical properties of water vary in space and time and most SWBs are well integrated into the terrestrial environment. Thus, multiple automated algorithms are required to identify SWBs amongst other earth features (Carroll *et al.*, 2009, Jain *et al.*, 2005 and Song and Civco, 2002). There is also a problem of replication over a large area because of the diverseness of water body features and the complications of the techniques. Therefore, automated approaches have only been restricted to a small area or regional scale (Ryu *et al.*, 2002 and Verpoorter *et al.*, 2012). However, Verpoorter *et al.* (2014) utilised automated thresholding, classification, vectorisation and shadow removal from Landsat-7 ETM+ to identify water body abundance. The database was further extrapolated to determine global water body (GLOWABO) abundance down to 0.002 sq. km in surface area. McDonald *et al.* (2012) also utilised the Pareto distribution to extrapolate water body abundance using data from the United States of America National Hydrography Dataset (NHD) and these data had varying resolutions.

Section 2.1 also reviewed the Downing *et al.* (2006) Pareto extrapolation of a global water body database using Lehner and Dolls (2004) GLWD. The strength of the GLWD database is the combination of multiple classical map sources, regional and global databases (at varying resolutions) to generate lakes, reservoirs, wetlands and SWBs abundance. However, the dataset only shows a strong correlation in identifying water bodies >10 sq. km, and there are uncertainties in identifying water bodies <10 sq. km which created an overestimation using the Pareto distribution (Seekell and Pace, 2011 and Seekell *et al.,* 2013).

Diverseness of water body features and multiple techniques are further complicated by imagery quality. But remote sensing of water bodies has been improving from World Atlases to 250 m resolution of Shuttle Radar Topography Mission (SRTM), MODIS 250 (Carroll *et al.,* 2009), Lidar (Ma *et al.,* 2006), MSSL Global Lakes database (MGLD), Global Land Cover Facility water body database, Landsat-7 ETM (Verpoorter *et al.,* 2012) and Landsat-8 (Sheng *et al.,* 2016) amongst others. With each passing satellite imagery dataset over the years, there have been improvements in imagery resolutions. Landsat-8 carries an Operational Land Imager (OLI) sensor with 30 m resolution that provides multi-temporal and multispectral imagery for identifying earth's features (Roy *et al.,* 2014), with global coverage. It has a higher resolution compared to Terra/Aqua MODIS which has 0.25 – 1 km resolution (McCullough *et al.,* 2012), and NOAA/AVHRR (Natural Oceanographic and Atmospheric Administration Advanced High Resolution Radiometer) which has ~1 km resolution (Bryant, 1999).

Landsat-8 provides an alternate approach to identifying water bodies if an automated approach is applied but it is classed as coarse compared to Google ™ Map and World

Imagery on ArcGIS. In identifying water bodies on Landsat-8, multiple band combinations are used to define water body features. Landsat-8 multispectral imageries can identify water bodies in natural colour, land/water interface and infrared (Loveland and Irons, 2016 and Roy *et al.,* 2014). The limitation of Landsat-8 and other temporal satellite imagery sources are shadow cast, cloud cover and vegetation cover and inability to distinguish diverse water surfaces (Sheng *et al.,* 2016). But water body identification can be improved by specific spectral indices or ratioing which eliminate dark shadows by dividing one image band with another (Xu, 2006). Also, Normalised Difference Water Index (NDWI) and Modified Normalised Difference Water Index (MNDWI) (Xu, 2006) are models that identify the water body's spectral signature on land (Liao *et al.,* 2014) using Landsat-based surface reflectance at 30m resolution (Feng *et al.,* 2016). This is achieved by creating an equation that combines different bands (Sarp and Ozcelik, 2016). Lidar and high resolution UAV imagery have been adopted in identifying earth features such as large lakes, vegetation cover, glaciers and rock fall (Ma *et al.,* 2006 and Turner *et al.,* 2015). A similar approach could be used in identifying SWBs. UAVs have been found to identify earth features at resolutions up to 0.02 m and can be set up for spatial and temporal imaging at fine details. But airplane based Lidar and UAV imagery are greatly limited by coverage area and set up cost factor. A more realistic and cost effective approach would be using Landsat-8, Google ™ Map and World Imagery on ArcGIS which have regional and global coverage and are publicly available data. Google ™ Map and World Imagery on ArcGIS stitch the best imagery quality from multiple dates to avoid of cloud cover. While Landsat-8 provides multi-temporal identification of water bodies which cannot be obtained in stitched imageries (Loveland and Irons, 2016, Lu *et al.,* 2011 and Roy *et al.,* 2014). Thereafter, sub-regional Lidar and UAVs data could be used to update pre-existing databases at sub-regional levels. These new developments could improve the level of details processed.

Also, it would be interesting to compare the GLWD (Lehner and Doll, 2004), which is one the most widely acceptable database, with current high resolution imagery maps for Northumberland in determining its regional water body abundance.

3.1.2.2 Ground Survey Approach

In Northumberland, ground surveys have been applied in ecology to determine wildlife and ecological interactions (Jeffries, 1998, Jeffries, 2005 and Jeffries, 2008), land use management and classical map survey (Elliott, 2011, Jeffries, 2008, Lunn, 2004 and Waddington, 2010). Identifying water body abundance and surface area flux is only a recent area of the ground survey study. Jeffries (2016) showed that there is a relationship between regional precipitation levels and SWBs abundance in Druridge Bay, Northumberland and this had implications for carbon capture potentials. Like Lidar and UAV, ground surveying is limited to sub-regional/regional coverage. Jeffries (2016) was only able to survey ~1 sq. km of Druridge Bay, Northumberland compared to a Northumberland regional area of ~5,000 sq. km.

3.1.3 Northumberland Landscape and water body distribution

Section 2.7 reviewed the Northumberland geology, habitats and influence of coal mining subsidence in creating ponds (Jeffries, 2008 and Waddington, 2010). The general topography of Northumberland is an Upland West, lowland East towards the North Sea coast and transitional landscapes in between (Figure 3.1). Northumberland elevation ranges from Cheviots (815m) to Wool Meath (652m) to the North Pennines being around 300m and Kielder Moor generally around 200 m, and down to the coast at sea level. The landscape is cut by river valleys that drain eastwards to the North Sea. The valleys drain into the Rivers Till, Tweed, Tyne and their tributaries (Charlton *et al.*, 2003, Lunn, 2004, Passmore and Waddington, 2009 and Waddington, 2010). The soils of Northumberland are dominated by glacial till and drift derived soil types and they cover most of the farm land which are seasonally waterlogged loamy and clay soils. Some areas of Northumberland have peat-like soil, flood plains and eroded soils (Gilbert *et al.,* 2014; Gilbert, 2016; Jeffries, 1998; Jeffries, 2008; Lunn, 2004; Taylor, 2017 *unpublished*; and Waddington, 2010).

Usually, water migrates from higher land elevations to the lowlands with its end points being inland water bodies, rivers and oceans (Calder, 1996 and Fan *et al.*, 2013). SWBs collect rainfall/snow and vary in capacity, shape and elevation. Upon SWBs saturation, water flows (water shedding) to larger lakes and streams through soils and these alter volume transfer and water chemistry (Boix *et al.,* 2012, Céréghino *et al.,* 2013, Fritz, 1996 Jeffries, 2016 and Oertli *et al.,* 2002). Also, the fluctuations and variability of these processes through time is a significant show of lakes and SWBs response to short/long term climate change respectively. The general water shedding pattern in Northumberland are from the western upland and undulating hills towards the eastern lowland soils and river plains (Wilkinson *et al.,* 2010). However, it is unclear if this causes more abundant water bodies in the lowland area compared to the upland. This is because precipitation, evaporation, landscape, land use, water transport and soil type are factors that influence the seasonal distribution of water bodies but it is expected that more water bodies are found on flat lands irrespective of land elevation compared to undulating or sloping land.

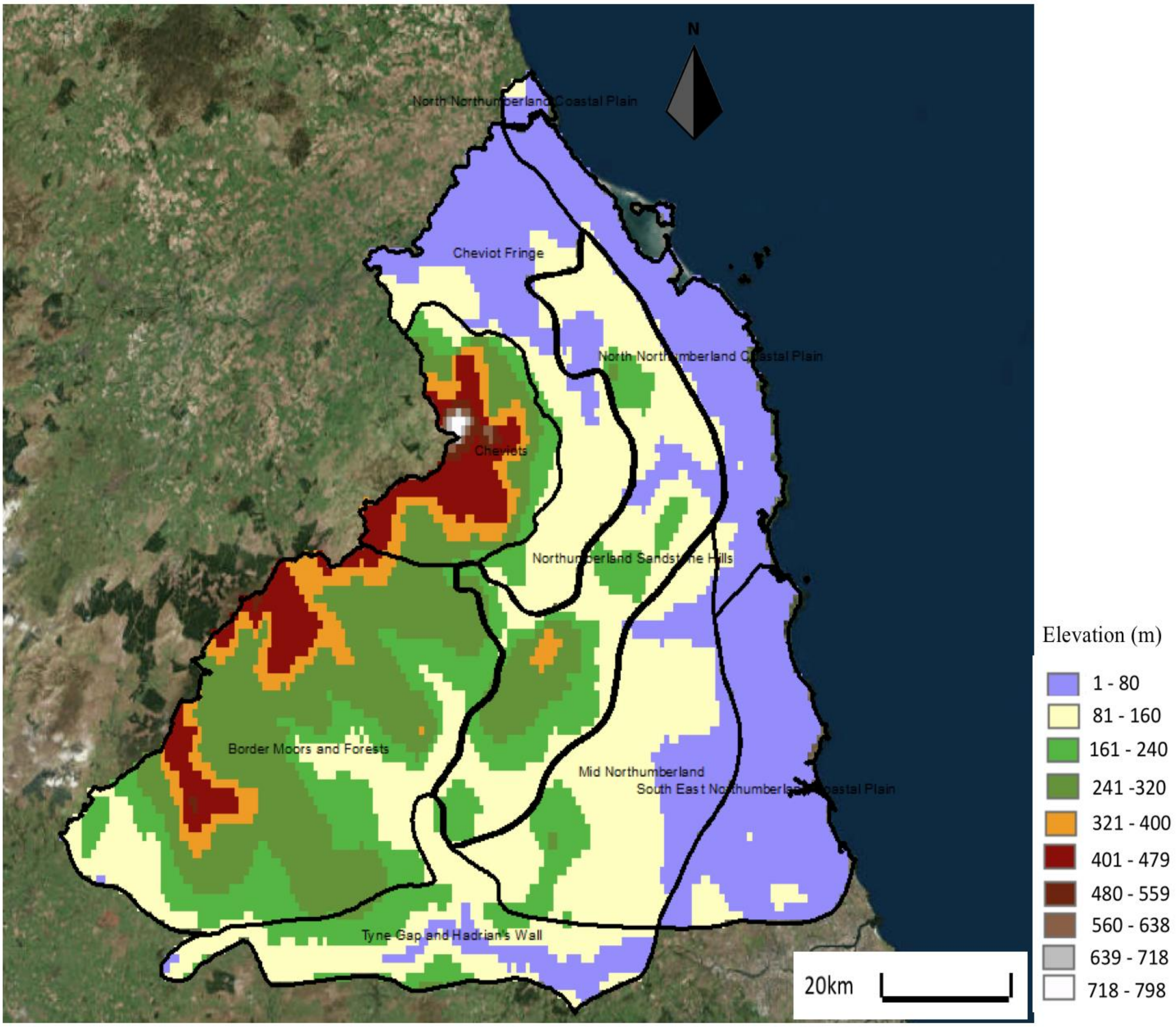

Figure 3.1: Northumberland elevation (DEM) showing upland West and lowland East varying elevations in between (World Imagery)

3.2.1 Research Aim

The aim of the research in this chapter is to quantify the number and surface area of water bodies in Northumberland by:

- Mapping water bodies using two types of imagery
- Analysing the effect of mapping scales in identification of water bodies
- Assessing the seasonal variability of number and surface area of water bodies
- Investigating different approaches to upscaling number and surface area of water bodies

The results of this research are later used in estimating the regional carbon stock in Northumberland in Chapter 6.

3.2.2 Research Questions

1. a. What is the total number and surface area of water bodies in Northumberland at 1:20,000 scale?

   b. What is the upscaled number and surface area of water bodies in Northumberland at 1:2,000 scale?
2. How does number and surface area of water bodies vary based on mapping scale and imagery type?
3. What are the effect of landscape and seasonal variations in number and surface area of water bodies?
4. What are the differences in water body metrics between coastal and inland and between National Character Areas (NCAs) of Northumberland?
5. How effective is the satellite imagery approach to mapping water bodies?

3.3 Method

3.3.1 Imagery Analysis

Northumberland water body distribution (NWBD) was analysed by manually drawing polygons around water bodies identified by eye on Landsat-8 and World Imagery for Northumberland at two scales using ArcGIS 10.0, with the exclusion of lotic systems (rivers, streams and their tributaries). The perimeter of water bodies identified in Northumberland was manually digitised at 1:2,000 scale (World Imagery) and 1:20,000 scale (World Imagery and Landsat-8). The scales were often adjusted to accommodate and improve details during digitising. Water body abundance was represented in logarithmic intervals or size ranges starting from 10 – 100 sq. m. Results were compared to the GLWD extract for North East, England.

World Imagery with higher resolution (0.3 m) allowed for detailed digitising of water bodies but the imagery provided no information on temporal changes because it is a stitch of different imageries collected at different dates. Landsat-8 data with resolution 15 – 30 m provided temporal changes with data collected from 2013 – 2015 on days with <20% cloud cover (Table 3.1). Landsat-8 imageries were downloaded for July and September 2013, July and December 2015, and April 2015. The Landsat-8 imageries were displayed in ArcGIS 10.0 as natural colour (4-3-2), infrared (5-4-3) and land/water (5-6-4) band combinations. The imageries were further pan-sharpened using the Panchromatic Band-8 but this had little effect on water body visibility. World Imagery was void of cloud cover but, as with Landsat-8 imagery, it had vegetation cover and shadow cast that hindered water body identification.

The GLWD comparison was developed by using a map outline of North East, England to extract water bodies within the North East of England.

Table 3.1: Maps and Imagery features used for identifying inland water bodies of Northumberland

Imagery Feature	*Map*		
	Landsat-8	World Imagery	World Imagery
Scale	1:20,000	1:20,000	1:2,000
Resolution	Low Resolution	High Resolution	High Resolution
Cloud Cover (%)	<20	0	0
Vegetation Cover and shadow cast	Present	present	present
Imagery Date	2013 - 2015	Various dates combined into one stitched image	
Northumberland (Area Analysed)	All	All	Selected NCAs

3.3.2 Northumberland National Character Areas (NCA)

For ease of digitising water bodies, Northumberland's National Character Areas (NCA) were reclassified from eight to six. The reclassification was done based on NCAs with similar vegetation, elevation, landscape, geo and biodiversity (Natural England, No Date). The reclassification combined Cheviots with Cheviots Fringes and Mid Northumberland with Northumberland Sandstone Hills. The other NCAs are South East Northumberland Coastal Plain, North Northumberland Coastal Plain, Tyne Gap and Hadrian Wall and Border Moors and Forests (Table 3.2 and Figure 3.2).

World Imagery and Landsat-8 were displayed at 1:20,000 scale to digitise all NWBD. Then, at 1:2,000 scale to digitise water bodies in National Character Area (NCA) test areas and this was upscaled using three different approaches to determine extrapolated NWBD.

Approach 1: Northumberland is assumed to have a uniform distribution of water bodies based on the hypothesis that Northumberland has uniform vegetation and elevations in all NCAs.

Approach 2 - Northumberland is divided into two zones - Coastal Plain and Border Upland. And assumed that these elevation zones have different distributions of water bodies, due to geological, vegetation and land use differences. The Border Upland (BU) includes Tyne Gap and Hadrian Wall, Border Moors and Forests, Cheviots/Cheviots Fringes and Mid Northumberland/Northumberland Sandstone Hills, and the Coastal Plain (CU) are South East Northumberland Coastal Plain and North Northumberland Coastal Plain (Figure 3.2).

Approach 3: Distribution of water bodies is upscaled for each of the six reclassified National Character Areas (NCA)

Table 3.2: Northumberland NCA Classification and Test Area

NCA	*NCA size (X 10^6 sq. m)*	*Test Area (X 10^6 sq. m)*	*Percentage test area (%)*
South East Northumberland Coastal Plain	437	40	9.15
Mid Northumberland *Northumberland Sandstone Hills*	637 726	50	3.67
Border Moors and Forests	1271	33	2.63
Cheviots *Cheviot Fringe*	364 515	45	5.11
Tyne Gap and Hadrian's Wall	434	37	8.64
North Northumberland Coastal Plain	376	22	5.84
Northumberland Area	4760	227	4.78

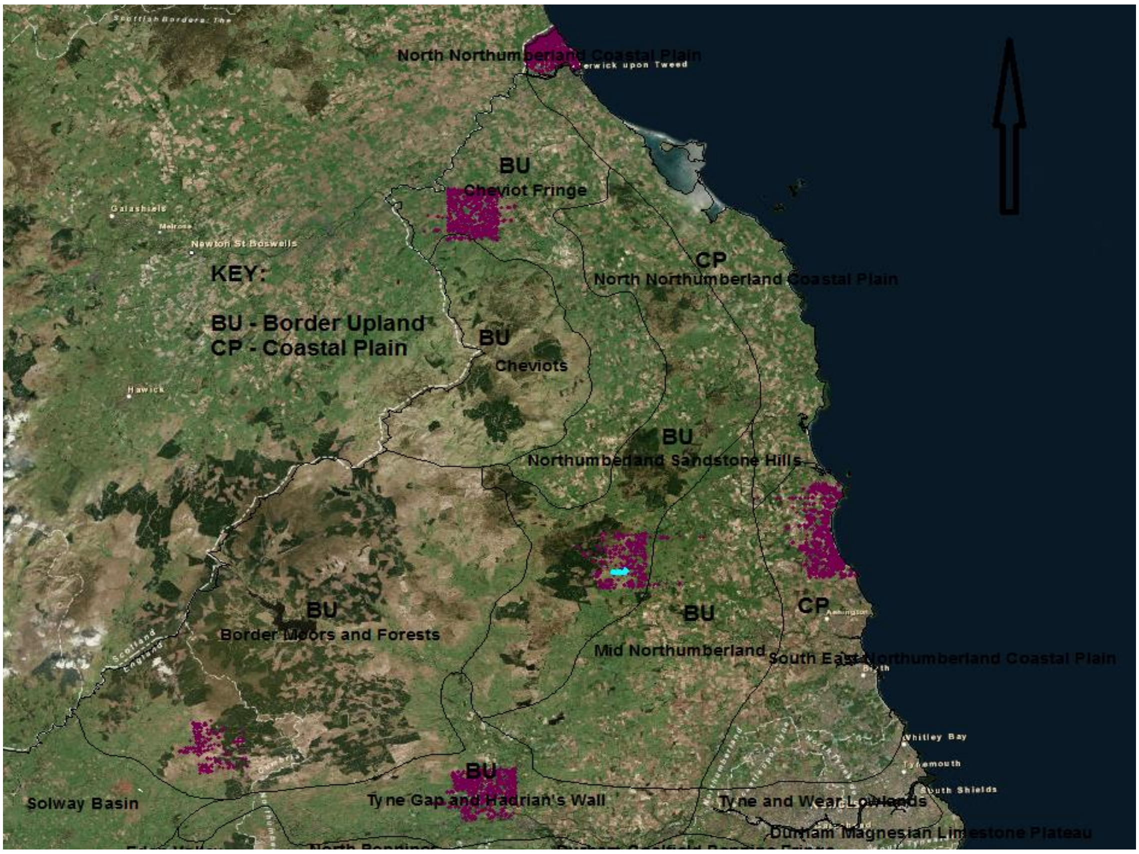

Figure 3.2: Northumberland NCA and test area for 1:2,000 scale water body distribution (World Imagery)

3.3.3 Rainfall and Temperature

Rainfall and temperature were analysed as climatic factors that control water body distribution in Northumberland. These climatic factors were assessed from July 2013 to April 2015 using data from the Met Office UK (Accessed – August 2015). The dataset includes monthly, seasonal and annual rainfall, temperature and sunshine. The climate data were compared with water body size and abundance digitised from the Landsat-8 image collected at the same time.

3.3.4 Accuracy Assessment

Accuracy assessment was carried out in six NCAs by counting number of water bodies in six ~1 sq. km sites in fine detail from World Imagery and comparing the results with a reference map (Google ™ Maps). The accuracy assessment of the South-East

Northumberland NCA, that is the number of water bodies identified in the World Imagery and Google ™ maps in Blakemoor Farms, Druridge, were further compared with ground survey data collected by Jeffries (2016) for the same site. Jeffries (2016) collected water body abundance data from November 2010 to November 2013 for the purpose of identifying the temporal variations in SWBs at Blakemoor farms (Figure 3.3). In this research, Jeffries (2016) assessment was used to approximate upper and lower limits of water body abundance based on the influence of precipitation.

Figure 3.3: Position of Druridge Bay, South East Northumberland, B is the Blakemoor site and “NR” labelled Nature Reserves sites between Amble and Cresswell, Northumberland (Jeffries, 2016).

3.4 Results

3.4.1 Accuracy of water body digitisation

Number of water bodies in six ~1 sq. km sites in the Northumberland NCAs varied identified on World Imagery and Google™ map. On World Imagery, the general trend showed an increase in number of water bodies with decreasing size range with more water bodies found in size range 10 – 100 sq. m compared to other size ranges except in Tyne Gap and Hadrian's Wall and South East Northumberland NCAs (Figure 3.4 and Figure 3.5). On Google Map, the number of water bodies identified was fewer compared to World Imagery and the distribution varied in each NCA. Mid Northumberland / Northumberland Sandstone Hills and North Northumberland NCA showed a decrease in number of water bodies with increasing surface area (Figure 3.4). There were no significant differences in number of water bodies identified on World Imagery and Google Map in all accuracy assessment sites of ~1 sq. km, as detailed in Table 3.3

Table 3.3: Test of difference in accuracy of water body digitisation on World Imagery and Google Map

NCA	*Wilcoxon Signed Rank Test Value*	*N*	*P*
South East Northumberland Coastal Plain	0.000	4	0.317
Mid Northumberland / Northumberland Sandstone Hills	2.000	4	0.655
Border Moors and Forests	2.000	4	0.593
Cheviots / Cheviot Fringe	0.000	4	0.109
Tyne Gap and Hadrian's Wall	2.000	4	0.655
North Northumberland Coastal Plain	0.000	4	0.157

Jeffries (2016) showed that there was variation over time in the number of water bodies in Blakemoor Farm Druridge Bay, South-East Northumberland. In the period of low precipitation, Jeffries' water body counts were less than World Imagery and Google™

map counts (Figure 3.5). During high precipitation, Jeffries' water body counts were higher. Therefore, in comparison to Jeffries (2016), this research seems to be representing an average number of water bodies between the wet and dry phase of Jeffries (2016). Also, Jeffries (2016) identified water bodies in size range of 1 – 10 sq. m which are not easily identified using satellite imagery.

There were no significant differences in the number of water bodies on World Imagery for South East Northumberland NCA and number of water bodies in Jeffries (2016) high rainfall (Wilcoxon Signed Rank Test Value = 6.000, N= 4, P = 0.109 and Jeffries (2016) low rainfall (Wilcoxon Signed Rank Test Value = 0.000, N = 4, P = 0.109, Pearson Correlation = 0.969). Also, there was strong positive correlation between World imagery and Jeffries (2016) (High rainfall: Pearson Correlation = 0.939 and Low rainfall: Pearson Correlation = 0.969).

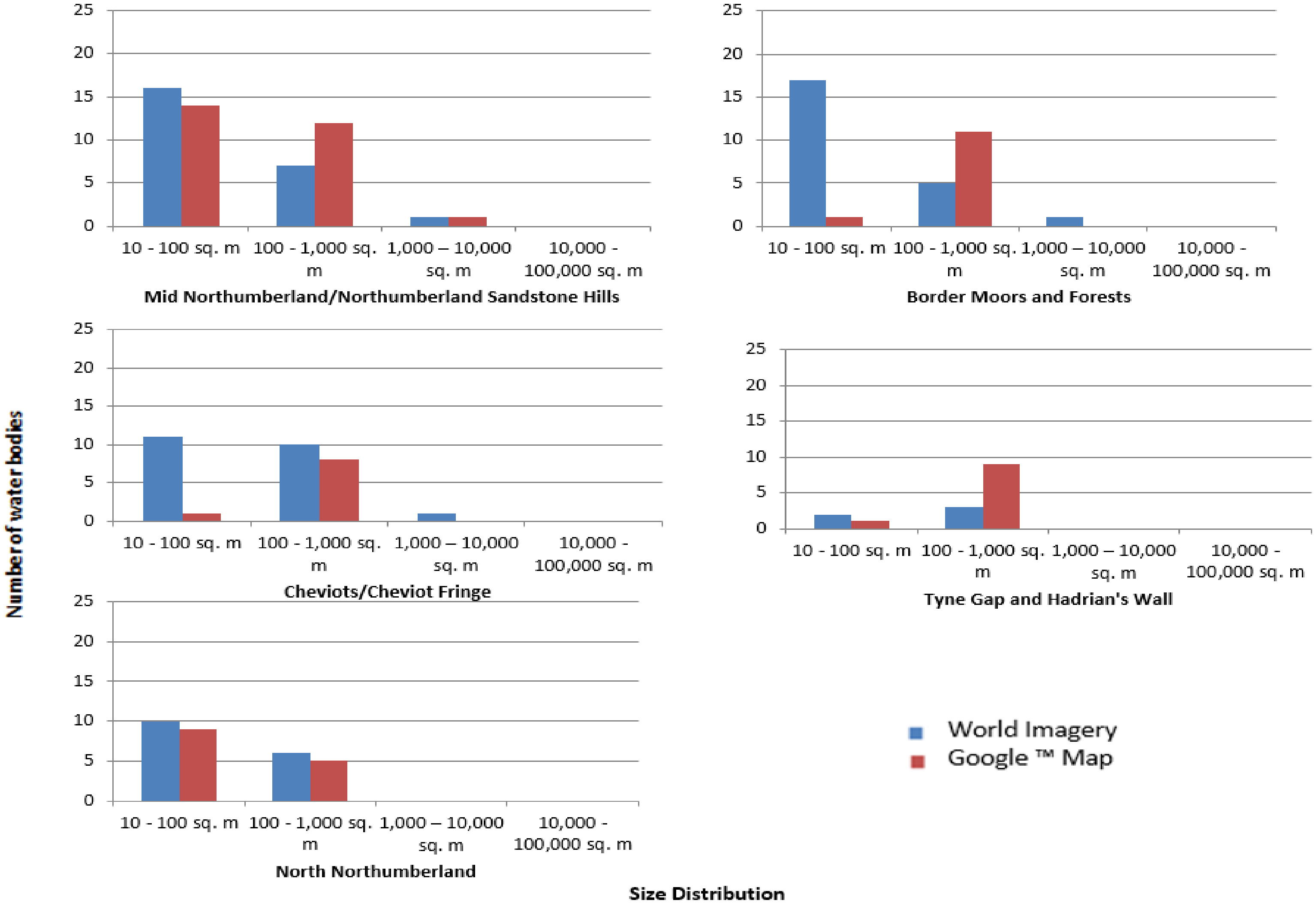

Figure 3.4: Accuracy assessment of water bodies' count in Northumberland comparing World Imagery and Google ™ maps

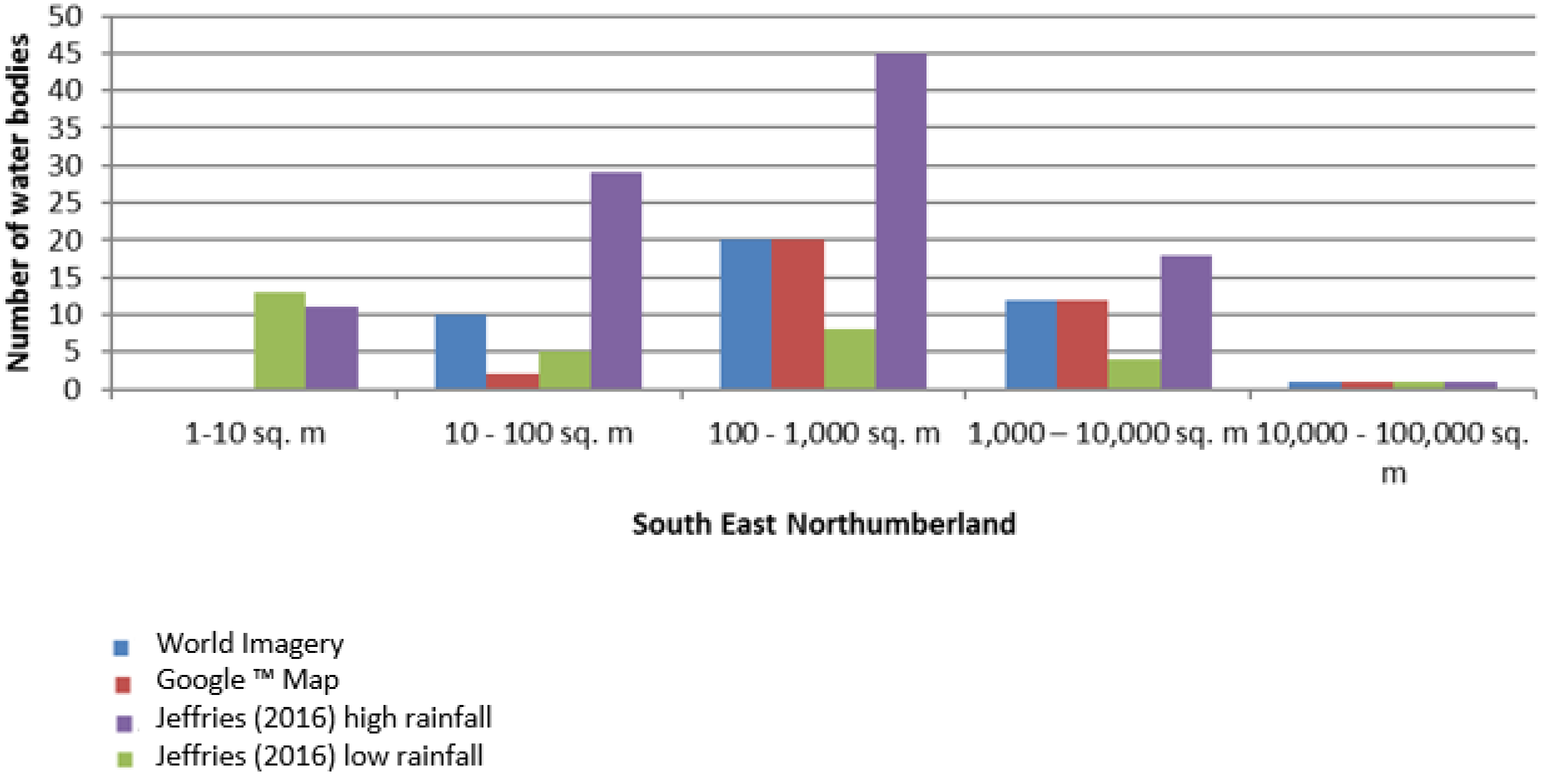

Figure 3.5: Comparison between Jeffries (2016) and satellite imagery counts of water bodies

3.4.2 Imagery Analysis

3.4.2.1 Northumberland Water Body Distribution (NWBD) using Landsat- 8 multi-temporal imagery at 1:20,000 scale

Across all of Northumberland mapped at 1:20,000 scale, only one water body was >10 sq. km but its surface area varied (Figure 3.6a & b) owing to seasonal change. The range 10,000 – 100,000 sq. m accounted for the most number of water bodies in all dates analysed (Table 3.5). There were more water body counts in spring of April 2015 and summer of July 2013 (Figure 3.6a & b) compared to other dates of analysis with July 2013 accounting for 1144 water bodies. The distribution showed fewer water body counts from range 100 - 1000 sq. m at 1:20,000 scale with no water bodies identified below 100 sq. m. Despite more water bodies counted in spring and summer, water bodies surface area was larger and expanded in the winter time of December 2014. This covered over 93.7 sq. km (Table 3.5) compared to July 2013 which covered 30.1 sq. km. The increased surface area in December 2014 is a function of expansion of surface area in ranges 10,000 – 100,000 sq. m from 262 counts in September 2013 to 495 (in December 2014).

Also, more water bodies were seen in summer due to reduced cloud cover. Winter time contains large or expanded water body areas that are often covered in cloud, as seen from December 2014 to April 2015 where ranges 0.1– 10 sq. km reduced in number and surface area but number and surface of area in SWBs in ranges 0.001 – 0.1 sq. km increased (Table 3.5). Similarly, there was a reduction in the overall surface area on this date by over 50% from 93.7 sq. km to 45.2 sq. km. Despite the temporal change influence by seasonality, there were no significant differences in water body abundance and changes in surface area of water bodies as shown in Table 3.4.

During the period of analysing water body abundance, seasonal rainfall in Northumberland ranged from ~150 mm to ~280 mm with winter of 2014 showing the highest rainfall. Summer and spring of 2013 and 2015 respectively had the lowest rainfalls (Figure 3.7a). Temperature was highest in summer 2013 and lowest in winter 2014 (Figure 3.7b). However, there was no clear relationship between water body abundance and weather parameters (rainfall and temperature). Typically, with increasing temperature and decreasing rainfall from winter to spring, the sizes and number of water bodies reduce.

Table 3.4: Test of difference in multi-temporal distribution of water bodies in Northumberland using Landsat-8 at 1:20,000 scale (mean with standard error), where N = 6

Landsat-8: 1:20,000 scale	*Surface Area*	
	P- value	Wilcoxon Signed Rank Test Value
Jul-13 to Sep-13	0.917	11.000
Sep-13 to Jul-14	0.116	18.000
Jul-14 to Dec-14	0.249	5.000
Dec-14 to Apr-15	0.249	5.000
	Number of water bodies	
	P- value	Wilcoxon Signed Rank Test Value
Jul-13 to Sep-13	0.345	4.000
Sep-13 to Jul-14	0.345	11.000
Jul-14 to Dec-14	0.686	6.000
Dec-14 to Apr-15	0.893	7.000

Table 3.5: Multi-temporal distribution of water bodies in Northumberland using Landsat-8 at 1:20,000 scale (mean with standard error)

Landsat-8: 1:20,000 scale	*Surface Area (X 10^6 sq. m)*					
RANGES	Jul-13	Sep-13	Jul-14	Dec-14	Apr-15	**Mean Area**
100 - 1,000 sq. m	0.015	0.007	0.003	0.004	0.001	**0.01 ± 0.00**
1,000 – 10,000 sq. m	3.420	0.783	0.354	0.260	1.113	**1.15 ± 0.55**
10,000 - 100,000 sq. m	11.170	7.802	18.136	21.525	17.777	**15.28 ± 2.51**
100,000 - 1,000,000 sq. m	3.730	4.328	55.002	40.010	10.379	**22.69 ± 10.45**
1 – 10 sq. km	0	3.756	43.749	19.438	2.556	**13.90 ± 8.21**
10 – 100 sq. km	11.970	12.322	13.905	12.429	13.392	**12.80 ± 0.36**
Total Surface Area	30.290	28.999	131.151	93.669	45.218	**65.83 ± 20.12**
	Number of water bodies					
RANGES	Jul-13	Sep-13	Jul-14	Dec-14	Apr-15	**Mean Number**
100 - 1,000 sq. m	21	10	5	8	1	**9 ± 3**
1,000 – 10,000 sq. m	586	146	58	40	169	**200 ± 99**
10,000 - 100,000 sq. m	526	262	431	495	605	**464 ± 58**
100,000 - 1,000,000 sq. m	10	22	218	183	48	**96 ± 43**
1 – 10 sq. km	0	3	18	8	2	**6 ± 3**
10 – 100 sq. km	1	1	1	1	1	**1 ± 0**
Total number bodies	1144	444	731	735	826	**776**

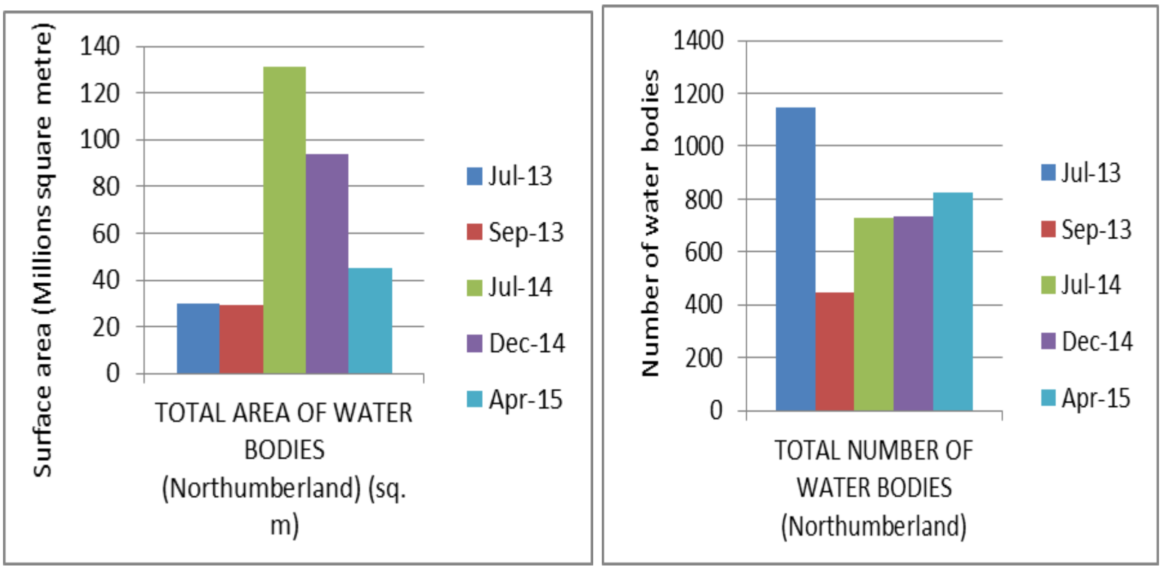

Figure 3.6a & b: Low resolution Distribution (a) area (b) number of water bodies in Northumberland (temporal distribution) at 1:20,000 scale

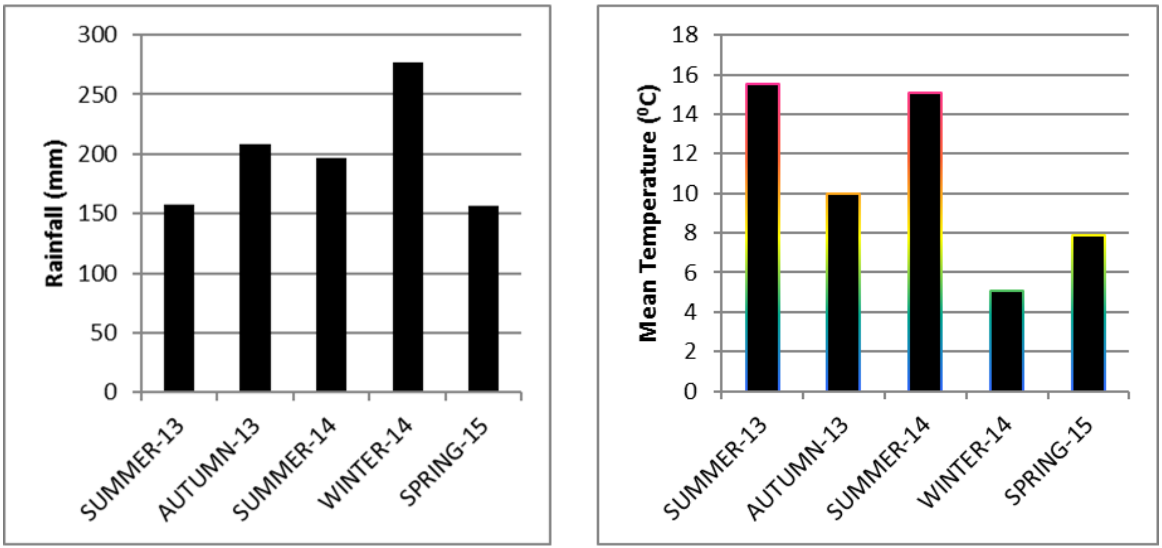

Figure 3.7a & b: Rainfall levels (mm) and Mean temperature (^{0}C) in East and North East England

3.4.2.2 Northumberland Water Body Distribution using World Imagery at 1:20,000 scale

Mapping water bodies using World Imagery allowed for digitising more water bodies down to size range 10 – 100 sq. m. There were more water bodies digitised at size range 100 – 1,000 sq. m (361) and 1,000 – 10,000 sq. m (832) (Table 3.6) compared to Landsat-8 multi-temporal average (Table 3.5). The distribution of water bodies at this scale also showed one water body >10 sq. km and two water bodies were identified in size range 1

– 10 sq. km. Using World Imagery at 1:20,000 scale, the total number of water bodies counted was 1395 (Table 3.6) which covered a total surface area of over 26 sq. km of the entire Northumberland. The most water body counts of 832 were identified in size range 1,000 – 10,000 sq. m (Figure 3.8a & b). Despite identifying water bodies down to 10 sq. m, there was a sharp reduction in surface area <1,000 sq. m and imagery analysis at 1:20,000 scale reduced what is identified as water bodies, causing the negligible size-distribution at below 100 sq. m (Figure 3.8a & b).

At 1:20,000 scale, there were more water bodies counted on World Imagery compared to Landsat-8 on average. The average surface area was higher on Landsat-8 (65.83 ± 20.12 sq. km) compared to World Imagery (26.243 sq. km) (Table 3.5 and 3.6) as well as in each size ranges (Figure 3.8). Test of difference analysis showed that there was no significant difference in World Imagery (average) and Landsat-8 (Surface area: Wilcoxon Signed Rank Test Value = 17.000, N= 6, P = 0.173, and number of water bodies: Wilcoxon Signed Rank Test Value = 6.000, N= 6, P = 0.686)

Table 3.6: Distribution of water bodies in Northumberland using World Imagery at 1:20,000 scale

RANGES (sq. m)	*Surface Area (X 10^6 sq. m)*	*Number of water bodies*
1 -10 sq. m		
10 - 100 sq. m	0.00	4
100 - 1,000 sq. m	0.21	361
1,000 – 10,000 sq. m	2.92	832
10,000 - 100,000 sq. m	4.81	178
100,000 - 1,000,000 sq. m	4.69	17
1 – 10 sq. km	2.15	2
10 – 100 sq. km	11.47	1
Total	**26.24**	**1395**

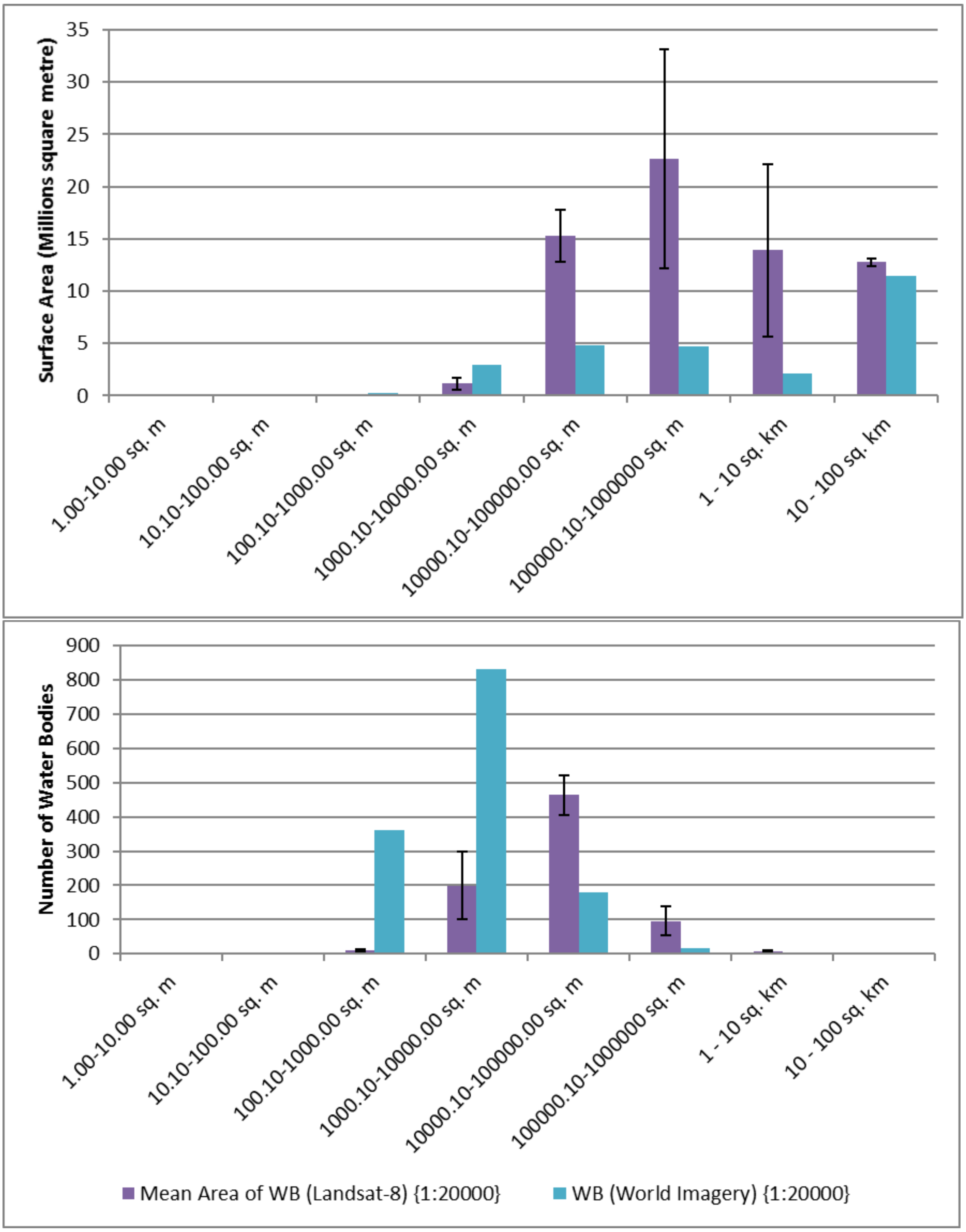

Figure 3.8a & b: High resolution distribution (a) area (b) number of water bodies in Northumberland using World Imagery

3.4.2.3 Northumberland Water Body Distribution (NWBD) using World Imagery at 1:2,000 scale (Test Area)

To identify water bodies <100 sq. m, viewing scale was adjusted from 1:20,000 to 1:2,000. Water bodies' count was 4202 with sizes ranging from 1 sq. m to 1 sq. km. The sum of the test area amounted to 4.78% of Northumberland and more water bodies were identified in the 1 – 1000 sq. m size range compared to 1:20,000 scale. The distribution did not follow Pareto or any other distribution pattern in all NCAs. Inland uplands of Cheviots and Border Moors and Forest (with higher elevations/undulating hills) had fewer counts compared to lowland areas (North Northumberland and South East Northumberland Coastal Plains). At 1:2,000 scale, total surface area of water bodies in size range 1 – 10 sq. m were <1000 sq. m (Table 3.7).

Table 3.7: Distribution of water bodies in Northumberland using World Imagery at 1:2,000 scale

RANGES (sq. m) *1:2,000 scale*	*Surface Area (X 10^6 sq. m)*	*Number of water bodies*
1 - 10 sq. m	0.001	152
10 - 100 sq. m	0.094	2016
100 - 1,000 sq. m	0.517	1693
1,000 – 10,000 sq. m	0.748	303
10,000 - 100,000 sq. m	0.889	26
100,000 - 1,000,000 sq. m	2.323	12
Total	**4.572**	**4202**

3.4.3 Upscaling approaches for estimating water bodies at World Imagery 1:2,000 scale

3.4.3.1 Approach 1: Entire Northumberland

Approach 1 summed the number and surface area of water bodies in all NCA test areas (which was 4.78% of the entire Northumberland area (Table 3.2)) and extrapolated it across Northumberland to determine NWBD, given the assumptions of uniformity in Northumberland vegetation and elevations in all NCAs. Thus, distributions were like the test area with negligible sizes and area below 10 sq. m. (Figure 3.9a & b). The estimate had no error values.

3.4.3.2 Approach 2: Coastal Plain and Border Upland

Approach 2 involved reclassifying Northumberland into Coastal Plain and Border Upland. The sum of water bodies (number and surface area) in South East Northumberland Coastal Plain and North Northumberland Coastal were extrapolated to determine number and surface area of water bodies in Coastal Plain. Similarly, the sum (number and surface area) of water bodies in Tyne Gap and Hadrian Wall, Border Moors and Forests, Cheviots/Cheviots Fringes and Mid Northumberland/Northumberland Sandstone Hills were extrapolated to determine number and surface area of water bodies in Border Upland. The upscaled water body abundance covered over 30.447 sq. km of Coastal Plain (813 sq. km) with more water bodies in range 100 – 1,000 sq. m and 10 – 100 sq. m compared to sizes >1,000 sq. m and no water bodies >100,000 sq. m. The Border Upland (3,950 sq. km) contained 52.268 sq. km of water bodies between 1 sq. m – 1 sq. km, with the lowest count in range 100,000 – 1,000,000 sq. m (Table 3.8).

Coastal Plain and Border Uplands upscale accounted for 39,138 ± 2062 water bodies in range 10 – 100 sq. m and 34,139 ± 1654 in range 100 – 1000 sq. m., while ranges 100,000 – 1,000,000 sq. m and 10,000 – 100,000 sq. m contained water body surface area >29 million sq. m respectively. Despite containing the largest water body counts, ranges 10 – 100 sq. m and 100 – 1,000 sq. m totalled around 1.92 million and 8.10 million respectively. Overall, there was a gradual increase in surface area from range 1- 10 sq. m to 100,000 – 1,000,000 sq. m in approach 2 with a corresponding inverse relationship in water body counts (except in range 1 - 10 sq. m) (Figure 3.9a & b). Standard error was based on variations in respective NCA test areas that were covered within Coastal Plain and Border Upland.

Table 3.8: Coastal Plain and Border Upland distribution of water bodies in Northumberland using World Imagery at 1:2,000 scale

	2 Coastal Plain		*4 Border Uplands*	
RANGES (sq. m) *1:2,000 scale*	Surface Area (x 10^6 sq. m)	Number of water bodies	Surface Area (x 10^6 sq. m)	Number of water bodies
1-10 sq. m	0.00 ± 0.00	228 ± 45	0.01 ± 0.00	1,953 ± 203
10-100 sq. m	0.28 ± 0.02	5,267 ± 44	1.63 ± 0.13	33,872 ± 1,620
100 - 1,000 sq. m	2.85 ± 0.23	8,513 ± 59	5.23 ± 0.48	25,625 ± 1,069
1,000 – 10,000 sq. m	6.62 ± 0.98	2,410 ± 32	7.29 ± 0.67	5,346 ± 334
10,000 - 100,000 sq. m	20.69 ± 2.87	266 ± 35	8.48 ± 1.38	546 ± 91
100,000 - 1,000,000 sq. m	0	0	29.62 ± 8.38	74 ± 21
Total	30.45	16,684	52.27	67,417

3.4.3.3 Approach 3: National Character Area (NCA) distribution of water bodies

Approach 3 involved extrapolating number and surface area of water bodies from NCA test areas to their respective NCA area. All NCAs contained water bodies ranging from 1 sq. m – 10,000 sq. m, but water bodies >10,000 sq. m could only be extrapolated in South East and North Northumberland Coastal Plain, Mid Northumberland and Northumberland Sandstone Hills. Approach 3 showed an inverse relationship between number and surface area of water bodies (except in size range 1 – 10 sq. m) (Table 3.10 & Figure 3.9). Standard error was based on variations in respective NCA test areas in Northumberland. There were no significant differences in the test of differences between South East Northumberland Coastal Plain Water body distribution against other NCAs in Northumberland using Approach 3 as shown in Table 3.9.

Table 3.9: Test of difference of South East Northumberland Coastal Plain Water body distribution against other NCAs in Northumberland using Approach 3

NCA	*Surface Area*		
	P- value	Wilcoxon Signed Rank Test Value	N
Tyne Gap and Hadrian's Wall	0.465	3.000	4
Border Moors and Forests	0.144	1.000	4
Mid Northumberland / Northumberland Sandstone Hills	0.225	3.000	5
Cheviots / Cheviot Fringe	0.715	6.000	4
North Northumberland Coastal Plain	0.800	1.000	5
	Number of water bodies		
	P- value	Wilcoxon Signed Rank Test Value	N
Tyne Gap and Hadrian's Wall	1.000	5.000	4
Border Moors and Forests	0.465	3.000	4
Mid Northumberland / Northumberland Sandstone Hills	0.686	9.000	5
Cheviots / Cheviot Fringe	0.273	8.000	4
North Northumberland Coastal Plain	0.138	2	5

In all, total count in Approach 1 is over 87,000 water bodies with over 95 million sq. m in upscaled surface area (Figure 3.9c & d). Approaches 2 and 3 contained 81 million sq. km and 82 million sq. km and water bodies counts were 70,745 and 84,102 respectively (Figure 3.9c & d). The approaches confirm that 1.3 – 2% of Northumberland contains water bodies in the size range 1 sq. m to 1 sq. km. This is with the exclusion of rivers, water bodies >1 sq. km and un-digitised areas covered in vegetation and shadow.

Table 3.10: Surface area and number of water bodies in Northumberland NCAs

NCA name	*Tyne Gap and Hadrian's Wall*	*Border Moors and Forests*	*Mid Northumberland / Northumberland Sandstone Hills*	*Cheviots / Cheviot Fringe*	*North Northumberland Coastal Plain*	*South East Northumberland Coastal Plain*
NCA area	434 x 10^6 sq. m	1,272 x 10^6 sq. m	1,360 x 10^6 sq. m	881 x 10^6 sq. m	377 x 10^6 sq. m	437 x 10^6 sq. m
Surface area of water bodies						
1-10 sq. m	2,889	2,915	2,996	3,827	1,339	428
10-100 sq. m	272,277	140,819	351,951	635,653	79,568	185,127
100-1,000 sq. m	1,082,606	958,340	1,614,562	2,633,787	732,253	1,901,049
1,000-10,000 sq. m	1,037,401	1,165,513	3,023,302	1,723,630	1,257,162	4,708,613
10,000-100,000 sq. m			6,479,087		1,493,042	16,278,394
100,000-1,000,000 sq. m			33,951,075			
Number of water bodies						
1-10 sq. m	407	397	512	550	212	55
10-100 sq. m	6,269	3,338	7,418	11,725	1,648	3,333
100-1,000 sq. m	3,881	2,782	4,859	9,303	2,331	5,600
1,000-10,000 sq. m	624	397	1,279	991	447	1,721
10,000-100,000 sq. m			341		47	191
100,000-1,000,000 sq. m			85			

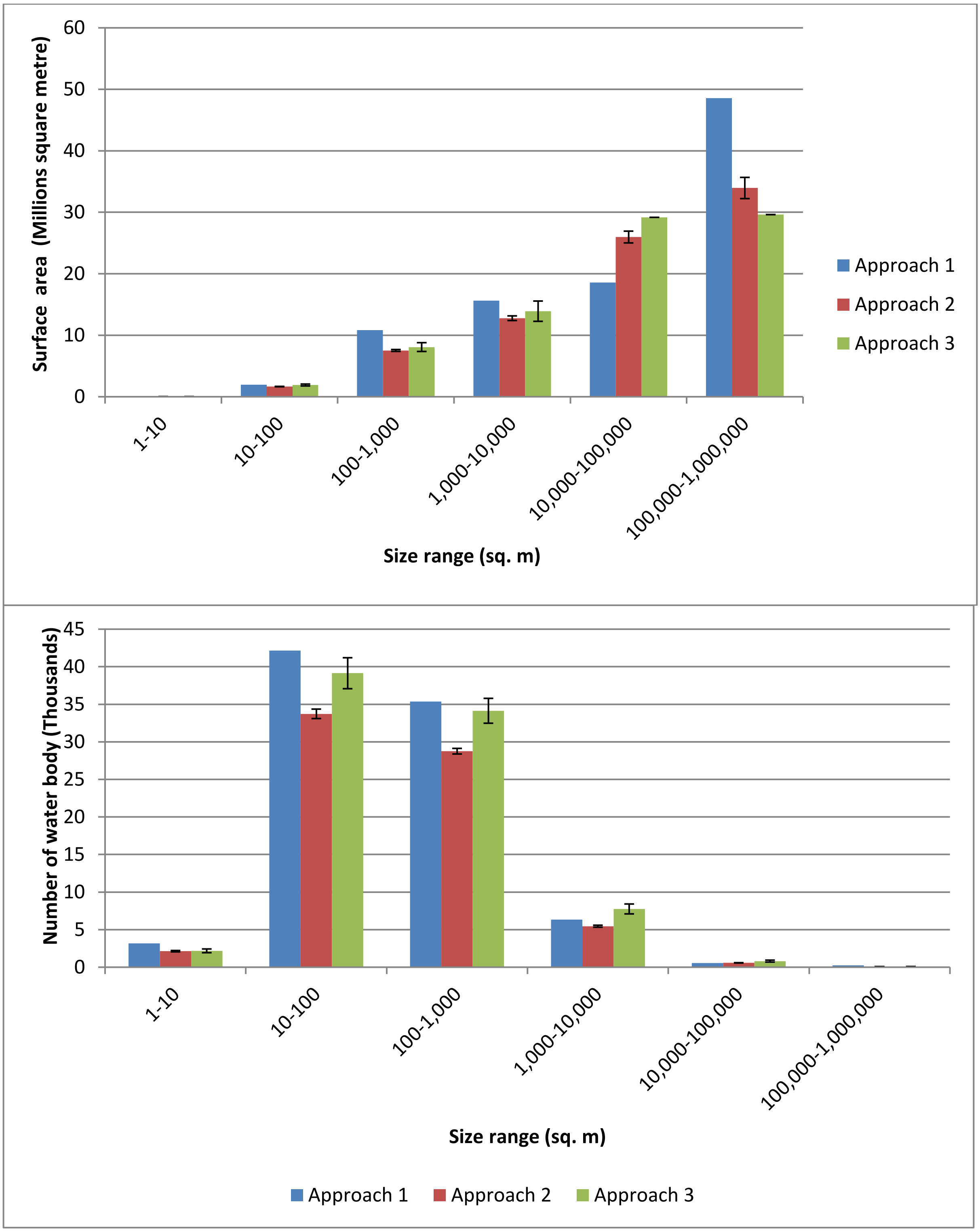

Figure 3.9a & b: Number and surface area of water bodies in Northumberland using World Imagery at 1:2,000 scale

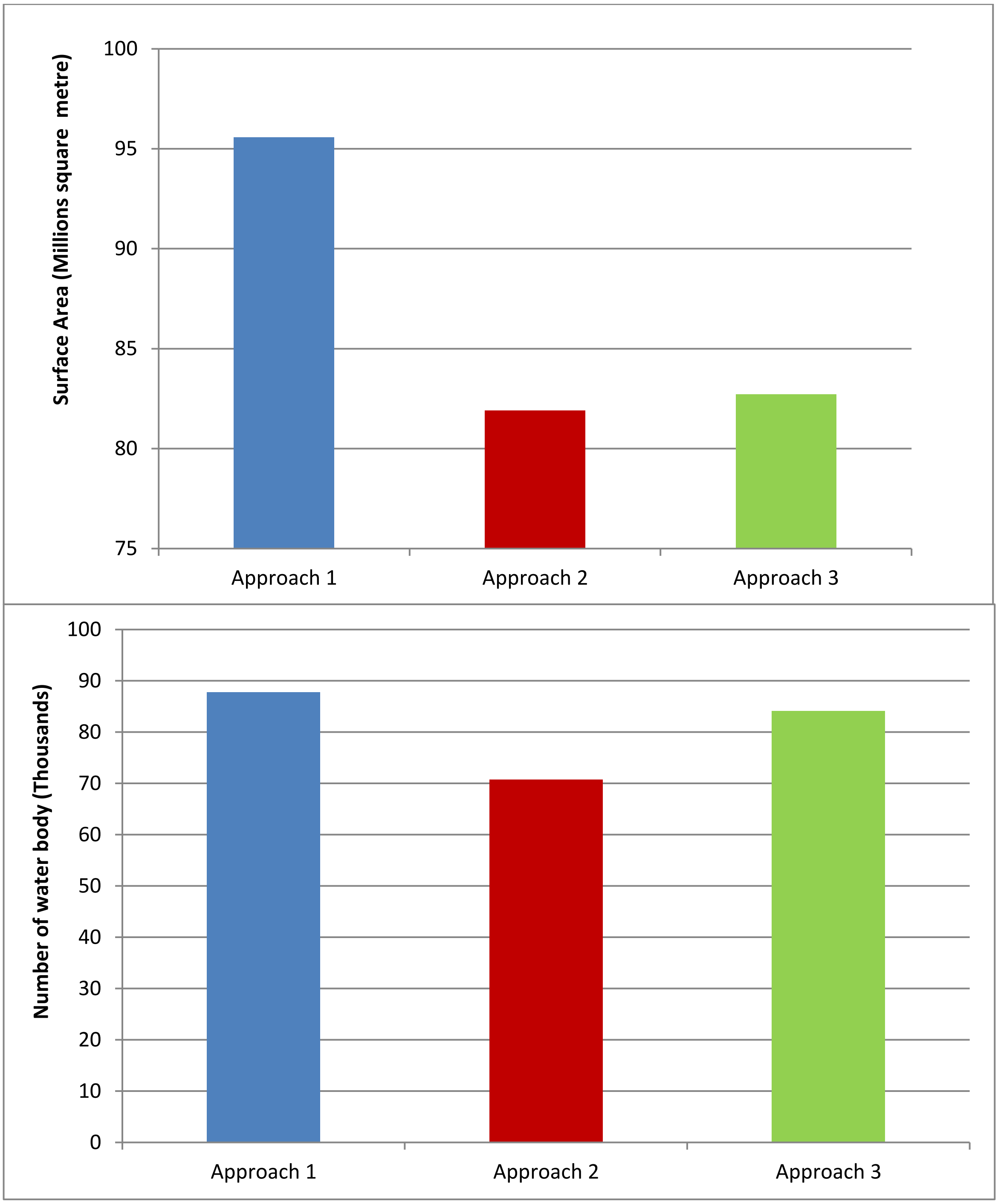

Figure 3.9c & d: Comparing approaches for NWBD using World Imagery at 1:2,000 scale (c) surface area (d) number

3.4.4 Global Lake and Wetland Database (GLWD) for North East England

In the North East database of the GLWD developed by Lehner and Doll (2004), water bodies were identified from 0.1 – 10 sq. km to 10 – 100 sq. km. Water bodies <1 sq. km in surface area were greater in number. And there was no Pareto distribution as suggested by Downing *et al.* (2006) (Table 3.11). As expected the database was limited in identifying water bodies <0.1 sq. km.

Table 3.11: Inland water bodies distribution of North East England (GLWD)

Size range (sq. km)	*Surface area (sq. km)*	*Number of water bodies*
10.1 - 100	27	2
1.1 - 10	54.1	21
0.1-1	30.7	57

3.5 Discussion

3.5.1 Underpinning Northumberland Water Body Distribution (NWBD)

Knowledge of the distribution of water bodies in size range <0.1 sq. km has been greatly flawed by the high level of uncertainties in calculating their abundance. The flaw is further encouraged by using parameters that control distributions of larger water bodies and lakes to extrapolate SWBs abundance as described by the GLWD and Downing *et al.* (2006). This chapter's results clearly indicate a change in trends from postulates by Schuiling (1977), Wetzel (1990) and Downing *et al.* (2006) of an existing relationship between the increase in number of lakes and decrease in surface area. Although these relationships are true in larger lakes and other large water bodies, it is not applicable in the estimation of SWBs' abundance because of the diversity and inter-dynamics of natural and anthropogenic factors that control SWB abundance. The results also showed that SWBs are more abundant than the larger water bodies at a regional and sub-regional scale with no clear patterns in distribution relationship. However, there is a strong limitation in identification and quantification of SWBs as it relates to the quality of imagery and temporal variations. The GLWD showed water bodies >0.1 sq. km increase in number of water bodies from large to small size ranges. NWBD showed similar relationships for its large water bodies but in the SWBs the pattern varied and these can be related to strong influences of regional climate and hypsometry on water bodies distribution (Seekell and Pace, 2011 and Seekell *et al.*, 2013). Furthermore, precipitation has been known to play a significant role in carbon capture in collaboration with the size of water bodies (Jeffries, 2016). This is evident in the abundance change of water bodies in Northumberland identified in this research (multi-temporal distribution of water bodies in Northumberland using Landsat-8 at 1:20,000 scale).

Water bodies <0.1 sq. km on NWBD showed no mathematical relationship with the GLWD database because its coverage starts from 0.1 sq. km. GLWD for North East England were closely related to Landsat-8 except for two water bodies identified >10 sq. km. This can be related GLWD to the database identifying other waters outside the Northumberland area. Overall, Landsat-8 and World Imagery databases confirm that there are more water bodies <0.1 sq. km than previously estimated globally and their impacts in climate change scenarios are being missed out (Boyd *et al.,* 2010). Their inclusion in the current climate change models poses an interesting outlook to understanding how much SWBs are defending the planet from increased atmospheric CO_2 and CH_4. Also, the global water body distribution is skewed towards the north of the equator between 35 - 70°N where land space is more abundant (Lehner and Doll, 2004). Thus, SWB influences on carbon capture have broader global significance than previously assumed.

3.5.2 Accuracy assessment of water bodies abundance in Northumberland

More water bodies were identified using the World Imagery maps compared to the Google™ maps. The accuracy of pond assessments using different imageries are subject to the image quality used for identification and the temporal variations in the water bodies based on landscape, precipitation flux and other environment influences (Jeffries, 2012). Each NWBD site had a varying number of water bodies because of the nature of the landscape, sediment types and localised processes that control natural and anthropogenic water body formation such as climate/weather and land use. Although, these keep SWBs abundance in a continuous flux, there were no statistically significant difference in utilising World Imagery or Google Map in identifying water bodies in each NCAs.

Ground survey data by Jeffries (2016) provided an alternate means of identifying water bodies and this confirmed the flux mechanisms of SWB abundance. It also confirmed the presence of water bodies in size range 1 – 10 sq. m that were not abundantly seen in satellite imagery in this research. Advancement in image quality could potentially improve identification of water bodies in size range 1 – 10 sq. m but water bodies of these sizes have quick wetting and drying phase and may be difficult to identify because of their close similarity with surrounding soils. Also, Jeffries (2016) ground survey data on precipitation showed that this research's water body distribution was at a median abundance and water body distribution can change based on precipitation influence. In high precipitation periods, more water bodies are expected to be formed but the geographical distribution of these water bodies are unclear because of the variation in landscape. However, the general conception remains that flat landscapes will be expected to have more water bodies than undulating landscape (Calder, 1996, Fan *et al.,* 2013 and Wilkinson *et al.,* 2009). It is also worth noting that in this research, there were no significant differences between World Imagery water body count in South East Northumberland Coastal Plain NCA and Jeffries' (2016) high and low precipitation water body counts. This confirms that this research's water body distribution bis at the median abundance.

Upscaling at 1:2,000 scale showed elevated counts of water bodies (>28,000) in size ranges 10 – 100 sq. m and 100 – 1000 sq. m compared to all other ranges. Upscaling or extrapolating water bodies using the test areas could be overestimating water body abundance in inland Northumberland which has undulating hills that cannot hold water bodies but this may be appropriate in lowland landscapes such as coastal and river flood plain areas of Northumberland. These areas are also influenced by the land use which contributes to the flux of NWBD. Factors such as image resolution and scale change also influence the variations in NWBD from an automated analysis approach in extrapolating water bodies. However, the variations in NWBDs reduce with increase in water body size range. Thus, results acquired are a function of how efficient the data processing and constrained applied to determine water body abundance.

3.5.3 Water bodies in Northumberland

At 1:20,000 scale, more water bodies were counted on World Imagery compared to Landsat-8 on average. But the average surface area was higher on Landsat-8 (65.83 ± 20.12 sq. km) compared to World Imagery (26.243 sq. km) in all size ranges. Like Jeffries (2016) at a sub-regional level, Landsat-8 also confirmed the continuous flux state of water body abundance at a regional level. This further confirmed that a single estimate is not tenable and a range should be given which reflects the strong control by regional influences. Also, water body abundance flux will not allow for establishing a fixed distribution pattern such as Pareto (Seekell and Pace, 2011, Raymond *et al.*, 2013 and Verpoorter *et al.*, 2014) regionally or globally.

3.5.4 Extrapolating Northumberland water body distribution

Approaches 1, 2 and 3 showed that 1.3 – 2% of Northumberland's area contains water bodies in the size range 1 sq. m to 1 sq. km. This is with the exclusion of rivers, water bodies >1 sq. km and un-digitised water bodies covered in vegetation, shadow cast and cloud cover. Global water body distribution is around to 2 – 3% of global land cover (Lehner and Doll, 2004 and Downing *et al.*, 2006), therefore, upscaling approaches are in line with literature from a global context. None of the estimates given by Approaches 1, 2 and 3 give a Pareto distribution because of the reduction in the size range 1 – 10 sq. m. Pareto distribution could have been achieved in Northumberland if the <10 sq. m were excluded from results. However, flux state of water bodies being controlled by regional climates, temporal changes and landscape meant that results may not be replicable in a different location or the same location in a different season (Jeffries, 2016). Image resolution, vegetation cover, and high evaporation in shallow water depths were also factors that potentially accounts for the inability to identify water bodies in size range 1 – 10 sq. m. Therefore, natural processes in Northumberland and data processing make it less likely for water body abundance to follow a Pareto distribution or any defined distribution pattern.

3.5.5 Temporal variation of Northumberland water bodies and carbon flux

This research showed that water body abundance does not directly correspond with weather parameters (temperature and rainfall) because filling up of a water body is an accumulation of precipitations and evaporations from previous months or seasons. Also, high precipitation also does not always directly relate to more water bodies because water supply generally feeds regional water bodies, soils, groundwater and river systems (Boix *et al.*, 2012, Céréghino *et al.*, 2008, Céréghino *et al.*, 2013 and Fritz, 1996). For large water bodies with deeper depth, precipitation flux is noticed in the reduction/increase in water volume. Most SWBs experience pond disappearance by the loss of water cover and become similar with the surrounding soil environment or become extremely shallow systems as a result of the seasonal accumulation of high evaporation and low precipitation (Jeffries, 2016). Pond reappearance occurs as result of an accumulation of seasonal low evaporation and high precipitation. This further explains why temperature and rainfall data for July and September 2013, July and December 2015, and April 2015 did not relate with Landsat-8 water body abundance collected from the same months.

Temporal analyses of water bodies showed a wide variation in the size-abundance of lentic systems in the region. Water body abundance flux can provide information on source and sink potentials. Pond disappearance and water body size reduction signify carbon sinks and burial of carbon in sediment respectively. While pond reappearance and the increase in water body surface areas could relate to the formation of new natural and anthropogenic ponds, the age of ponds, carbon sink/source flux and precipitation levels (Raymond *et al.,* 2013 and Gilbert *et al.,* 2014).

In July 2013 and July 2014, there were more water bodies counted in Northumberland. Without factoring water body depth, increase in surface area of water bodies can play significant roles in CO_2 and CH_4 flux viz-a-viz carbon cycling in water bodies (Cole *et al.,* 2007, Downing, 2010 and Raymond *et al.,* 2013). The increase in water bodies' surface area also allows for easier entry of terrestrial carbon into water systems (Boyd *et al.,* 2010). However, it is difficult to determine the general water bodies' behaviours with increasing total surface area as there is potential for heterogeneous behaviours of different water bodies at different size ranges in Northumberland (Gilbert *et al.,* 2014). From December 2014 to April 2015, there was a decrease in surface area of water bodies from 93.7 sq. km to around 45 sq. km. This represents over 50% drop in water bodies surface area and consequently burial of accumulated terrestrial and aquatic carbon (Raymond *et al.,* 2013 and Clow *et al.,* 2015). During this period, there was a massive reduction in water body sizes between 0.1 – 10 sq. km which could be related to surface evaporation (that is, accumulation of seasonal temperature increase and reduced rainfall), land sparing (Lamb *et al.,* 2016) and other agro-based techniques. In the winter, there is more DOC settling at pond bottoms due to slowed microbial actions in cold weather (Dunalska *et al.,* 2003). Also, increase in precipitation and pond size expansion favour water transport of allochthonous and autochthonous carbon.

It should be noted that at 1:20,000 scale, no water bodies were identified between size range 1 - 10 sq. m, 10 - 100 sq. m and few water bodies were identified at 100 – 1,000 sq. m. But temporal changes are significant in identifying the importance of environmental and climatic controls in water body distribution, hence, the continued conversation on improving imagery to further understand SWBs' carbon burial (Clow *et al.*, 2015, Downing *et al.*, 2006, Downing 2010, Lehner and Doll, 2004 and Verpoorter *et al.*, 2014) and to identify water bodies <1,000 sq. m.

3.5.6 Small water bodies abundance in Northumberland and carbon capture

With improved imagery quality and no cloud cover, the error in digitising imagery was highly reduced for water bodies' identification on World Imagery compared to Landsat-8. World Imagery provided no information on the temporal change in water body distribution or effect of seasonal variation. At 1:20,000 scale, there was only one water body >10 sq. km (like Landsat-8) and two water bodies in size range 1 - 10 sq. km. With decreasing size range, more water bodies were identified compared to low resolution Landsat-8 even in size range 10 – 100 sq. m. This is in-line with researches by Downing *et al.* (2006), Cole *et al.*, 2007, Seekell *et al.* (2013) and Verpoorter (2014) that more water bodies abound in surface area <0.1 sq. km. However, 1:20,000 scale is limited in identifying water bodies <1,000 sq. m accurately.

To achieve identification to 1 sq. m, the scale was adjusted in 6 selected areas to 1:2,000. This identified water bodies' sizes between 1 sq. m to 1 sq. km. In the selected areas, more SWBs were identified in finer details in each NCA. The distribution of water bodies coincided with a downhill distribution of water bodies in Northumberland, that is, South East and North Northumberland Coastal Plains and other lowland areas in Northumberland contained more water bodies compared to areas of higher elevations.

Consequently, SWBs contribute significantly to carbon flux in flat lands (Seekell *et al.,* 2013 and Clow *et al.,* 2015). Agricultural activities also play a significant role in the carbon burials in these areas (Lamb *et al.,* 2016) as Gilbert *et al.* (2014) showed that there is heterogeneity in the carbon storage in Coastal Northumberland with carbon storage of up to 20% in organic carbon sequestered in ponds. This confirmed land use as a significant influencer of small water distribution (Downing *et al.,* 2006).

To understand the significance of SWBs to the carbon cycle and abundance down to 1 sq. m, three approaches were developed to extrapolate the number of ponds in Northumberland at 1:2,000 scale. The three approaches showed more water bodies counted between 1 – 10,000 sq. m which has not been previously estimated in literature. But the estimated water body count in the size range 1 – 10 sq. m were <3200 with a very negligible surface area. Therefore, at size range <10 sq. m, there is need to improve imagery quality, identification techniques that distinguish water bodies from surrounding soil and eliminate vegetation cover that may hinder digitising water bodies at 1:2,000 scale. Water bodies in size range 1 – 10 sq. m are more subject to climatic effects compared to the other size ranges because of their quick wetting and drying. Hence, carbon fluxes are more rapid and significant compared to large water bodies (Gilbert *et al.,* 2014).

3.6 Limitation to Northumberland Water Body Distribution Research

World Imagery is not available for multiple dates so temporal change cannot be investigated at higher spatial resolution but this was partly compensated by the Landsat-8 data which provided images from different dates. Landsat-8 images were limited in the identification of SWBs compared to larger water bodies, due to the pixel size. Also, in the accuracy assessment, field survey data came from only one source (Jeffries, 2016) and from one site in Northumberland. But alternative accuracy assessment of water bodies for all NCAs was achieved by analysing Google ™ Map. There is a limitation in field surveys of other NCA of Northumberland as this could not be completed within the timescales of this PhD research.

Extrapolated data needs further methods for constraining it as land features in Northumberland vary within an NCA. Therefore, there is a chance of overestimation of water body abundance in undulating land and upland features, despite using three approaches and the localised data source for estimating water body abundance.

The research is also limited in absence of an accurate means to measure pond expansion and reduction in surface area. Although Landsat-8 provided a means of identifying surface area flux, the level of detail produced can be further enhanced through improved methodologies around actual field and high resolution imagery data.

However, the similarity in the results with other works goes to show the plausibility of the research methods/approaches as well as plausibility of the extrapolations.

3.7 Conclusion

There are multiple methods for identifying water body distribution and each method has strengths and weaknesses in estimating SWB distribution. This research used map-based identification of water bodies to estimate water bodies abundance and distribution. There were variations in the identification of water bodies distribution based on imagery, precipitation change and mathematical extrapolations. The reality is water bodies abundance and distributions are in continuous flux and their identification is dependent on the accuracy of the data utilised, imagery quality and scale of analysis.

SWBs and their abundance flux are important in carbon capture and understanding of their size and abundance behaviour with corresponding environmental control will help unlock new knowledge in carbon capture. Therefore, researching constrained automated methods of quantifying water bodies (including Pareto distribution) should be further considered in determining regional and global estimates. However, Pareto distribution seems to be only correct for larger water bodies.

Chapter 4: Carbon Stock in Northumberland Water Bodies

4.1 Background

Water bodies have been found to behave differently in terms of their source or sink potentials (Cole *et al.,* 2007, Gilbert *et al.,* 2014 and Seekell *et al.,* 2013). These broad source/sink potentials are affected by numerous factors including regional climate, surrounding vegetation type and terrestrial inclusions, age of water body and pond succession, water body biochemistry and flux processes within the ponds, water body depth and hypsometry (Downing *et al.,* 2006, Gilbert *et al.,* 2014, Raymond *et al.,* 2013 and Seekell *et al.,* 2013). These and other environmental/physical, biological and geochemical/chemical factors control carbon stocks in water bodies.

Estimates of SWBs' carbon stocks are important for quantifying inland water body contributions to the carbon cycle (Boyd *et al.,* 2010 and Ntengwe and Edema, 2008). Knowledge of this and the regional number and surface area of water bodies further define the total carbon stocks in SWBs at a regional scale. In comparison to the well-defined atmospheric and ocean carbon sinks, there is a need to more accurately estimate the various sub-compartments of land or terrestrial carbon cycle (Cole *et al.,* 2007, Gilbert *et al.,* 2014 and Tranvik *et al.,* 2009). The majority of the land sub-compartments are not well understood especially the SWBs compartment and SWBs have been described as the potential missing carbon sink or a missing link in the global carbon cycle (Aufdenkampe *et al.,* 2011, Cole *et al.,* 2007 and Lal, 2004).

This chapter focuses on quantifying carbon that is stocked in sedimentary depositions of ponds. This was achieved by quantifying carbon stock deposited from allochthonous and autochthonous sources in selected ponds at Druridge Bay, South East Northumberland. The ponds were categorised into four pond types (similar to Gilbert *et. al.,* 2014) and three water body size ranges. The results of the carbon analyses are upscaled to determine the carbon stock across Northumberland water bodies in Chapter 6.

4.2 Literature Review

There are numerous environmental/physical, biological and geochemical/chemical factors that combine to influence the variation of carbon stocks within ponds. These flux mechanisms are closely similar to other flux systems within the global carbon cycle such as lakes, rivers and streams which process and stock carbon in the water column and bottom sediments. However, in ponds, there is highly reduced fluvial transport which contributes to increasing carbon loss to the atmosphere (Cole *et al.,* 2007). The reduced fluvial transport also means that SWBs do not have well-graded sediments, as is found in oceans, with materials well graded from coarse to fine, from ocean shore to deep continental shelf (Demaison and Moore, 1980, Gilbert *et al.,* 2014 and Grabowski *et al.,* 2011). This is significant because aggregation proportions of fine and coarse materials contribute to the efficiency for carbon burial in sediments or carbon remobilisation into the water column and atmosphere.

For SWBs, the interplay of sedimentary material aggregation and other sub-factors within physical/environmental, biological and geochemical/chemical factors influence carbon source/sink potentials. This explains why highly porous or loose sediments, which are expected to easily remobilise carbon, can stock more net carbon than impermeable sediments (Gilbert *et al.,* 2014). These varying influences also create a flux pattern of carbon exchange with the atmosphere and carbon burial. Thus, the efficiency of pond carbon flux determines net carbon source or sink (Gilbert, 2016 and Taylor, 2017). Although these factors are numerous, varying and environment specific, this research focused on:

- Sediment wetness

- Organic carbon concentration
- Carbon Nitrogen Ratio (C:N)
- Dry bulk density
- Carbon stock

And the roles of each of these factors are reviewed below.

The microbial community inherent in the pond sediment and how it affects carbon stock is researched in Chapter 5.

4.2.1 Sediment wetness

Pond sediment composition can either be the interaction of gas, solid and liquid materials which are mostly prevalent in shallow SWBs or solid and liquid materials which occur prevalently in larger water bodies (Avnimelech *et al.,* 2001). These can be described as temporary or permanent ponds respectively (Gilbert *et al.,* 2014 and Gilbert, 2016). The solid components of water body sediment are usually organic and inorganic matter (Grabowski *et al.,* 2011) and the liquid phase is water. In a permanent pond system, the top layer of sediment with high sediment wetness or moisture content is referred to as soft sediment (Yuvanatemiya and Boyd, 2006) because of its continuous exposure to the pond water column (Munsiri *et al.,* 1996) (Figure 4.1). Also, some permanent ponds are known to contain gasses as a result of microbial methane production in pond sediment (Gebert *et al.,* 2006 and Sanders *et al.,* 2007) or sediment aeration in sand dune pond sediments.

Sediment wetness or moisture penetrates more efficiently in unconsolidated sediments which have high porosity and the wetness reduces down the core profile as a result of increased compaction in sediment (Grabowski *et al.,* 2011). Sediment wetness also has a direct effect on organic matter concentration (Gilbert, 2016) and microbial communities/biochemistry within pond sediment (Grabowski *et al.,* 2011). Increased porosity in sediment allows development of biofilm that attach themselves to the inorganic matter within sediment pore spaces for mineral extraction and transport of nutrients through pond sediments. As sediment compaction increases, there is a reduction in pore spaces which hinders the development of microbial biofilms and moisture penetrations in sediments (Grabowski *et al.,* 2011). Within the biofilms, the nature of microbial loads varies and microbial variability is controlled by carbon source, pond permanence/temporariness and pond environment amongst other factors. Sediment wetness is also known to influence the nature of the microbial community in SWBs and microbial communities of ponds are further explored in Chapter 5.

4.2.2 Organic Carbon Concentration

Percentage organic carbon in small pond sediments has been found to range from 1 - 19% in lowland sites in Northumberland (Gilbert *et al.*, 2014). This is within the ranges that were previously recorded by Dean and Gorham (1998), Downing *et al.* (2008) and Boyd *et al.* (2010). Although this confirms the carbon sink potentials of small ponds, small ponds are generally in a flux state of carbon preservation and oxidation with oxidative by-products of CO_2, H_2S and CH_4. Gilbert *et al.* (2014) showed the highest organic carbon concentrations were recorded in loose naturally vegetated permanent pond sediments in Blakemoor Northumberland while the lowest were in arable field ponds. This goes to show that carbon depositions can vary with pond type and are largely controlled by the capacity to bury particulate carbon or remobilise carbon for redox. Also, temporary ponds in Hauxley, Northumberland, accumulated carbon concentration by creating anoxia and a dampening layer of moss (Jeffries, 1998) and this further identified the diversity of influences on carbon buried in sediment.

4.2.3 Carbon Nitrogen Ratio (C:N)

Carbon in pond sediment can be sourced from either allochthonous or autochthonous material inputs. Allochthonous carbon (with heavy molecular weight), like coarse sediment, has a quick sedimentation time to pond bottom with minimal microbial interaction in the water column. Allochthonous materials are usually characterised by complex molecular structures (Dean and Gorham, 1998) that are sometimes difficult to degrade by pond microbes. Thus, they continuously accumulate in the pond bottom and are slowly degraded by microbes penetrating through wet sediments. They include mainly plant and animal matter from the terrestrial environment, and make up the bulk of particulate organic carbon (POC) in water bodies. Autochthonous carbon quantities in ponds are functions of biologic interactions within the pond and are mostly associated with in-situ microbes. They are characterised by typically less complex organic structure, slower sedimentation time compared to heavy terrestrial carbon and can easily be oxidised within the pond water column (Boyd, 1995). They include esters, amino acids, lipids and metabolic substrates of microbes, and they make up a large proportion of Dissolved Organic Carbon (DOC) found in ponds.

Knowledge on the source of organic matter is important in understanding and building relationships between the various sub-compartments of the land or terrestrial carbon cycle. Although carbon mineralisation alters the identity of organic matter and makes it difficult to define organic matter source especially after extreme microbial degradation, the origin of organic matter can be described by measuring the ratio of carbon and nitrogen content (Meyers and Ishiwatari, 1993 and Cole *et al.,* 2007). Pond sediment rich in terrestrial input or allochthones are characterised by complex organic compounds with C:N ratio typically >10:1. Vascular Plants are identified by C:N ratio between 20-30:1.

Pond sediment dominated by autochthonous carbon are characterised by C:N ratios <10:1 (Ishiwatari and Uzaki, 1987, Meyers and Ishiwatari 1993 and Prahl *et al.,* 1994).

C:N ratio analyses in water bodies provides an avenue for down core profiling of carbon processes and origin. This is needed to understand aquatic carbon oxidation, pond succession and processes related to terrestrial input over time. Pond succession reflects the depositional environmental conditions and the changes in vegetation that supply materials into water bodies' bottom sediments (Biggs *et al.,* 1994, Gilbert *et al.,* 2014, Jeffries, 2008). Ponds in Northumberland have been identified to reflect changes in depositional material based on seasonal changes and land use. C:N ratio can be used to identify these depositional changes (Gilbert, 2017), which reflect changes from algae rich, plant rich or vascular plant depositions into ponds.

It is expected that the level of information from C:N ratio in a stable lentic system (e.g. ponds) will be more easily interpreted compared to river systems that are influenced by highly oxidative and continuous material mixing and transport. High oxidation of terrestrial organic matter can lower C:N ratios (Emerson and Hedges, 2003). These cause inland water bodies (rivers and lakes) surrounded by terrestrial plants to have C:N ratio <10:1 which complicate C:N ratio analyses.

4.2.4 Dry Bulk Density

In understanding the variation of sediment wetness in different ponds, there is also a need to understand the porosity in pond sediment and porosity is usually tied to the sediment type and grain size. Fine clay soil clumps together and has low sorption for water compared to coarse sediment that allows for water absorption. Therefore, highly porous sediments have lower dry bulk density after removal of moisture or sediment wetness. Sediment cohesion is a function of the existing weathering, transport and microbial processes that are inherent in the pond sediment (Grabowski *et al.,* 2011). The size aggregation and chemical interaction of sedimentary minerals bring about cohesion, and the proportions of fine or coarse grains and their potential chemical bonding or interactions cumulate to create material density (Bennett and Hulbert, 2012).

In pond sediments' down core profile, compaction and porosity tend to control the dry bulk density. At the top sediment, materials tend to unevenly aggregate coarse and fine sediment laterally. Thus, high porosity and moisture penetration contribute to low dry bulk density and the reverse occurs in pond bottom sediment because of increased compaction of sediments and overburdens overlying sediments. Gilbert *et al.* (2014) identified that permanent natural ponds in Northumberland had a lower dry bulk density in sediment compared to arable, pasture and sand dune ponds. This is because of the continuous sediment wetness or moisture penetrating through natural pond's top sediment which causes low compaction or sediment looseness. Dry bulk density increased down core profile at different rates depending on sediment type (Munsiri *et al.,* 1995). Also, ponds that hold gas bubbles cause a reduction in dry bulk density in that portion of sediment (Avnimelech *et al.,* 2001, Grabowski *et al.,* 2011 and Gilbert, 2016).

4.2.5 Carbon stock

Allochthonous and autochthonous carbon inputs are the main contributors to carbon stock in pond sediment. Figure 4.1 typifies the deposition of sediment in pond bottom over a 34-year period. The figure shows accumulation of materials from allochthonous and autochthonous deposition of carbon into bottom sediments based on prevailing physical/environmental, biological and geochemical/chemical factors that control carbon stock or development of soft sediments in the pond bottom.

The sources of the materials are usually from surrounding and in-situ biochemical matter decomposition, which causes bio-accumulation and geo-accumulation of materials by erosion and sedimentation (Yuvanatemiya and Boyd, 2006). The flux of organic carbon (burial and re-mobilisation) and nutrients are functions of temperature, oxygen penetration, soil type, compaction, moisture penetration, primary productivity, the age of pond and seasonal wetting/drying of water body amongst other factors. High carbon preservation is favoured by anoxia while high oxygen penetration into pond bottoms leads to more CO_2 emissions. Overall, carbon stocks are expected to be high in top pond sediment and reduce down core profile as result of terrestrial input deposited in the top sediment. Autochthonous organic carbons in a highly oxidised environment are susceptible to microbial degradation. However, when they reach the pond bottom as DOC or colloidal DOC, their fate is dependent on sediment wetness and the pre-existing factors that control flux of organic carbon (Dunalska *et al.*, 2003, Yuvanatemiya and Boyd, 2006, Cole *et al.*, 2007, Ntengwe and Edema, 2008, Boyd *et al.*, 2010 and Gilbert *et al.*, 2014).

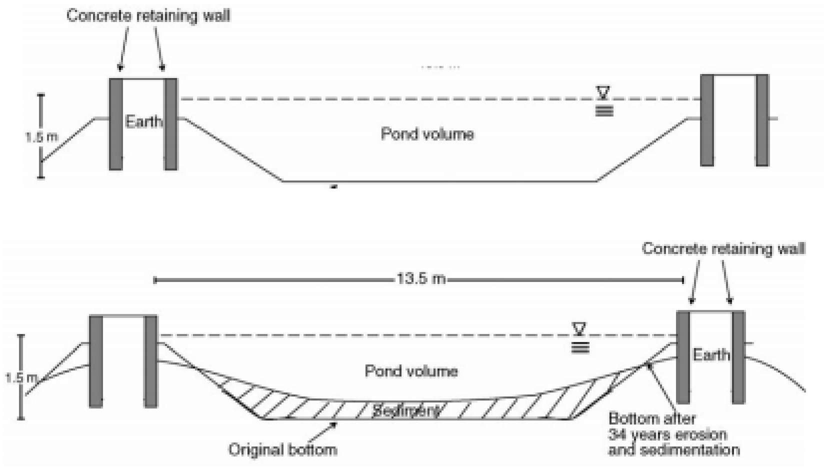

Figure 4.1: Accumulation of soft sediment in pond (Yuvanatemiya and Boyd, 2006)

4.2.6 Background Summary

The dry bulk density of pond sediment is a function of sediment type and aggregation, porosity, sediment wetness and material compaction. And irrespective of pond types, pond size or any other factor, high carbon burial in pond sediments are tightly related to the appropriate aggregation, dry bulk density and sediment wetness (Gilbert *et al.*, 2014). Therefore, there is a relationship between carbon stock and dry bulk density of pond sediments, that is, carbon stock in sediments are a component of total sediment composition.

Although, there are multiple influences acting to varying degrees on carbon stocks in a pond, the origin of carbon can be tied to allochthonous (terrestrial) input and aquatic microbial processes.

4.3.1 Research Aim

This chapter is aimed at quantifying the carbon stock in small water bodies (SWBs), its variation across and within pond sediments. This is to contribute to the limited research in this field and it continues works by Gilbert *et al.* (2014) at Northumbria University on carbon capture efficiencies in Blakemoor, Northumberland (Research Title: The Power of Ponds: Sequestering organic carbon in small natural water-bodies). The results of this chapter are to be integrated into estimating regional carbon stock in Northumberland water bodies in Chapter 6.

The research aims are achieved by analysing the variabilities of carbon stocks and carbon stock parameters of different ponds in Northumberland. These parameters are:

- Pond size (10 - 100 sq. m, 100 - 1,000 sq. m and 1,000 - 10,000 sq. m)
- Pond types (sand dune ponds, arable ponds, pasture ponds and natural ponds)
- Down core profile parameters of:
    - Sediment wetness
    - Organic carbon concentration (%C)
    - C:N Ratio
    - Dry bulk density
    - Carbon stock

4.3.2 Research Questions

1. a. How much organic carbon is stored in the pasture, natural, sand dune and arable ponds in Northumberland per square metre?
    b. What is the average amount of carbon stored per square metre of water bodies in Northumberland?
2. a. What is the effect of size ranges on carbon storage in all pond types?
    b. What is the effect of size ranges on carbon storage within pasture pond?

3. a. How does carbon storage vary with depth?

   b. How significant are pond depth, bulk density and sediment wetness to carbon storage?

4.4 Methods

4.4.1 Research design

The research focussed on validating and further enhancing the existing body of knowledge in understanding carbon stock in small water bodies. The sample site was Druridge Bay, South East Northumberland with core samples collected from selected water bodies that were previously analysed by Gilbert *et al.* (2014) and Gilbert (2016). The selected ponds were chosen to account for:

Water body size ranges:

- 10 – 100 sq. m,
- 100 – 1,000 sq. m and
- 1,000 – 10,000 sq. m

Pond types:

- sand dune,
- arable,
- pasture and
- natural ponds

A pilot study collected sediment cores in February 2014 for initial analyses of carbon stock in Northumberland ponds. Thereafter, 18 pond sediment cores were collected from ten (10) ponds in June 2016 to analyse variability in terms of pond size ranges, pond types and down core profile. Weather conditions during sampling days were clear summer skies and calm breeze with temperature ~20^0C.

4.4.2 Sample Collection

Samples were collected from Druridge Bay using a cylindrical corer that collects 45 cm of pond sediment with a 4.70 cm diameter (similar to corer used in Gilbert, 2016). The coring technique involved manually pushing in the corer to collect pond sediment beneath the water layer. Then, the corer is carefully turned in a circular motion and guarded out of the sediment to prevent loss of bottom sediment and breakage of the sediment core. Thereafter, the sediment core was carefully pushed out (with minimum force) and placed on a clean sheet of aluminium foil. On-site, the sediment core was sliced into 1-3 cm thick sediment discs, wrapped and bagged.

Table 4.1 shows the number of ponds sampled and their size range. The cores were collected at different depths of the pond, starting at the pond edge. For pasture ponds with size range 10 – 100 sq. m and all arable ponds, triplicate cores were collected at the edge, middle and centre of the ponds. In pasture ponds of size range 100 – 1,000 sq. m and 1,000 – 10,000 sq. m, triplicate cores were collected at the pond edge and at intervals of 3 metres further away from the pond edge. For natural and sand dunes ponds, duplicate cores were collected at pond edge and at knee-deep depths of the pond.

Table 4.1: Pond type and size range of cores collected for carbon analysis

Pond type	*Size ranges (sq. m)*		
	10 -100	100 -1,000	1,000 – 10,000
Sand dune pond		2	1
Arable Pond	1	1	1
Pasture Pond	3	3	3
Natural pond		1	2

4.4.3 Laboratory Analysis

The freshly collected sediment samples were unwrapped, weighed and placed in small open-air containers. Samples (sliced discs) were homogenised and 0.25 g of each wet sample was further analysed for its microbial communities. The remaining sediments were re-weighed and placed in a dry cabinet at room temperature to limit loss of labile organic carbon from sediments. The samples were weighed at 3 day intervals and after three weeks of drying the samples' dry weight became stabilised for a week and this was considered as the dry weight of the sediment.

4.4.3.1 Sediment Wetness

Sediment wetness was calculated as the percentage difference in wet and dry weight:

Sediment Wetness (%) = $\frac{wet\ weight - dry\ weight}{wet\ weight} * 100\%$

Dry Bulk Density of pond sediment was defined as the dry weight of sediment divided by the volume of the pond sediment disc. This is similar to the empirical calculation of density (that is mass/volume).

Volume = πr^2h, (r= radius of corer and h = slice thickness)

Thus, Dry Bulk Density = $\frac{Dry\ weight}{Volume}$ (in g/cm^3)

4.4.3.2 Grinding and Sieving

After the drying phase, the samples (disc slices) were ground in a mortar with a pestle and large stones, vegetation and roots manually removed. Thereafter, the sediments were sieved (to 5µm) and kept in 10 ml vials for carbon and nitrogen analysis. In between grinding and sieving of each sediment slices (samples), the equipment was cleansed with acetone.

4.4.3.3 TEA Analysis of Pond Sediment

Percentage carbon and nitrogen were analysed in each pond sediment samples using the Total Elemental Analyser (TEA Brand: Thermo Scientific FLASH 2000 Series Organic

Elemental Analyser™ - Manufactured by Thermo Fisher Scientific Inc.). The combustion system worked by helium carrier gas (flow rate 130 ml/min) carrying 5 mg (approximate value recorded to 0.01 mg) of combusted dry sediment samples through a copper sulphate filter (which removes water from the combusted sediment) into a Gas Chromatography (GC) column and through magnesium perchlorate filter (which is a drying agent that dries the gas from the combusted sediment). The result was then detected by a thermal conductivity detector (TCD). The runtime per sediment sample was set at 360 seconds and two oven temperatures were set at 980 ^{0}C and 680 ^{0}C. This allowed for detection of nitrogen and carbon peaks.

After every 100 samples run in the TEA, the filters were changed and the system was recalibrated for a new run using aspartic acid as a known standard for instrumental calibration and an unknown standard (which is aspartic acid) to confirm instrumental calibration. Blanks were also run to check for any issue around TEA detection. Within each core and/or in every 10 samples, triplicates of that sample were run to check for the limit of quantification of the results.

4.4.3.4 Carbon and Nitrogen concentration (%)

The results of the TEA analyses were presented as percentage carbon and nitrogen, which were calculated from the 5 mg of combusted pond sediment. The results were collected for analysing carbon concentration, C:N ratio and carbon stocks.

4.4.3.5 Carbon Dry Bulk Density

Carbon dry bulk density in the pond sediment core was calculated as a percentage of the dry bulk density of pond sediment that contains only carbon.

Carbon Dry Bulk Density = $\frac{\%\,Carbon}{100} * Dry\ Bulk\ Density\ of\ Pond\ Sediment$ (in g/cm^3)

4.4.3.6 Carbon Nitrogen Ratio (C:N)

The C:N ratio was calculated from the percentage carbon and nitrogen (concentration) from the TEA analysis.

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

4.4.3.7 Carbon Stock

Given the dry bulk density in a pond core, carbon stock per square metre was calculated for ponds based on differences in pond types, size range and changes down core profile. Therefore, carbon stock per cm of depth per square metre of surface area was represented as the mass of carbon to volume ratio within a square metre of sediment.

Carbon Stock per cm depth per square metre pond = $\text{Carbon Dry Bulk Density} * \frac{10{,}000}{1{,}000}$

(in Kg C per sq. m)

Results were also presented as the sum of 5 x 1 cm depths in the down core profile analysis of carbon stock. Total carbon stock per core was the sum of all carbon stock per sediment disc in the entire core.

4.5 Results

4.5.1 Depth of Cores

The cores included 9 pasture ponds cores collected as triplicates from three size ranges and 9 other cores from the sand dune, arable and natural pond types as described in Table 4.2 and they also covered size range 10 – 100 sq. m, 100 – 1,000 sq. m and 1,000 – 10,000 sq. m. The depth of the cores varied but was longest in natural ponds and shortest in arable ponds.

Table 4.2: Depth of pond sediment cores analysed

Depth of Pond Cores

pond type	Number of Core analysed	Size Range		
		10 - 100 sq. m	100 - 1000 sq. m	1,000 - 10,000 sq. m
sand dune	3		12.00 cm, 12.00 cm	10.00 cm
arable pond	3	13.00 cm	7.00 cm	10.00 cm
natural pond	3		17.50 cm	22.00 cm, 22.00 cm
pasture pond	9	15.00 cm, 10.50 cm, 9.00 cm	19.50 cm, 17.00 cm, 22.00 cm	17.50 cm, 16.50 cm, 12.50 cm
total cores depth	18	47.50 cm	107 cm	110.5 cm

4.5.2 Descriptive assessments of ponds and pond cores

Temporary ponds include all ponds in size range 10 – 100 sq. m and all arable ponds.They were shallow in depth and mostly dried out. Permanent ponds included all ponds in size range 100 - 1,000 sq. m and 1,000 – 10,000 sq. m except for arable ponds. They are characterised by water cover all year round with potential wetting and drying of the pond edges.

Arable pond cores contained mainly fine-grained sediment with mainly earthenware red colour clay in the bottom sediment. The top sediment contained debris interwoven with fine grain sediments. Sand dune pond cores contained loosely brittle dark sediment interwoven with sandy sediments. They also contained macrophyte roots and other debris/detritus (Figure 4.2a). Pasture pond sediments were richly dark loamy sediments, with slightly sticky clayey texture and a smaller quantity of sand compared to sand dune sediments (Figure 4.2b). Natural pond sediment cores were similar to the pasture pond cores with rich dark loamy and slightly clayey sediments.They also contained macrophyte roots, sandy sediments and debris. During the sample collection, natural and pasture ponds in size range 1,000 - 10,000 sq. m had gas bubbles trapped in the sediment. These gas bubbles were not collected but lost to the atmosphere.

Figure 4.2a: Typical photo of a sand dune pond’s sediment core collected from Northumberland

Figure 4.2b: Typical photo of a pasture pond's sediment core collected from Northumberland

4.5.3 Sediment wetness

Generally, sediment wetness decreased from top to bottom of sediment cores and it was lowest in the bottom core sections of most ponds analysed. The topmost sediment in the natural pond (size range 1,000 – 10,000 sq. m) contained the highest sediment wetness (83.74%) of all 190 sediment discs from all ponds. Arable ponds (size range 1,000 - 10,000 sq. m) at depth 3 cm accounted for the lowest sediment wetness of 14.21% (Figure 4.3b). Overall, sediment wetness followed a similar pattern of high to low from top to bottom of the core except in arable pond sediments (Table 4.3, Table 4.4 and Figure 4.3 a, b, c and d). The arable pond sediments contained the lowest sediment wetness of all pond types and sand dune pond sediment were highest followed by pasture and natural ponds respectively (Table 4.3). Most of the sediment cores analysed showed an inverse graph-like reduction (negative, often statistically significant, correlation with depth) in sediment wetness down the core profile except for arable ponds 100 – 1,000 sq. m and

1,000 – 10,000 sq. m (which gave a positive correlation with depth Table 4.4). A non-parametric Kruskal-Wallis test showed f Significant differences between Pond Types with Kruskal-Wallis test value = 33.71, DF = 3, P = 0.000, N = 198. Statistical analysis was performed for only pond types (Table 4.4) as there were not enough core replicates to analyse differences between pond size ranges of each pond type for all variables studied in this chapter.

Table 4.3: Average sediment wetness across pond types

Pond Type	*Average Sediment wetness (%) across pond types*	*Number of sediment disc per pond type*
Sand Dune	47.64 ± 4.41	24
Arable	21.81 ± 1.28	18
Pasture	36.74 ± 1.04	107
Natural	35.84 ± 2.36	41

Table 4.4: Correlation of pond depth with sediment wetness

Correlation of Pond Depth with Sediment Wetness (Pearson Correlation, P value, N)

pond type	Size Range 10 - 100 sq. m		
	Pearson Correlation	P value	N
sand dune			
arable pond	-0.880	0.004	8
natural pond			
pasture pond a	-0.872	0.002	9
pasture pond b	-0.878	0.001	10
pasture pond c	-0.987	0.000	12
pond type	Size Range 100 - 1000 sq. m		
sand dune A	-0.893	0.003	8
sand dune B	-0.755	0.030	8
arable pond	0.797	0.107	5

natural pond	-0.802	0.002	12
pasture pond a	-0.932	0.000	15
pasture pond b	-0.911	0.000	13
pasture pond c	-0.244	0.380	15
pond type	Size Range 1,000 - 10,000 sq. m		
sand dune	-0.958	0.000	8
arable pond	0.946	0.015	5
natural pond 2A	-0.897	0.000	14
natural pond 2B	-0.662	0.007	15
pasture pond a	-0.816	0.001	12
pasture pond b	-0.869	0.000	13
pasture pond c	-0.833	0.000	15

Given the randomness of factors that control sediment wetness, the variations seem mainly controlled by the current weather state (precipitation and evaporation), water supply, sediment type and pond depth.

In the size range 10 – 100 sq. m, sediment wetness was strongly influenced by seasonal weather changes that come with quick seasonal drying and wetting of ponds. The pasture and arable ponds were sampled dry with no water cover. The arable pond recorded 30.50% and 21.77% at the topmost and bottom sediments respectively (Figure 4.3b). The pasture pond contained 29 – 58% from bottom to top sediment. Also, all pasture ponds, irrespective of the size range, showed similar trends in sediment wetness (Figure 4.3c). Thus, the variations in sediment wetness based on pond type showed that water supply were significant influencers of sediment wetness in ponds.

In size range 100 – 1,000 sq. m, the natural pond reduced from 47.39% to 26.61% from top to bottom and the reduction was gradual. In sand dune ponds, sediment wetness dropped at depth 1.5 cm but increased at 2.5 cm depth showing that moisture can change within short depths (Figure 4.3a). The variations seemed to be more dependent on localised factors within the pond sediments such as porosity, sediment grain size, sediment mixture and compaction.

In size range 1,000 – 10,000 sq. m, ponds were permanent except the arable pond. The top sediment recorded high sediment wetness then reduced down core profile except in the arable pond. Sediment wetness changes in pasture ponds were similar with size range 100 – 1,000 sq. m. The arable pond within this size range was linear from top to bottom.

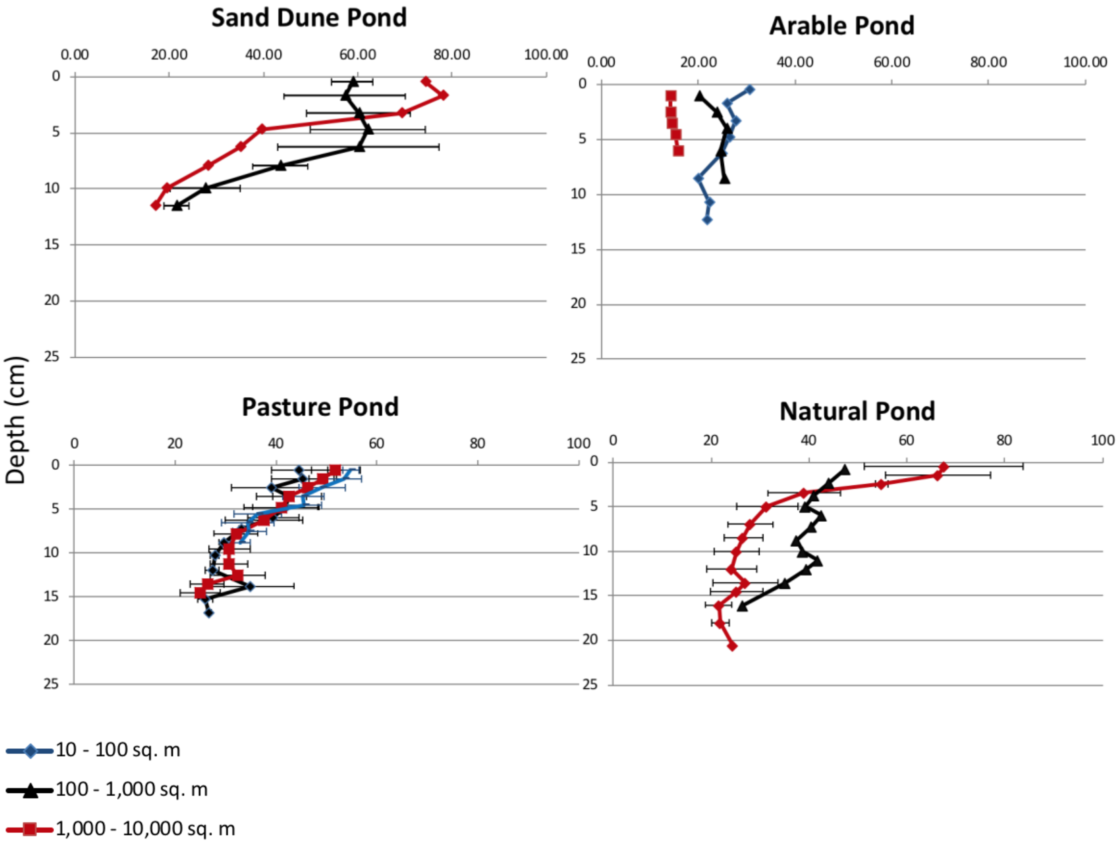

Figure 4.3: Sediment wetness in ponds (a) Sand Dune (b) Arable (c) Pasture (d) Natural ponds (where pasture ponds were triplicate cores and natural pond 1,000 – 10,000 sq. m and sand dune pond 100 – 1,000 sq. m were based on two samples collected at the edge and deeper part of the ponds)

4.5.4 Carbon Concentration

4.5.4.1 Carbon concentration across pond types

In all pond sediments, the overall average carbon concentration was 3.97 ± 0.26 %C (range 0.58 %C – 26.63 %C). The top layer of the sand dune pond (1,000 – 10,000 sq. m) recorded the highest carbon concentration in all samples but carbon concentration in the pond's bottom core was similar to sand dune pond (100 - 1,000 sq. m) (Figure 4.4a). Natural ponds ranged from 0.66 %C – 16.48 %C. The change down core in 100 – 1,000 sq. m was not linear (Figure 4.4d). All arable ponds showed a linear decrease of carbon concentrations with depth (Figure 4.4b).

Carbon concentration in pasture ponds are shown in Figure 4.4c, size range averages include 10 – 100 sq. m (6.65 ± 0.59 %C, range = 2.81 %C – 12.78 %C), 100 – 1,000 sq. m (2.41 ± 0.23 %C, range = 1.14 %C – 6.71 %C) and 1,000 – 10,000 sq. m (3.29 ± 0.29 %C, range = 1.27 %C – 7.83 %C), with more carbon concentrated in size range 10 – 100 sq. m (Figure 4.5).

Similar to sediment wetness, most of the sediment cores analysed showed an inverse graph-like reduction (negative, often statistically significant, correlation with depth) in carbon concentration down the core profile (Table 4.5). A non-parametric Kruskal-Wallis test showed Significant differences between between pond types with Kruskal-Wallis test value = 22.08, DF = 3, P = 0.000, N = 198.

Table 4.5: Correlation of pond depth with carbon concentration

Correlation of Pond Depth with Carbon Concentration (Pearson Correlation, P value, N)

pond type	Size Range 10 - 100 sq. m		
	Pearson Correlation	P value	N
sand dune			
arable pond	-0.514	0.193	8

natural pond			
pasture pond a	-0.747	0.021	9
pasture pond b	-0.730	0.016	10
pasture pond c	-0.904	0.000	12
pond type	Size Range 100 - 1000 sq. m		
sand dune A	-0.524	0.183	8
sand dune B	-0.878	0.004	8
arable pond	-0.066	0.916	5
natural pond	-0.340	0.916	12
pasture pond a	-0.734	0.002	15
pasture pond b	-0.781	0.002	13
pasture pond c	-0.748	0.001	15
pond type	Size Range 1,000 - 10,000 sq. m		
sand dune	-0.343	0.405	8
arable pond	0.209	0.736	5
natural pond 2A	-0.540	0.046	14
natural pond 2B	0.028	0.921	15
pasture pond a	-0.764	0.004	12
pasture pond b	-0.756	0.003	13
pasture pond c	-0.578	0.024	15

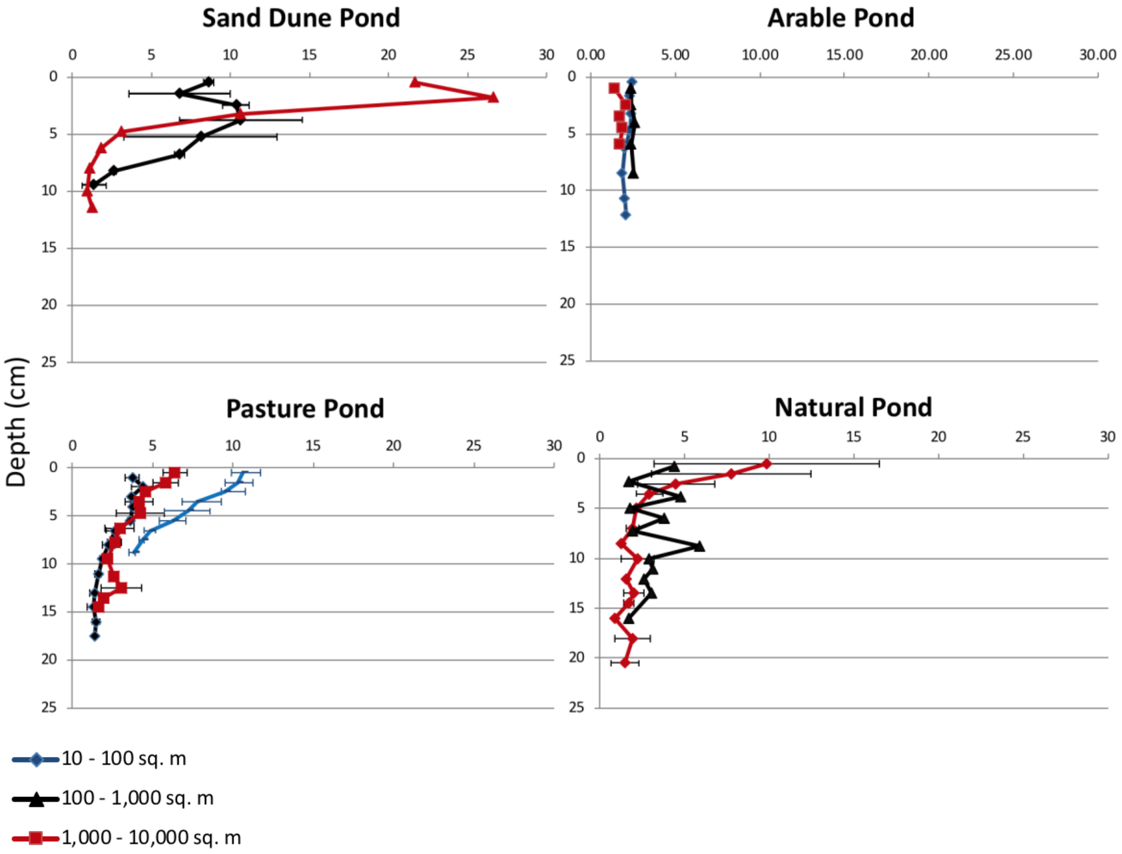

Figure 4.4: Carbon concentrations in ponds (a) Sand Dune (b) Arable (c) Pasture (d) Natural ponds (where pasture ponds were triplicate cores and natural pond 1,000 – 10,000 sq. m and sand dune pond 100 – 1,000 sq. m were based on two samples collected at the edge and deeper part of the ponds)

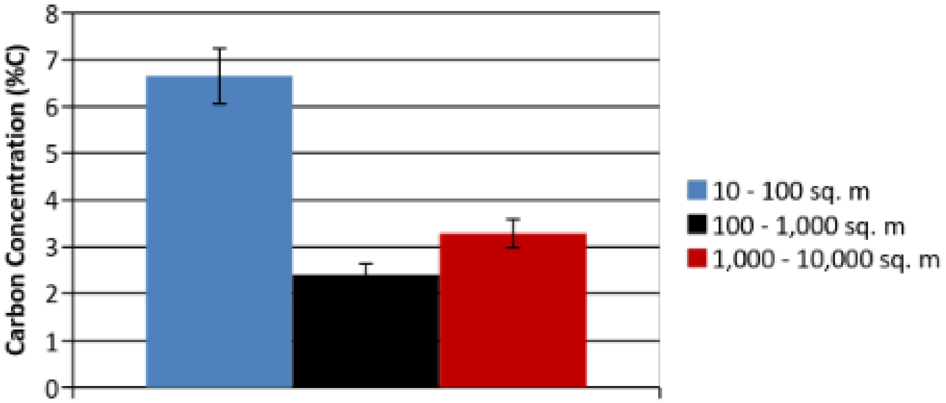

Figure 4.5: Average carbon concentration in pasture pond in size ranges 10 - 100 sq. m, 100 - 1,000 sq. m and 1,000 - 10,000 sq. m

4.5.4.2 Carbon concentration across size ranges

In all size ranges, carbon concentration decreased down the core profile (Figure 4.6) and concentration varied in all pond sediment cores. Ponds within the size range 10 – 100 sq.

m recorded the highest carbon concentrations. The topmost sediment averaged 8.78 ± 2.16 %C while the bottom sediment contained 2.96 ± 2.09 %C. Size range 100 - 1,000 sq. m recorded lower carbon concentration compared to 10 – 100 sq. m and 1,000 – 10,000 sq. m. Ponds within size range 1,000 – 10,000 sq. m have the highest carbon concentration in top sediment averaging 8.77 ± 2.44 % but their overall average was less than 10 – 100 sq. m (Figure 4.6). The trend showed a gradual reduction in carbon concentration from top to bottom of pond sediments.

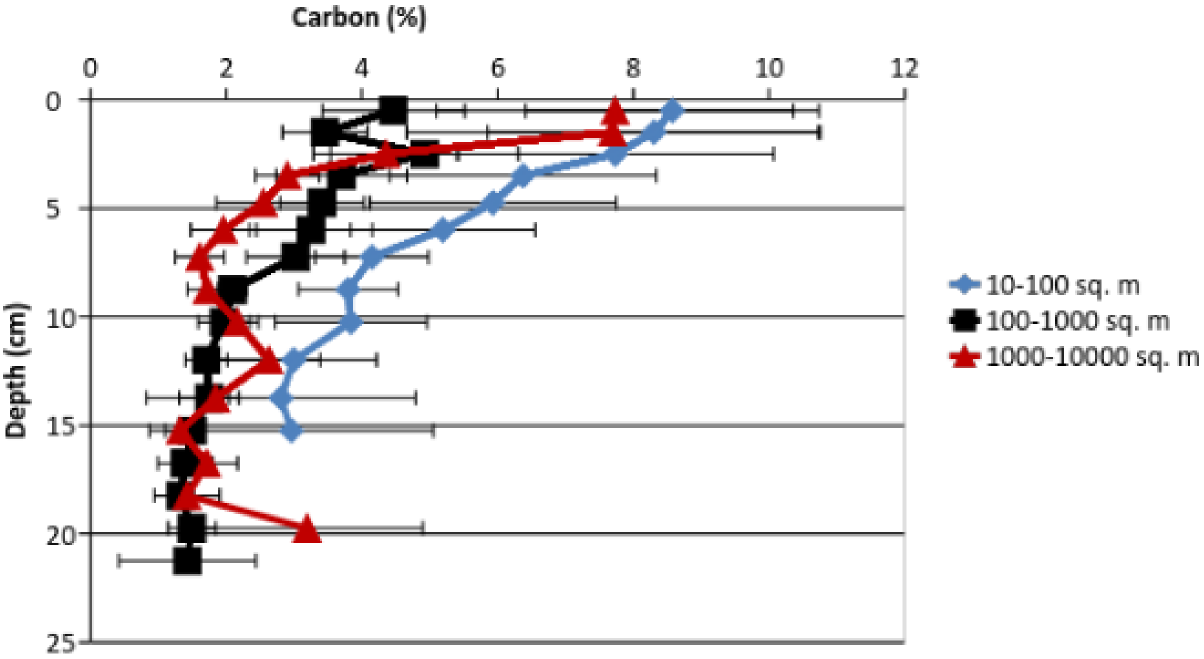

Figure 4.6: Carbon concentration across size ranges

4.5.5 Carbon Nitrogen Ratio (C:N ratio)

From all ponds, maximum and minimum nitrogen concentrations were 1.61% and 0.09% and they were recorded in sand dune ponds (size range 1,000 – 10,000 sq. m). The overall average C:N ratio was 10.64 ± 0.12 (range 6.69 – 16.55). The C:N ratio decreases with depth except in all arable ponds and natural pond (100 – 1,000 sq. m). Arable ponds remained at a roughly constant level down the core profile, ranging from 8.86 – 10.93 in all size ranges (Figure 4.7b). Sand dune ponds in all size ranges ranged from 8.44 to 16.55 (Figure 4.7a). The C:N ratio in natural ponds ranged from 6.69 – 14.12. C:N ratio averages based on size range included 10.15, 9.30 and 9.87 in size range 10 – 100 sq. m, 100 – 1,000 sq. m and 1,000 -10,000 sq. m respectively. Pasture pond size range averages include 10 – 100 sq. m (10.23 ± 0.09), 100 – 1,000 sq. m (9.51 ± 0.14) and 1,000 – 10,000 sq. m (10.88 ± 0.17).

Similar to sediment wetness and carbon concentration, most of the sediment cores analysed showed an inverse graph-like reduction (negative correlation with depth) in C:N ratio down the core profile, with many having statistically significant correlation P <0.05 (Table 4.6). A non-parametric Kruskal-Wallis test showed significant differences between pond types with Kruskal-Wallis test value = 41.98, DF = 3, P = 0.000, N = 198.

Table 4.6: Correlation of dond depth with C:N ratio

Correlation of Pond Depth with C:N ratio (Pearson Correlation, P value, N)

pond type	Size Range 10 - 100 sq. m		
	Pearson Correlation	P value	N
sand dune			
arable pond	-0.608	0.110	8
natural pond			
pasture pond a	-0.758	0.018	9
pasture pond b	-0.029	0.938	10
pasture pond c	-0.705	0.010	12
pond type	Size Range 100 - 1000 sq. m		
sand dune A	-0.685	0.061	8
sand dune B	-0.921	0.001	8
arable pond	0.782	0.118	5
natural pond	-0.096	0.767	12
pasture pond a	-0.791	0.000	15

pasture pond b	-0.902	0.000	13
pasture pond c	-0.629	0.012	15
pond type	Size Range 1,000 - 10,000 sq. m		
sand dune	-0.347	0.399	8
arable pond	0.301	0.622	5
natural pond 2A	-0.923	0.000	14
natural pond 2B	-0.471	0.077	15
pasture pond a	-0.738	0.006	12
pasture pond b	-0.761	0.003	13
pasture pond c	-0.600	0.018	15

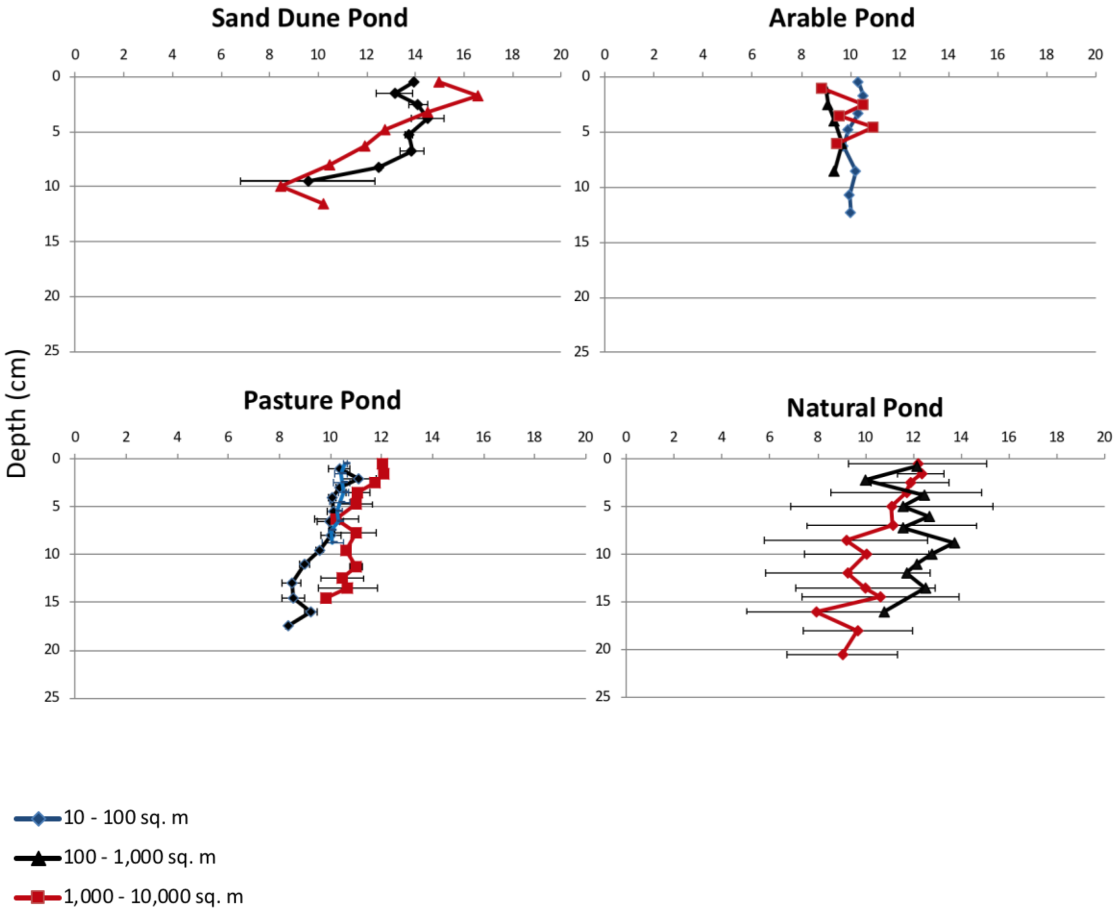

Figure 4.7: C:N ratio in ponds (a) Sand Dune (b) Arable (c) Pasture (d) Natural ponds (where pasture ponds were triplicate cores and natural pond 1,000 – 10,000 sq. m and sand dune pond 100 – 1,000 sq. m were based on two samples collected at the edge and deeper part of the ponds)

4.5.6 Dry Bulk Density

4.5.6.1 Sediment Dry Bulk density

Ponds' sediment dry bulk density increased from top to bottom of the ponds except in arable ponds (Figure 4.8a). For sand dune, natural and pasture pond >100 sq. m with water cover, sediment dry bulk density was low in the top sediment and increased down core. In the top sediment of ponds <100 sq. m (all of which have no water cover) and all arable ponds, sediment dry bulk density was higher compared to the ponds with water cover. Dry bulk density >1 g/cm^3 was associated with low sediment wetness and <1 g/cm^3 relates to high sediment wetness or a mixture of low moisture and soil air in temporary ponds. In the top 3 cm of pond sediment, averages of size range 100 - 1,000 sq. m and 1,000 - 10,000 sq. m were 0.91 ± 0.13 g/cm^3 and 0.81 ± 0.15 g/cm^3 respectively. As they were <1 g/cm^3, it connoted low sediment dry bulk density (Figure 4.8a). However, sediment dry bulk density increased with depth and it was characterised by lowered sediment wetness. Size range 10 - 100 sq. m was characterised by a gradual increase in sediment dry bulk density from the top sediments towards the bottom. In the bottom core, sediment dry bulk density was highest in size range 1,000 - 10,000 sq. m (Figure 4.8b).

Unlike statistical analyses of sediment wetness, carbon concentration and C:N ratio which showed more negative correlations, most of the sediment cores analysed for sediments dry bulk density showed more positive, often significant, correlations with pond depth (Table 4.7). A non-parametric Kruskal-Wallis test showed significant differences between pond types with Kruskal-Wallis Test value = 20.46, DF = 3, P = 0.000, N = 198.

Table 4.7: Correlation of pond depth with sediment dry bulk density

Correlation of Pond Depth with sediment dry bulk density (Pearson Correlation, P value, N)

pond type	Size Range 10 - 100 sq. m		
	Pearson Correlation	P value	N

sand dune			
arable pond	0.700	0.053	8
natural pond			
pasture pond a	0.619	0.075	9
pasture pond b	0.797	0.006	10
pasture pond c	0.954	0.000	12
pond type	Size Range 100 - 1000 sq. m		
sand dune A	0.776	0.024	8
sand dune B	0.966	0.000	8
arable pond	-0.251	0.684	5
natural pond	0.303	0.338	12
pasture pond a	0.467	0.079	15
pasture pond b	0.911	0.000	13
pasture pond c	-0.016	0.956	15
pond type	Size Range 1,000 - 10,000 sq. m		
sand dune	0.756	0.030	8
arable pond	0.001	0.999	5
natural pond 2A	0.685	0.007	14
natural pond 2B	0.496	0.060	15
pasture pond a	0.649	0.023	12
pasture pond b	0.829	0.000	13
pasture pond c	0.840	0.000	15

4.5.6.2 Carbon Dry Bulk Density

Carbon dry bulk density changed down core profiles in all pond sediments and across ponds. The general trend for all pond types decreased down core profile except in arable ponds. The smaller ponds (10 - 100 sq. m) contained higher carbon dry bulk density compared to larger ponds (100 - 1,000 sq. m and 1,000 - 10,000 sq. m). Also, the samples with high sediment wetness had higher carbon dry bulk density. Arable ponds were characterised by very low sediment wetness and consequently very low carbon dry bulk density.

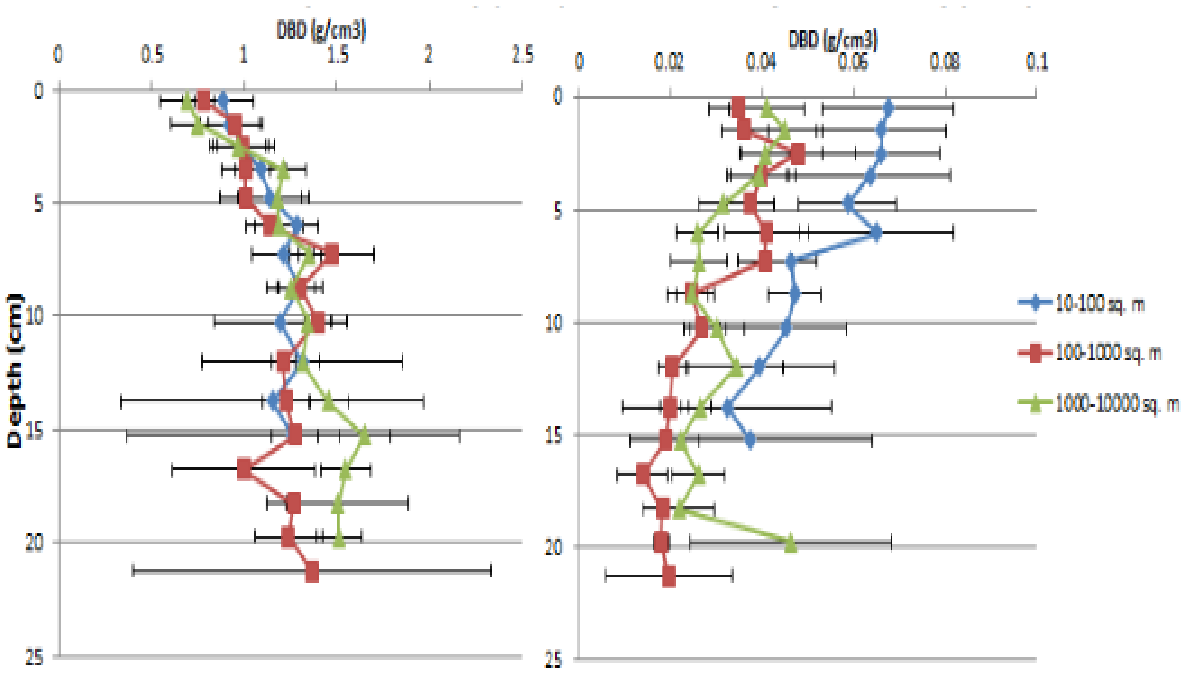

Figure 4.8: Dry Bulk Density of (a) Pond Sediment (left) and (b) Carbon (right)

4.5.7.1 Carbon Stock in Ponds

The average carbon stock per sq. m varied between ponds and within ponds. Table 4.8 shows carbon stock in different ponds and size ranges, and Table 4.9 showed correlation of pond depth with carbon stock in ponds. Based on the depth of each pond core analysed, the overall pond average for size ranges 10 - 100 sq. m, 100 – 1,000 sq. m and 1,000 – 10,000 sq. m were 4.48 ± 2.07 kg C/sq. m, 3.04 ± 0.61 kg C/sq. m and 3.64 ± 0.88 kg

C/sq. m respectively (Table 4.8). The changes in size range showed carbon stocks in pond sediment did not decrease with increasing size ranges but are controlled by the prevailing factors that support or limit carbon stocking in individual pond sediments. Carbon stock was highest in size range 10 – 100 sq. m. On average, size range 10 - 100 sq. m accumulated more carbon than 100 – 1,000 sq. m and 1,000 – 10,000 sq. m (Figure 4.9). The pond type averages include sand dune ponds (3.75 ± 0.84 kg C/sq. m), arable ponds (1.65 ± 0.38 kg C/sq. m), natural ponds (3.74 + 0.01 kg C/sq. m) and pasture ponds (5.26 ± 0.73 kg C/sq. m). The lowest carbon stocks per sq. m were arable ponds, probably as they were relatively shallow and dry.

Table 4.8: Carbon stock of ponds in Northumberland (average value taken for sand dune pond (100 - 1,000 sq. m), natural pond (1,000 - 10,000 sq. m) and averages for all pasture ponds)

Pond Type	*size range (sq. m)*			
	10-100 sq. m (kg C/sq. m)	100-1,000 sq. m (kg C/sq. m)	1,000 - 10,000 sq. m (kg C/sq. m)	Pond Average (kg C/sq. m)
sand dune		2.92 ± 0.17	4.59	3.75 ± 0.84
arable pond	2.41	1.36	1.17	1.65 ± 0.38
natural pond		3.84	3.64 ± 1.49	3.74 ± 0.01
pasture pond (Average)	6.55 ± 0.53	4.04 ± 0.23	5.18 ± 0.63	5.26 ± 0.73
Size Range Average	4.48 ± 2.07	3.04 ± 0.61	3.64 ± 0.88	

Arable and natural ponds showed positive, nut non-significant, correlations of carbon stock with depth, and most pasture ponds showed negative, sometimes significant correlations except for one pasture core analysed at size range 10 – 100 sq. m (Table 4.9). A non-parametric Kruskal-Wallis test showed significant differences between pond types with Kruskal-Wallis Test value = 13.31, DF = 3, P = 0.004, N = 198.

Table 4.9: Correlation of pond depth with carbon stock

Correlation of Pond Depth with carbon stock in ponds (Pearson Correlation, P value, N)

pond type	Size Range 10 - 100 sq. m		
	Pearson Correlation	P value	N
sand dune			
arable pond	0.397	0.330	8
natural pond			
pasture pond a	-0.530	0.142	9
pasture pond b	0.292	0.413	10
pasture pond c	-0.768	0.004	12
pond type	Size Range 100 - 1000 sq. m		
sand dune A	0.080	0.850	8
sand dune B	-0.858	0.006	8
arable pond	0.703	0.185	5
natural pond	0.101	0.754	12
pasture pond a	-0.150	0.594	15
pasture pond b	-0.562	0.046	13
pasture pond c	-0.409	0.130	15
pond type	Size Range 1,000 - 10,000 sq. m		
sand dune	-0.087	0.838	8
arable pond	0.163	0.794	5
natural pond 2A	0.032	0.914	14
natural pond 2B	0.423	0.116	15
pasture pond a	-0.730	0.007	12
pasture pond b	-0.591	0.330	13
pasture pond c	-0.431	0.109	15

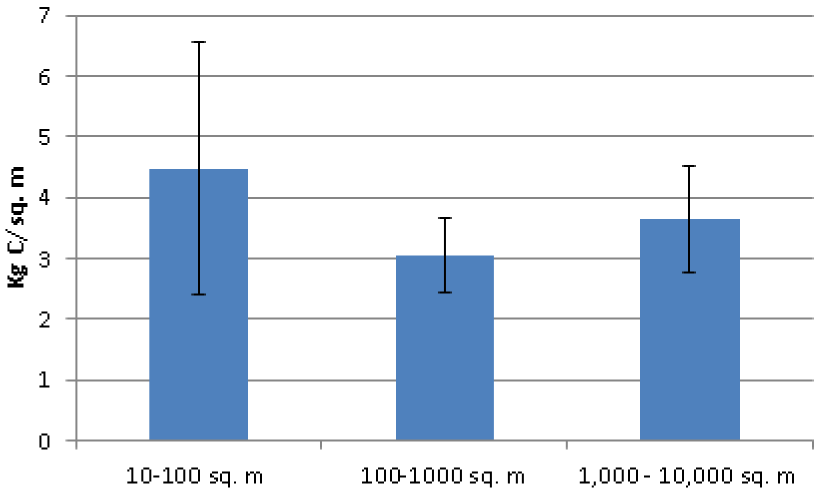

Figure 4.9: Average carbon stock per sq. m across size ranges

4.5.7.2 Carbon stock in pasture ponds

Overall average carbon stocks within pasture ponds were 5.26 ± 0.73 kg C/sq. m and it was the highest amongst all pond types. Size range 10 – 100 sq. m had an average of 6.55 ± 0.53 kg C/sq. m (range = 5.49 – 7.22 kg C/sq. m) and this is the highest recorded in all pond size ranges and pond types. Furthermore, carbon stocks vary within pasture ponds and size range as shown in Table 4.10.

Table 4.10: Carbon stock of pasture ponds in Northumberland in size range 10 - 100 sq. m, 100 - 1,000 sq. m and 1,000 - 10,000 sq. m

Total Carbon Stock per sq. m in Pasture Pond

Pond core	size range (sq. m)		
	10 - 100 sq. m (kg C/sq. m)	100 - 1,000 sq. m (kg C/sq. m)	1,000 - 10,000 sq. m (kg C/sq. m)
c1	6.94	3.59	6.35
c2	5.49	4.30	4.21
c3	7.22	4.25	4.96
Size Range Average	6.55 ± 0.53	4.04 ± 0.23	5.18 ± 0.63

4.5.8 Average carbon stock down core profile

In the down core analysis at 5 cm intervals, results showed a general decrease in carbon stock down core in both size range and pond type, with all cores analysed being at least 5 cm deep.

4.5.8.1 Top 5 cm of pond depth

This is the most biologically active layer of pond sediments, given it has low sediment dry bulk density and high sediment wetness except in arable ponds. Table 4.11 shows the carbon stock average in the top 5 cm. The overall carbon stock averages were higher in size range 10 - 100 sq. m (Table 4.11). Carbon stocks were highest in pasture pond followed by the sand dune, natural and arable ponds respective (Figure 4.10).

Table 4.11: Carbon Stock in the top 5 cm of pond sediment

Pond Type	*size range (sq. m)*		
	10-100 sq. m (kg C/sq. m)	100-1,000 sq. m (kg C/sq. m)	1,000 - 10,000 sq. m (kg C/sq. m)
sand dune		2.04 ± 0.01	2.81
arable pond	1.15	0.82	0.97 ± 0.40
natural pond		0.99	1.24
pasture pond (Average)	3.58 ± 0.44	1.84 ± 0.31	2.32 ± 0.10
Size Range Average	2.36 ± 1.21	1.42 ± 0.30	1.84 ± 0.43

4.5.8.2 5 – 10 cm of pond depth

This contained the second highest carbon stock on average in the down core profile analysis of ponds. Similar to the top 5 cm, pasture pond accumulated the most carbon stock on average and pasture pond in size range 10 – 100 sq. m stocked the most carbon in all pond size ranges. Carbon stocks were lowest in arable ponds. Based on size range, carbon stock accumulation was highest in 10 – 100 sq. m and lowest in 100 – 1,000 sq. m (Table 4.12 and Figure 4.10).

Table 4.12: Carbon Stock in 5 cm - 10 cm depth of pond sediment

Pond Type	*size range (sq. m)*		
	10-100 sq. m (kg C/sq. m)	100-1,000 sq. m (kg C/sq. m)	1,000 - 10,000 sq. m (kg C/sq. m)
sand dune		0.72 ± 0.27	1.77
arable pond	0.65	0.54	0.20
natural pond		1.56	0.76 ± 0.34
pasture pond (Average)	2.40 ± 0.04	1.06 ± 0.07	1.40 ± 0.28
Size Range Average	1.53 ± 0.88	0.97 ± 0.22	1.03 ± 0.35

4.5.8.3 10 – 15 cm of pond depth

Maximum core depth for sand dune (1,000 – 10,000 sq. m) and arable ponds (100 – 1,000 sq. m and 1,000 – 10,000 sq. m) was under 10 cm. Therefore, no data were recorded for those ponds. However, pasture ponds in all size ranges had the most carbon stock per square metre on average. Natural pond (size range 100 - 1,000 sq. m) stocked the most carbon stock based on size range (1.15 kg C/sq. m). Overall, the size range averages were 0.59 kg C/sq. m, 0.64 kg C/sq. m and 0.90 kg C/sq. m of size ranges 10 – 100 sq. m, 100 – 1,000 sq. m and 1,000 – 10,000 sq. m respectively (Figure 4.10).

4.5.8.4 15 - 20 cm of pond depth

All the cores from ponds within size range 10 – 100 sq. m were under 15 cm in length. Therefore, only ponds in size range >100 sq. m and <10,000 sq. m were analysed. The pond types were pasture and natural ponds with each being in size ranges 100 – 1,000 sq. m and 1,000 – 10,000 sq. m. They stocked averages of 0.27 kg C/sq. m and 0.44 kg C/sq. m respectively (Figure 4.10).

4.5.8.5 20 – 25 cm in pond depth

Only pasture pond (100 – 1,000 sq. m) and natural pond (1,000 – 10,000 sq. m) had depths >20 cm and they averaged 0.83 kg C/sq. m and 0.41 kg C/sq. m respectively (Figure 4.10).

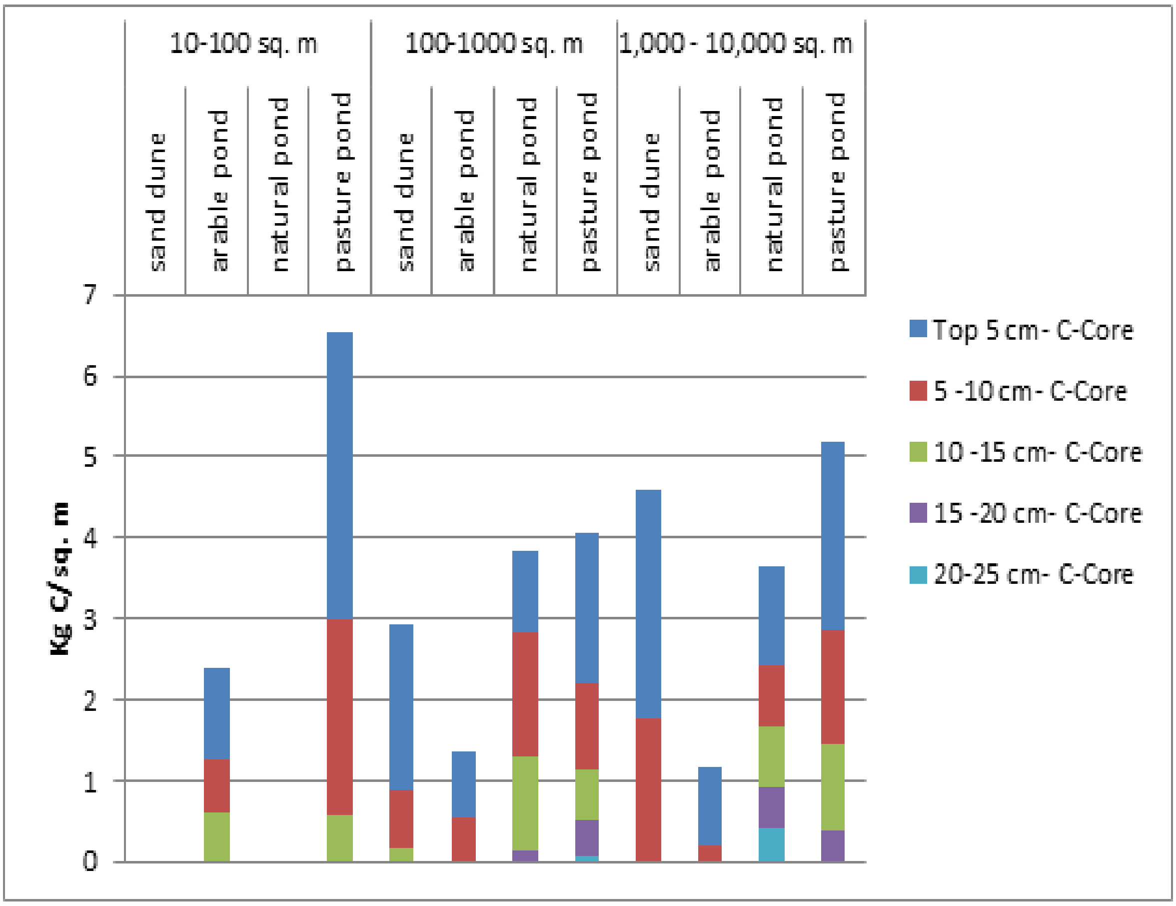

Figure 4.10: Down core profile of carbon stock in Northumberland

4.6 Discussions

4.6.1 Pond sediment core

Core depth in Northumberland ponds is strongly controlled by the underlying pond sediment types, texture, composition and water penetration. In the sand dune pond, the sediment trend was from soft sandy sediment at the top to rocky-clayey bottom soils. Arable pond sediments were characterised by tough compacted clay sediment with no water cover and low sediment wetness. The natural and pasture ponds were mainly permanent ponds, except the pasture ponds in size range 10 - 100 sq. m, and were easier to sample compared to temporary ponds due to high sediment wetness. And low sediment wetness, high sediment compaction and/or clay content in pond sediment made it difficult to collect arable pond cores using a manual corer. Hence, they were short compared to other pond types. Each pond analysed exhibited diverse statistical relationships with pond depth in the analyses of sediment wetness, carbon concentration, C:N ratio, dry bulk density and carbon stock.

4.6.2 Sediment wetness, sediment and carbon dry bulk density as factors that control carbon stocks in pond sediment

In each pond, the relationship between sediment wetness and dry bulk density (of carbon and sediment) was different. Overall, there is an inverse relationship between sediment dry bulk density and sediment wetness. Whereby high sediment dry bulk density begets low sediment wetness. Carbon dry bulk density was inversely related to sediment dry bulk density. The relationship between sediment wetness, sediment and carbon dry bulk density can also be related to the area of microbial influence and carbon processing in pond sediment (Boyd *et al.,* 2010 and Grabowski *et al.,* 2011), as the moisture aids the movement of nutrient and microbes through pore spaces in sediments.

Despite the significant role sediment wetness play in creating a direct relationship with the carbon concentration of pond sediments within size range 1,000 - 10,000 sq. m, except in arable ponds, it has an unclear relationship with ponds <1,000 sq. m and this could potentially be as a result of multiple environmental, biological and geochemical factors that are required to be in play for efficient carbon stocking. The storage and flux of carbon in sediment is therefore a conglomerate effect of biological, geochemical and environmental factors inherent in the pond environment (Boyd *et al.,* 2010, Cole *et al.,* 2007, Gilbert *et al.,* 2014, Grabowski *et al.,* 2011 and Seekell *et al.,* 2013) of which sediment wetness or moisture plays a significant role in microbes and material transport. Irrespective of the size range of ponds or temporary or permanent ponds, natural, sand dune and pasture ponds showed high sediment wetness in the top sediment as a result of low compaction and high porosity (Grabowski *et al.*, 2011) and gradually declined down core profile due to increased sediment compaction. A good example was the top sediment of pasture pond (size range 10 - 100 sq. m) which was sampled with no water cover. It contained 58% sediment wetness in its pore spaces and these values were similar to moisture in permanent ponds. In the sediment bottom with high compaction, fewer pore spaces and fine clayey materials in sand dune pond, sediment wetness reduced to levels that were similar to arable ponds. Arable ponds of all size ranges were characterised by richly fine-grained clay soil with limited pore spaces for moisture penetration, hence, lowered sediment wetness. This also resulted in lowered carbon concentration compared to other pond types analysed.

Fine-grained bottom sediment and increased dry bulk density lower carbon concentration in sediment (Avnimelech *et al.,* 2001), that is, an inverse relationship exists. Variation in dry bulk density can be related to material transport in ponds. In large lakes, river systems and oceans, sedimentary material transport involves the quick settlement time of large coarse material whilst finer materials are transported for longer distances in the aquatic system (Grabowski *et al.*, 2011). This is different in small water body systems; the material transport occurs within a short distance and short sedimentation columns. It results in a mix of coarse and fine materials in the top sediment of ponds. Furthermore, it creates increased pore spaces for moisture penetration that permit the development of microbial biofilms. The microbial biofilms attach to the inorganic minerals in the sediment (Boyd *et al.*, 2010). This is potentially the consequence of the higher carbon concentration in the top sediment in all pond size range compared to the pond bottoms. However, carbon concentration decreased down core profiles as a result of increased dry bulk density and reduced porosity caused by sediment overburden.

4.6.3 C:N ratio analyses of carbon source in small water bodies

Gilbert *et al.* (2014) identified the variability in carbon storage within ponds. In term of the material transport, lentic water bodies are more effective in material sedimentation compared to rivers and streams and other lotic systems. River systems show transient variation in carbon storage and source (C:N ratio) at the different locations within the river and this is due to continuous changes triggered by material entry and transport (Kaushal and Binford, 1999). Despite variations in levels of carbon concentrations in the same pond sediment, pond sediments show close similarities in terms of carbon source at different depths within the sediment and different parts of the pond. This is a function of reduced physical perturbation rather than stream material flow that occurs in lotic systems. In the analysis of the pasture ponds, they showed a similar source of carbon in the top sediments with C:N ratio ranging from 10-12:1. This is significant as C:N ratio serves as a proxy for describing the source of carbon (Ishiwatari and Uzaki, 1987 and Kaushal and Binford, 1999). The similarities in C:N ratios are also associated with the closely similar physical, chemical and microbial processes that occur in the ponds. Although these influences and carbon source are closely similar, the variation in C:N ratio range between 10-12:1 could potentially be reflecting the slight changes in microbial community structure (Briée *et al.*, 2007), and non-uniform physical and chemical influences in different depths within the sediment and different parts of the pond. Knowledge on microbial community structure and its influence on carbon source identification are explored further in Chapter 5.

The C:N ratio down core profile showed the changes in terms of carbon sources that are buried within a pond during pond succession and the relationship between allochthonous and autochthonous carbon inputs. This can be related to flux in terrestrial carbon entry and aquatic primary productivity in ponds over time. The bottom sediment of the sand dune ponds contained C:N ratio <10:1 and this indicated that the ponds were predominantly producing aquatic carbon at that time or high oxidation of terrestrial carbon. Lowered C:N ratio could potentially be as a result of bacteria in the pond bottom which are nitrogen-limited using up the nitrogen from aquatic algae (Muller, 1977) and creating ammonia by-products which elevate nitrogen in the pond bottom and lower the C:N ratio in sediment. It can also be as a result of high oxidation and/or degradation of terrestrial carbon in the ponds' bottoms. The C:N ratio gradually increased towards the top sediment and this can be related to change in organic carbon source, that is, more terrestrial input possibly from a change in surrounding vegetation (pond succession). The two size ranges of natural ponds had similar C:N ratios at the top sediment ranging from 10-12:1 but carbon concentrations differed. Size range 1,000 - 10,000 sq. m (C:N ratio <10:1) graduated into a more predominantly aquatic carbon or highly oxidised C:N ratio with depth while 100 - 1,000 sq. m (C:N ratio >10:1) remained slightly more terrestrial carbon source. This suggests that the nature of organic carbon stored in ponds was controlled by the surrounding depositional environment and nature of microbial influence within the pond.

Arable ponds showed C:N ratios ranging between 8-11:1. This range of C:N ratio could be a result of low carbon concentration and reduced proliferation of microbes in clayey sediments. Other contributory factors to this level of C:N ratio in arable ponds are shallow depths, low moisture penetration from drying and wetting of sediment, high compaction and low pore spaces within the sediment. Arable pond sediments' carbon could be terrestrial or aquatic sourced but its nature may have been highly oxidised in the pond or during transport to the pond.

Overall, C:N ratio for pond types and size ranges ranged between 8-14:1. Organic carbon source varies between aquatic (autochthonous) carbon, terrestrial (allochthonous) carbon and highly oxidised carbon sources depending on the depositional environment or source. The results did not indicate a significant entry of vascular plant material as a carbon source. However, it is unknown if the vascular plant entry was undetected as a result of high oxidation during transport to the ponds or oxidation as a result of microbial utilisation in the pond (Emerson and Hedges, 2003).

4.6.4 Carbon Stock in Small Water Bodies

4.6.4.1 Carbon Stock based on Pond Types and Size Ranges

There were no direct relationships connecting carbon stocks in pond sediments and the pond size. This suggests that pond sizes are one of many other factors that control carbon stocking but its influence is not as significant compared to dry bulk density, sediment wetness or organic carbon source. Some of the other influencers could be the depth of water column where primary productivity occurs, and the concentration of nutrients that support carbon processing, amongst other influencers of carbon stock levels. Carbon stocks were highest in size range 10 - 100 sq. m because small size water bodies are favoured to capture more carbon due to their ability to quickly saturate themselves with material during the wetting phase of the ponds. And these materials are transported into pond bottoms as colluvium or alluvium. Thereafter, microbial processing, sediment compaction and pond inorganic materials determine the propensity for storage and preservation or mineralization. The small size of pond further enhances nutrients and bio-element concentration which favour continuous microbial processes. In the pasture pond 10 - 100 sq. m, 6.55 kg C/sq. m were stocked in sediments down core profile while in the arable pond (10 - 100 sq. m), 2.41 kg C/sq. m were stocked. The variation potentially ties to the microbial utilisation of carbon and access to nutrients, which are less likely to be accessed in arable ponds compared to pasture ponds, due to high sediment compaction that limits material movement within the fine sediments of arable ponds. Shirakova *et al.* (2013) identified a relationship between oxygenation in water bodies and carbon storage. The high carbon stock can be tied to the variations in oxygen levels within the two ponds. Arable ponds with very shallow depths are most likely exposed to more surface interactions that favour mineralisation processes of carbon compared to deeper pasture depths which favour stocking of more carbon; alongside creating anoxic bottoms as a result of high terrestrial inflow from the surrounding environment (Kortelainen *et al.,* 2004, Kortelainen *et al.,* 2006, Mulholland and Elwood, 1982 and Shirokova *et al.,* 2013).

The reality is that increased carbon stock in pasture ponds in size range 10 – 100 sq. m occurs as result of multiple factors that favour carbon stocking in ponds. They could include material transport, autochthonous microbes, nature of the pond and type of sediment minerals (clay, loamy or sandy sediment) amongst others. These influences vary widely and are also associated with the flux potential of the carbon stock in inland water bodies (Premke *et al.,* 2016) and their interactions determine if a pond is a net source or sink.

In the size range 100 - 1,000 sq. m, the pasture pond (4.04 kg C/sq. m) accounted for the highest carbon stock in this size range. Generally, pasture ponds are characterised by a mixture of predominantly dark loamy sediment laced together in clay and a small proportion of sandy sediment. This makes the pond sediments favourable for microbial processing of allochthonous and autochthonous carbon. The dry bulk density of pasture ponds generally trends from less compact sediment at the top which favours carbon processing and increased compaction down the core which favours lock-in of carbon in sediments. There was a similar pattern in the natural pond (100 - 1,000 sq. m) which stocked 3.84 kg C/sq. m. Carbon stock in the arable pond (1.36 kg C/sq. m) and sand dune (2.92 kg C/sq. m) ponds were low compared to the natural and pasture ponds in this size range for two distinctive reasons. Arable ponds sediment limited microbial penetrations, while sand dune ponds contain very loose sandy (coarse) sediments compared to the other pond types which allowed for moisture penetration. Also, the high sediment wetness in loose sediment can create uneven displacement of carbon in sediment and remobilizes carbon into the water column by leaching. However, the looseness of the sediment potentially allows for microbial proliferation through sediments and creation of biofilms with inorganic sediments (Grabowski *et al.*, 2011). The absence of or low percentage of fine grain sediment to lock in carbon favours displacement and remobilisation of carbon into the water column which is further utilised for oxidative processes.

In size range 1,000 - 10,000 sq. m, carbon stock varied and this could be related to variations in sedimentary transport. Compared to size ranges 10 - 100 sq. m and 100 - 1,000 sq. m, sedimentary material transport in size range 1,000 - 10,000 sq. m potentially follows the patterns of finer material travelling further away from the pond edge while coarse sediments settle at the pond edge. Although the distinction in sedimentary material distribution is unconfirmed in this research, it is expected to lie between larger lakes and oceans, and unconsolidated material distribution of ponds <1,000 sq. m. The variation in carbon stocks is controlled by factors such as sediment grain size gradient, microbial process and carbon source, and their diversity in different parts of the pond. Arable ponds (size range 1,000 - 10,000 sq. m - 1.17 kg C/sq. m) recorded the lowest carbon stock in all ponds analysed as a result of these factors and their highly compacted fine sediments. Also during the dry phase of arable ponds, they can become dense and caked sediments which limits microbial processes. The arable ponds in Northumberland were sampled in their dry state as fine sediment with low sediment wetness or moisture.

4.6.4.2 Variation in Carbon Stocks within ponds

Boyd *et al.* (2010), Gilbert *et al.* (2014), Pitman *et al.* (2013) and Sobek *et al.* (2005) have all identified variations in carbon storage across ponds and within pond systems. Also, results within this research confirm the carbon stock variations within and across pond types and pond size ranges. In the analysis of the triplicate pasture pond cores in all three size ranges, carbon stock in each pond had a varying degree of deviation (standard errors) from the average value and none of the ponds showed similar carbon stock. This is also confirmed in the natural pond (1,000 - 10,000 sq. m) with carbon stocks of 2.14 kg C/sq. m and 5.14 kg C/sq. m within the same pond. The variation within the ponds could be the function of the various in-situ microbial activities and material processing/material inflow (transport) in the ponds. Also, the carbon stock levels could vary as a result of anthropogenic activities occurring within the pond such as animal waste inclusion in pasture ponds and pond ecosystem perturbation by humans, cattle, other domestic animals and wild animals.

Individual cores analysed in pasture ponds in all three size ranges ranked amongst the highest carbon stocks in Northumberland ponds. Their carbon stock levels did not vary as widely as in the natural ponds. But variations occurred in pasture ponds maybe as a result of the uneven and poor dispersal of sedimentary material into and within the ponds.

4.6.4.3 Temporary and Permanent Ponds

Seasonal variations affect the carbon levels in SWBs. For example, increased rainfall permits material flow into water bodies, increased allochthonous carbon deposition and development of primary productivity of DOC and colloidal DOC (Shirakova *et al.*, 2013). Generally, ponds in Northumberland are characterised by seasonal drying and wetting. Temporary ponds dry out completely at some point in a year while permanent ponds lose moisture but do not dry completely. When these ponds are filled and over-capacitated, there is an overflow that causes the expansion of the pond area. Thus, shallow small water bodies are dynamic in terms of their surface area. The overflow of SWBs allow for the collection of materials that are deposited in the pond bottoms. This can trigger anoxia, and carbon processing leading to sequestration or atmospheric loss. Depending on pond type, depths and surface area, small ponds accumulate organic and inorganic materials which include vegetation, detritus, animal waste, agricultural and non-agricultural inputs (Boyd *et al.*, 2010) by erosion or direct input. These materials are further processed by autochthonous pond microbes and the microbial communities vary depending on the wetness or dryness of the pond, amongst other factors.

During the pond wetting phase, temporary ponds have increased DOC exchanges with the atmosphere as CO_2 from oxidative processes. More DOCs are utilised for oxidative processes because it takes longer to reach thepond bottoms compared to POC and colloidal DOC and this exposes them to microbial utilisation (Shirokova *et al.*, 2013). During the drying phase, DOC production, sedimentation and storage improve as the water column becomes shorter and concentration of nutrients increases alongside anoxia. For temporary ponds in size range 10 - 100 sq. m, their small size allows them to accumulate materials quicker than larger water bodies. Hence, they store more carbon and process sedimentary material more efficiently. During the dry phase of temporary ponds, the top sediments are exposed to the atmosphere. Soil air (Boyd, 1995) and photolysis (photo-dissociation) cause the accelerated loss of labile organic matter (short chain organic carbon) in the exposed sediments (Inegbedion, 2013). This creates a layer of organic carbon with longer and denser molecular structure than the originally exposed sediments. The continuous seasonal overturns of drying and wetting further create stratified layers of carbon mats in the pond bottoms (Munsiri *et al.,* 1995). This stratification is significant in the efficient storage and chemical lock-in of carbon in SWBs with temporary pond characteristics (Boyd *et al.*, 2010). This further enhances higher carbon storage in ponds in size range 10 - 100 sq. m compared to 100 - 1,000 sq. m and 1,000 - 10,000 sq. m.

Permanent ponds are not stratified by the loss of labile organics as a result of the continued water cover but microbial stratification can occur by the development of Firmicutes and/or Chlorobi phyla in sediment (Baker *et al.*, 2015). These microbes are associated with anoxia in ponds and anoxic bottoms trigger improved carbon stock due to slowed levels of microbial oxidations to CO_2 while oxygen bottoms create a carbon source (Gilbert *et al.*, 2016). There is also a strong influence of temperature on permanent ponds with respect to carbon processing that was not covered in this research.

4.6.4.4 Carbon storage down core profile

Carbon stock in the top 5 cm of ponds was highest because it was the most biogeochemically and physically active layer of pond sediments. In the permanent pond, this layer is directly in contact with the spond water column. The sediment within this layer is significant in determining the source or sink potential of a pond. For permanent pond in a highly-oxygenated environment and sandy pond sediments with loose compaction, there is the probability that carbon oxidations will be prevalent in those sediments. Conversely, anoxia favours carbon preservation in the sediment. In a temporary pond, this layer is significant in the development of the stratified layer that preserves carbon in sediment. The top sediment layers are involved in the remobilisation of sediments into the water column. It is also active in the microbial processing of allochthonous carbon, which is further absorbed/buried in sediments.

At 5 cm - 10 cm of the core, carbon stock patterns were similar to the top 5 cm but the carbon stored at this depth was reduced compared to the top 5 cm. This is largely attributed to sediment being less loose compared to the top 5 cm with overburden and compaction beginning to build in sediment. This further starts to reduce the microbial interactions in sediment as microbes require less dense sediments and moisture to utilise carbon. Also, microbial interaction with allochthonous carbon is reduced at this depth compared to the top layer.

At 10 cm - 15 cm of the core, sediment wetness gradually reduces. Size range 10 - 100 sq. m had carbon stock of 0.59 kg C/sq. m and size range 100 - 1,000 sq. m had more average carbon stock at this depth compared to size range 10 – 100 sq. m and 1,000 - 10,000 sq. m. This is either as a result of reduced compaction occurring in size range 100 - 1,000 sq. m that reduced microbial activities or an isolated or unidentified external influence(s) on the ponds.

At depth 15 cm - 20 cm and 20 cm - 25 cm, the ponds are mainly characterised by core bottom sediments or no data. The carbon stocks were reduced as a result of lowered moisture penetration, reduced microbial activity and sediment compaction.

Natural ponds (100 - 1,000 sq. m) at depths 5 cm - 10 cm and 10 - 15 cm were found to be storing more carbon than the top 5 cm. This could be attributed to several reasons affecting the top 5 cm of the natural ponds. It could be that the top 5 cm is exhibiting a carbon source potential or different material entry and microbial processing which reduce carbon stocks, amongst other possible reasons. The pond could also be going through an ageing phase where carbon stock begins to slow down (Gilbert, 2016). However, this research does not have the data to efficiently answer this question. The most likely reasons lie within material transport (carbon entry), pond sediment type and aggregations, moisture penetration, compaction (dry bulk density) and microbial processing interacting at optimum to enhance storage at lower depths compared to any other pond types at similar depths.

4.7 Limitation to carbon stock research

Data collected for carbon analyses were collected from sites at Druridge Bay in South East Northumberland. It was not possible to collect samples from inland Northumberland areas such as the Cheviots because of site access permission and logistics. Also, this research did not consider the temporary variation of carbon stock within ponds and there was a limitation of accessing water bodies >10,000 sq. m due to safety, access to the necessary equipment and logistics.

Also, the absence of data from other parts of Northumberland potentially reduces the range of the results. But the results clearly shows variability in carbon stock levels within ponds and across different ponds and this is similar to work by other authors in the region.

4.8 Conclusion

Carbon stock in ponds varies across pond types, size ranges and down core profile. Pond size ranges have no direct effect on the carbon stock but affect environmental factors contributing to the total variation. The variations in carbon stocks, however, are controlled by numerous environmental/physical, biological and chemical/biochemical factors. The effects of these factors also vary and are interdependent.

Various ponds have different sediment types and the aggregation of these minerals play an important role in carbon stock potentials of ponds. Highly compacted sediments have the most storage potential and loose compaction leads to loss of carbon. However, efficient stocking of carbon occurs in pond sediments with an efficient mix of loose and compacted sediment to allow for preservation, microbial processing and moisture penetration. Hence, highly porous sandy sediment ponds potentially have high productivity but a poor preservation of carbon while arable ponds can have low porosity and high sediment compaction that restricts microbial penetration, thus, high preservation.

In the determination of carbon source, the C:N ratio provided a broad overview of activities that influence allochthonous and autochthonous carbon. But there was not a clear distinction of the source type because of oxidation of organic carbon and organic matter mixing. Therefore, exploring the microbial metabolism or feeding preference of allochthonous or autochthonous carbon source will be a valuable tool in distinguishing the organic carbon source in SWBs.

In all, the capacity of SWBs to stock carbon increases with decreasing pond surface area in Northumberland. Temporary pasture ponds accumulate the most carbon in all ponds analysed as a result of its smaller size, aggregation of fine and coarse minerals, seasonal wetting and drying. And arable pond types accumulated the least carbon given as a result of different environmental/physical, biological and chemical/biochemical influences.

Chapter 5: Microbial Influence on Carbon Stock

5.1 Background

The interactions between microbes and carbon from allochthonous and autochthonous sources in ponds have been discussed in Chapter 2. Chapter 4 established the variation of carbon stocks in a pond as a result of physical/environmental, biological and geochemical/chemical factors.

This chapter explores the influence of microbial metabolisms on carbon stock in pond sediment. The findings will further enhance knowledge of microbes' contribution to the carbon stock in pond sediments. Metabolic processes in ponds are as diverse as the microbes living in the pond environment but there are broad metabolisms that occur in all ponds alongside pond specific metabolic processes (Ansola *et al.*, 2014, Briée *et al.,* 2007 and Shirakova *et al.*, 2013). This chapter focuses on broad metabolic features that occur across ponds and how they influence carbon stock in water bodies. This research covers:

- Microbial community structure
- allochthonous and autochthonous carbon processing
- and oxygenation in pond sediment,

to determine the relationship between carbon stock and microbes. The research involved PCR pyrosequencing in targeting the 16S rRNA gene of bacterial phyla and their relative abundance in the pond sediment used for carbon analyses.

5.2 Literature Review

5.2.1 Microbes interactions in pond sediments

Microbes are widely abundant in soils and pond sediments (Dunbar *et al.,* 2002, Falkowski and Raven, 2013 and Trebilco *et al.,* 2013). Their activities serve as important biogeochemical cycling markers for bio-elements such as carbon, nitrogen and sulphur (Ansola *et al.,* 2014, Baker *et al.,* 2015 and Briée *et al.,* 2007). The characteristics of the ecosystem are dependent on archaea, bacteria, fungi and other eukaryotes activities which have a wide diversity of biochemical processing (Bärlocher and Boddy, 2016 and Briée *et al.,* 2007). Gilbert *et al.* (2014) and Chapter 4 showed that the top sediment layer of ponds accumulates more carbon than bottom sediments because of lower dry bulk density, high carbon concentration, access to terrestrial material entry, quick POC sedimentation, anoxia and high microbial actions. In lower pond bottom sediments, high dry bulk density (compaction and overburden) reduce microbial action on organic matter by limiting microbial movement through sediment pores (Avnimelech *et al.,* 2001, Boyd *et al.,* 2010 and Grabowski *et al.,* 2011). Chapter 4 also discussed that carbon stocks were controlled by environmental factors such as water body size, heterogeneity of habitat and pond depth. Although these controlling factors vary widely and have different levels of influence on microbes in ponds, microbes generally utilise carbon as the main energy source. Therefore, factors that influence carbon stock will also influence microbial community structure, their metabolism and biologic interactions of competition, commensalism, symbiosis and predation (Jardillier *et al.,* 2005). Investigating microbial community structure further unlocks the individual metabolisms that cause the pond sediment to act as a carbon source or sink.

Microbiologists have a well-established principle that “everything is everywhere but the environment selects” (Baas-Becking, 1934). Microbial community structure can infer the prevalent environmental and nutrient conditions, such as halophiles and organism associated with halophiles are selectively able to survive in high salt environments (Dai *et al.,* 2013 and Oren, 2010). Some other environmental and nutritional controls on microbes are iron concentrations (Cornell and Schwertmann, 1996), anoxia in highly sulfate reducing pond sediment (Briée *et al.,* 2007), temperature and moisture (Liang *et al.,* 2003) amongst others.

Also, the abundance of certain microbial groups over others relates to microbial adaptations in that environment. Adaptation depicts the nature and types of microbes better suited for a specific environment (Lozupone and Knight, 2007 and Sims *et al.,* 2013). This is why autochthonous microorganisms are more successful in ponds than allochthonous microbes; they have adaptive features and genes that allow them to survive better because of their longer evolutionary adaptations and successful microbial relationships to the environment (Elena and Lenski, 2003). Although the presence of nutrients sustains the growth of a dominant microbial group in an ecosystem, their abundance is also associated with predation and antibiotics which limit the growth of other groups. Growth limitations of some microbial groups have been associated with excesses or lack of nutrient in the environment (Jiang *et al.,* 2006 and Briée *et al.,* 2007). In an extreme environmental change, some non-performing microbes adjust or adapt to a state of dormancy either as spores or seed banks. This is important in small ponds that undergo seasonal pond succession. Non-performing or stasis microbes that are limited by the environmental change such as a change from summer to winter can re-flourish when situations become more favourable (Biggs *et al.,* 1994, Gilbert, 2016, Gobet *et al.,* 2012 and Vymazal, 2005).

SWBs have been regarded as an extension of the forest biome and terrestrial carbon that holds allochthonous carbon depositions (Battin *et al.*, 2009). But this is not entirely true because microbes in ponds are capable of utilising allochthonous carbon and creating autochthonous carbon via photosynthetic and chemosynthetic pathways. These processes are more effective in SWBs than large lakes, rivers and oceans because of higher nutrient concentration and access to terrestrial material input (Battin *et al.*, 2009 and Cole *et al.*, 2007). Microbes in SWBs can be used to distinguish allochthonous and autochthonous carbon more effectively than in large lakes, rivers and oceans which are characterised by high oxidation and material transport (Battin *et al.*, 2009, Downing *et al.*, 2006, Cole *et al.*, 2007 and Tranvik *et al.*, 2009). This is because of small ponds' shallow depth, reduced current and perturbations. Also, allochthonous and autochthonous carbon producing/utilising microbes can show variation in down core pond succession.

5.2.2 Key microbial metabolism in small water bodies

The ubiquity of microbial metabolic activities in ponds is related to microbial redox potentials and bio-elements. Redox in ponds is generally in flux with an imbalance to favour carbon sequestration or atmospheric loss (Gilbert, 2016). The predominant microbe determines the material exchanges, substrate utilisation, growth limitations and the relationship between microbes in creating a predominantly reduced or oxidised environment (Chapelle, 2001, Coby *et al.*, 2011, Kluber and Conrad, 1998, and Weber *et al.*, 2006). And this redox potential further defines carbon sequestration which occurs in a predominantly reduced pond environment or carbon loss which occurs in an oxidised pond environment (Clarens *et al.*, 2010 and Mata *et al.*, 2010). While the less dominant metabolisms in the pond environment support processes to enhance dominant microbe redox or reflect microbes in stasis (DeLong, 2005 and Nyström, 2003).

There are multiple activities that occur in pond environments at various levels of metabolic dominance. But for the purpose of this research, the key microbial processes include microbial community structure and their interactions, oxygenation in ponds and allochthonous and autochthonous carbon sources for energy. The phyla described to achieve this purpose are Proteobacteria, Bacteroidetes, Fibrobacteres, Planctomycetes, Verrucomicrobia, Acidobacteria, Firmicutes Cyanobacteria, Chlorobi, Chloroflexi, and some methanogens. Other phyla described include some candidate phyla and superphyla relationships, amongst several microbial metabolisms in various pond environments.

5.2.2.1 Microbes influential to pond sediment processes

Proteobacteria are a very successful Gram-negative bacterial phylum in diverse natural environments and they are known to utilise diverse substrates for their metabolisms. They are divided into six subphyla of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria and Acidithiobacillia (Garrity *et al.,* 2005 and Williams and Kelly, 2013), and the subphyla aremonophyletic or a group of organisms that have similar ancestral descendants (Figure 5.1). Alphaproteobacteria have a large diversity and vary widely in functionality. Alphaproteobacteria are found to be more abundant in soil, pond and lake sediments compared to the water column and tend to be phototrophic especially in genus *Rhodobacter* (Briée *et al.,* 2007). Betaproteobacteria are found in freshwater lakes and are prevalent in anaerobic conditions. In an environment with abundant Betaproteobacteria, candidate phylum OD1 (Parcubacteria) are also found (Briée *et al.,* 2007). Betaproteobacteria include hydrogen-oxidising (*Hydrogenophilus* and *Aquaspirillum* species), hydrogen sulphide (H_2S) related (*Thiobacillus*), nitrifying (*Dechlorimonas*) and methylotroph (*Methylophilus*) bacteria. In stratified lakes, Betaproteobacteria (*Betaproteobacterium*) have been found to be in symbiotic relationships with phototrophs such as the green sulphur bacteria (Williams and Kelly, 2013), where they share symbiotic exchange of nutrients/metabolites for the cohesive development.

Gammaproteobacteria are known to oxidise reduced sulfate (*Thiocystis* and *Thiothrix*). They are parasitic on frogs and invertebrates in water bodies. Briée *et al.* (2007) described the genus *Chromatium* as rod-shaped purple sulphur bacteria with a mucous coating of intracellular sulphur granules. They use H_2S as an electron donor for CO_2 reduction and create granular sulphur as waste. Purple sulphur bacteria are mainly classified in the Chromatiaceae (a Gammaproteobacteria family) of which *Chromatium* is a genus (Imhoff, 2014). Deltaproteobacteria are mainly sulfate reducers that are prevalent in anoxic settings of stratified and anoxic lakes (Karr *et al.,* 2005). Myxobacteria are a group of Deltaproteobacteria which ferment and degrade organic carbon. Epsilonproteobacteria are naturally associated with the sulfidic environments of the deep-sea (Lindstrom *et al.,* 2005) and sulphur springs (Elshahed *et al.,* 2003). They are absent and rare in freshwater lakes and appear in the oxic and anoxic interface of the marine environment (Lin *et al.,* 2006).

Bacteroidetes are a diverse phylum that anaerobically digest carbon in freshwater lakes, deep sea sediment, sulfidic caves and aquifers. They are mainly hydrolysers and degraders of cellulose, chitin and fermenters under strict anaerobic conditions (Baker *et al.,* 2015 and Briée *et al.,* 2007). In association with Bacteroidetes, OD1 are found in the freshwater and marine environment and they are mainly anaerobic digesters (Chouari *et al.,* 2005). OD1 are oxygen sensitive and are found in anoxic or oxygen depleted environments (Elshahed *et al.,* 2003).

Fibrobacteres, Planctomycetes and Verrucomicrobia are also key bacteria phyla that influence metabolic activities in ponds to a varying degree dependent on environmental conditions. Genus *Fibrobacter* of Fibrobacteres are fibrolytic bacteria originating from ruminant animals and are capable of processing plant polysaccharide (Béra-Maillet *et al.,* 2004). Planctomycetes are found mainly in lakes, aquifers, marine sediment and anaerobic digesters. *Planctomyces* spp. are divided into two main functional groups based on metabolism, that is, facultative aerobic chemoorganotrophs which utilise carbon by respiration and fermentation, and strictly anaerobic autotrophs that carry out anaerobic oxidation of ammonia to nitrate (van Niftrik *et al.,* 2004). Verrucomicrobia are found in ponds, lakes and agricultural soil, groundwater systems and deep-sea sediments (Madigan, 2003 and Sangwan *et al.,* 2005). They are known to ferment various sugars and degrade organic matter. Depending on the environment Planctomycetes and Verrucomicrobia could be dominant, or be part of the dominant microbes influencing metabolism or be in stasis but Fibrobacteres can only be found in grazing ponds.

5.2.2.2 Oxygen and photolytic processes in ponds

Small ponds have potential to be oxic, suboxic and/or anoxic depending on the prevailing physical/environmental, biological and geochemical/chemical influences. This affects the nature of metabolism that occurs within the pond system. In oxygen depleted ponds, the abundance of Cyanobacteria, Chloroflexi and purple sulphur bacteria increases as the main phototrophic microbial community (Elshahed *et al.,* 2003). They perform anoxygenic photosynthesis using H_2S rather than H_2O as the electron donor and elemental sulphur is produced as the by-product. Chloroflexi are found in a wide array of environments and are known to be both aerobic and anoxygenic thermophiles (green non-sulphur bacteria). Madigan (2003) also described large filamentous cyanobacteria (*Oscillatoria* spp) which were characterised by anoxygenic photosynthesis using H_2S.

Chloroflexi are anoxygenic, phototrophic and filamentous bacteria. They have been known to grow in warm springs as thermophiles. In stratified lakes, they are characteristically the green non-sulphur bacteria in the anoxic layers (Briée *et al.,* 2007).

Chlorobi (green sulphur bacteria) are a group of anoxygenic photosynthetic bacteria frequently found in anoxic microbial mats and stratified lakes. Similar to Cyanobacteria, they perform photosynthesis by utilising H_2S as the electron donor. They have been known to be photoheterotrophic and in symbiosis with other heterotrophs (Glaeser and Overmann, 2004). Also, methanogenic archaea and sulphur reducing bacteria dominate oxygen depleted water bodies. Figure 5.1 shows the Order Methanomicrobiales of Euryarchaeota contains methanogenic species and they are predominantly associated with freshwater sediments (Abreu *et al.,* 2001, Barns *et al.,* 1996 and Boetius *et al.,* 2000). In an anaerobic pond sediment environment, Betaproteobacteria and Deltaproteobacteria are more abundant than Alphaproteobacteria and Gammaproteobacteria. Anoxia-inducing microbes create an environment that permits the development of other microbes that require anoxia for their survival (Baker *et al.,* 2015, Briée *et al.,* 2007, Madigan, 2003, Quaiser *et al.,* 2003 and Sangwan *et al.,* 2005). Bacteroidetes, Chloroflexi, and methanogens phyla are associated with metabolisms that function in almost exclusively anoxic environments, with the Euryarchaeota and Crenarchaeota being mainly methanogenic (Abreu *et al.,* 2001, Barns *et al.,* 1996 and Lundgren *et al.,* 2008).

Given the selectivity of anoxia in creating dominant microbes and other microbes that can survive in anoxia, carbon-utilising and carbon-producing microbes are also influenced by anoxia. This can be further used to identify predominant microbes that utilise allochthonous carbon and/or produce autochthonous carbon. At phyla level, Bacteroidetes are the main exclusive anaerobic digesters of cellulose and chitin degraders in anoxia. Elshahed *et al.* (2003) showed that Cyanobacteria and Chloroflexi proliferate in anoxia as the main phototrophs and Chlorobi have been found to be phototrophic in anoxic and suboxic environments (Briée *et al.,* 2007, Elshahed *et al.,* 2003 and Madigan, 2003). Thus, these two groups of microbial phyla can be used to infer allochthonous and autochthonous carbon in pond sediments.

Acidobacteria and Firmicutes are gram positive bacteria commonly found in freshwater ponds. Firmicutes are predominant in anoxic sediments or chemoclines in stratified lakes (Briée *et al.,* 2007). Acidobacteria are abundant in nature, especially in soil (Dunbar *et al.,* 2002 and Quaiser *et al.,* 2003). They are found in multiple environments including deep sea vents, aerobic and anaerobic environments. Similar to Acidobacteria, Verrucomicrobia and Proteobacteria have been found to be abundant in oxic and anoxic environments. Similar to Cyanobacteria, Chloroflexi and Chlorobi, in anoxia or environment where the predominant microbial metabolisms occur in anoxia, it is expected that Acidobacteria, Verrucomicrobia, Proteobacteria and other phyla containing both aerobic and anaerobic species will preferentially or predominantly elucidate more anoxia dwelling species than oxia dwelling species (Figure 5.1).

Ponds also contain phyla in varying abundance, whose growth is based on their interaction with other microbes in ponds. Notable examples are:

- OD1 – Parcubacteria, are abundant in anoxic sediments (Nelson and Stegen, 2015 and Rinke *et al.,* 2013).
- WS3 – Latescibacteria, which are a member of the superphylum Fibrobacteres-Chlorobi-Bacteroidetes (FCB) and are found in a wide range of habitats and anoxic sediments (Rinke *et al.,* 2013 and Youssef *et al.,* 2015).
- BRC1 – uncultured candidate bacteria found in soil, anoxic marine and sinkhole mat clones (Baker *et al.,* 2015)

Some other candidate phyla that exist include OP11 – Microgenomates (Rinke *et al.,* 2013), SR1 – Absconditabacteria (Hug *et al.,* 2016) and TM7 – Saccharibacteria

(Albertsen *et al.,* 2013), and they have been identified in diverse natural and cultured environments.

WS3 abundance has been associated with Fibrobacteres-Chlorobi-Bacteroidetes (FCB) superphylum as their growth is interdependent. The superphylum shares similar metabolic pathways and performs anaerobic fermentative metabolism of carbon (Youssef *et al.,* 2015). Their abundances are also influenced by microbial relationships, nutrient availability and favourable environmental conditions (Jardillier *et al.,* 2005, Lozupone and Knight, 2007, Rinke *et al.,* 2013, Sims *et al.,* 2013 and Youssef *et al.,* 2015).

Finally, terrestrial carbon entry into ponds is also known to trigger oxygen fluxes in ponds and permanent anoxia in certain ponds (Briée *et al.,* 2007). This indicates that microbes are not the sole influencers of pond oxygenation but a combination of physical/environmental, chemical and biological interactions in the pond environment. However, this has been understudied in SWBs.

5.2.3 Background Summary

Similar environmental factors influence carbon stocks and microbes in ponds. These influences determine the microbial community structure of pond sediments at any given time. The microbial utilisation of carbon as an energy source can also be used to determine a pond's carbon source and sink potentials. Within the microbial communities, dominant microbes can create environmental factors such as anoxia that enhance their growth and growth of other microbes that can tolerate anoxia. Therefore, adaptation provides an avenue for microbes to adjust to environmental changes and the flux of microbial abundance reflects succession in pond sediments, allochthonous and autochthonous carbon sources.

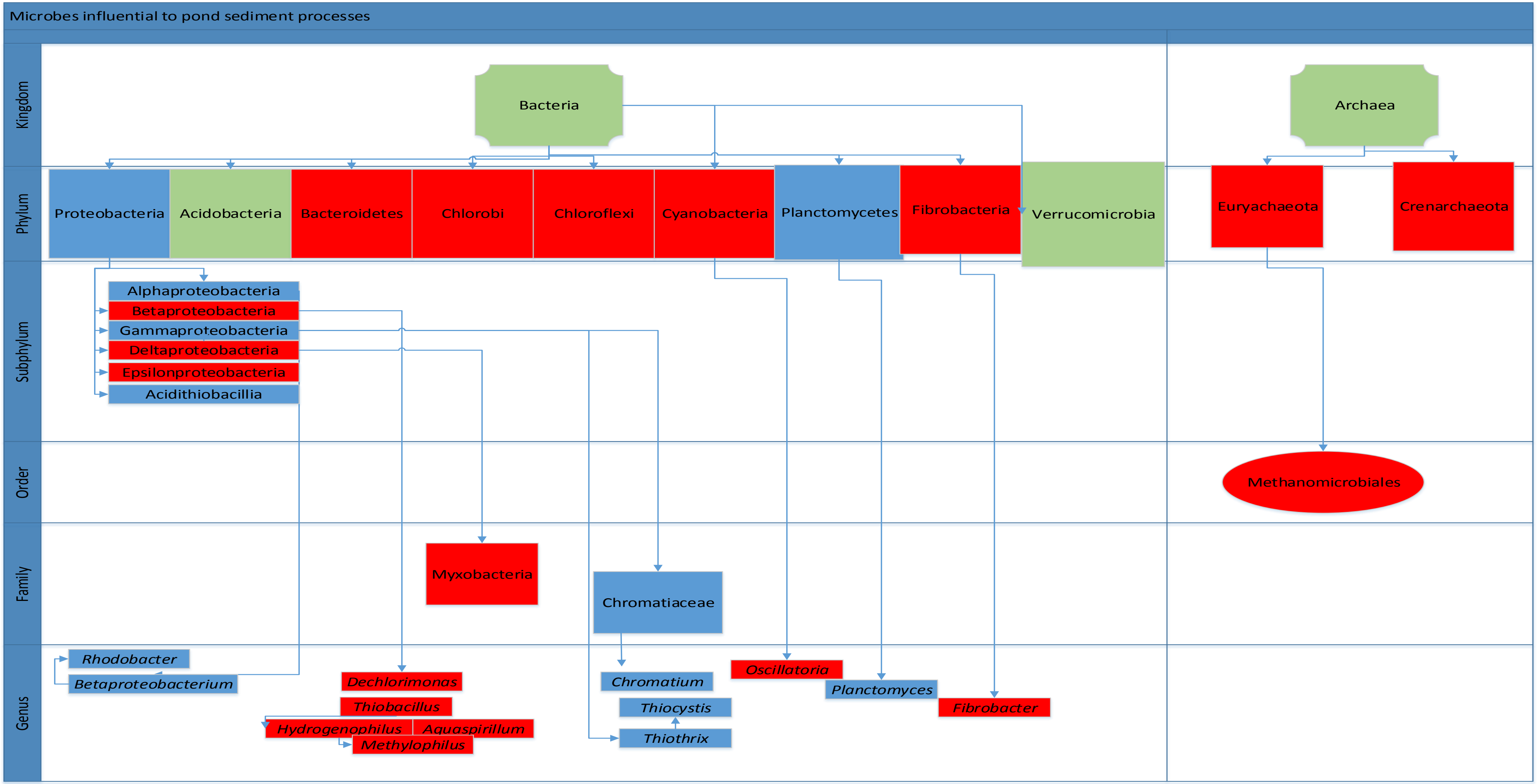

Figure 5.1: Summary of some key predominantly oxic microbes (blue), predominantly anoxic microbes (red), and microbes that express in oxia and anoxia (green) depending on environment, that are influential to carbon processing in ponds

5.3.1 Research Aim

The aim of this chapter is to identify key microbial processes occurring within pond sediments and how they drive carbon stock. This is achieved by describing the microbial community at phyla level and examining their influence on carbon stocks in pond sediment. Tying carbon stock variations to microbes unlocks new knowledge on oxia and anoxia, allochthonous and autochthonous carbon sources and other metabolisms in SWBs. This further defines the importance of small water bodies for carbon capture.

5.3.2 Research Questions

1. a. What are the broad variations in microbial community structure across all pond sediments?

   b. What are the abundant microbial phyla in the Northumberland pond sediments?
2. a. How does pond oxygenation influence microbial community structure?
3. a. How do allochthonous and autochthonous carbon influence microbial community structure in each pond sediment?

   b. How does this affect oxygenation and carbon source dynamics?

5.4.1 Methods

The methods were designed to identify bacteria within pond sediments. This was undertaken in ponds of various types and sizes. Polymerase Chain Reaction (PCR) was used to identify 16S rRNA genes. The results were defined at bacterial phyla level with the removal of Operational Taxonomic Units (OTU) <80%.

5.4.2 Sample collection and processing

5.4.2.1 Research site

The samples used were homogenised sub-samples of the cores used for carbon analysis. For microbial analysis, 12 ponds sediment cores were used from 10 of the ponds in Druridge Bay, Northumberland. The selected sediment cores for microbial analyses were designed to account for:

Water body within size ranges:

- 10 – 100 sq. m,
- 100 – 1,000 sq. m and
- 1,000 – 10,000 sq. m

Pond types:

- arable,
- natural,
- pasture and
- sand dune ponds

5.4.2.2 Core sampling

Table 5.1 below contains the number of cores used from each pond in Northumberland and their depths. Analyses for microbes in pond sediment were done at 1 cm intervals of homogenised sediments for the Top 5 cm. Beyond the 5th cm, sediments were homogenised at 2 cm intervals depending on the depth of the pond core.

Table 5.1: Depth of pond core, pond type and size range for microbial analysis

Pond type and depth of microbial analysis	*Size Ranges (sq. m)*		
	10 -100	100 - 1,000	1,000 – 10,000
Arable Pond	7 cm	5 cm	5 cm
Natural pond		10 cm	12 cm, 12 cm
Sand dune pond		8 cm, 8 cm	8 cm
Pasture Pond	10 cm	12 cm	12 cm

5.4.3 Laboratory Analysis – 16S rRNA Sequencing using Illumina Next Generation Sequencer

The 16S rRNA sequencing is a fast and informative way of identifying and comparing bacterial communities in environmental samples. It is a well-established method for phylogenetic and taxonomic tracing of bacteria in samples. Sequencing of 16s rRNA gene amplicons (rRNA fragments) on an Illumina Next Generation Sequencer uses domain level PCR reactions to amplify isolated genomic DNA and millions of sequences of genetic fragments are generated to provide more knowledge of microbial community structure in the environment (Kozich *et al.*, 2013 and Taylor, 2017). In 16S rRNA analysis, there are several variable genomic regions such as the V3, V4 and V45 regions which can be sequenced and this can be used to identify the dynamics of microbial community structure (Kozich *et al.*, 2013, Sanschagrin and Yergeau, 2014 and Taylor, 2017). In this research, the targeted region for microbial community structure analyses was the V4 region of 16S rRNA genes. It is worth noting that this type of sequencing allows for massive parallelisation of replications and clonal separation of templates without the need to insert gene fragment into a microbial host or community, which was what occurred in previous methodologies for microbial analysis (Taylor 2017 unpublished).

5.4.3.1 DNA Extraction and Isolation

Using the PowerSoil DNA Isolation procedure (By MO BIO Laboratory, Inc., Carlsbad, CA, USA) and following the manufacturer's protocols, 0.25 g of sediment was placed into a PowerBead Tube. It was gently vortexed to dissolve humic acids in the sediment, disperse sediment in the tube and protect DNA from degradation. Thereafter, 60 µl of Solution C1 (sodium dodecyl sulfate (SDS) and other disrupting agents) were added for cell lyses completion (SDS is an anionic detergent that dissolves lipid/fatty acids) and vortexed for 10 seconds. The solution was attached to a flatbed pad and vortexed for a further 10 minutes for mechanical lysis and solution homogenisation.

The solution was centrifuged at 10,000 x g for 30 seconds at room temperature and 2 ml of the supernatant was transferred into a tube of 250 µl Solution C2. Solution C2 (a patented Inhibitor Removal Technology (IRT)) was added for precipitating organic and inorganic materials to improve DNA purity. The tube was centrifuged at 10,000 x g for 1 minute and the 600 µl of supernatant was collected (avoiding the pellets) and transferred into a 2ml collection tube.

200 µl of Solution C3 (a patented Inhibitor Removal Technology (IRT)) was added to the solution, vortexed briefly and incubated at 4 ^{0}C for 5 minutes to remove organic and inorganic material which improved DNA purity. The tube was centrifuged at 10,000 x g for 1 minute. The supernatant was transferred to a clean 2 ml collection tube. Then, 1.2 ml of Solution C4 (a high salt concentration solution) was added and vortexed for 5 seconds, 675 µl loaded onto a spin filter and centrifuged at 10,000 x g for 1 minute. The flow-through was discarded and the process was repeated once more (DNA preferentially binds to the silica membrane of the spin filter in high salt concentration).

At this stage, 500 µl of Solution C5 (ethanol based wash solution for cleaning DNA bound to the silica membrane) was added to remove residual salts, inorganic and organic materials. The resulting solution was centrifuged at room temperature for 30 seconds at 10,000 x g. The flow-through was discarded and the spin filter was centrifuged at 10,000 x g for 1 minute to remove residual Solution C5.

The spin filter was carefully transferred into a clean 2ml collection tube and 100 µl of Solution C6 (sterile elution buffer) was carefully added to the centre of the spin filter membrane for efficient and complete release of DNA from the spin filter. The solution C6 passes through the silica membrane and DNA that was not bound to the silica (in high salt concentration) was released into the 2ml collection tube. The solution in the 2 ml collection tube was centrifuged at 10,000 x g for 30 seconds and the spin filter was discarded. The resulting solution was stored in a freezer for further analysis at -10^{o}C.

5.4.3.2 Quality Analysis – Nanodrop Spectrophotometry and Gel Electrophoresis

This procedure was performed by Northumbria University PCR/DNA Analysis Lab. The Nanodrop 1000 instrument was used for spectrophotometric determination of DNA concentration ratios of 260nm/280nm and 260nm/230nm. Humic substances contaminating DNA samples were represented by the 260nm/280nm ratios and protein-based material contamining DNA samples were represented by the 260nm/230nm ratios. Humic and protein based contaminants are inhibitors of PCR analysis by reducing yield and/or quality of results, therefore, establishing 260nm/280nm and 260nm/230nm ratios is important for assessing data quality produced from PCR sequencing.

Gel electrophoresis operates on the principle of size and charge based separation of DNA fragments' negative charge in ionic buffer solution, due to the release of phosphate groups. In this research, gel electrophoresis was used to determine the quality of extracted DNA and ionic buffers used for these experiments were Tris Borate Ethylenediamine tetra-acetic acid EDTA (TBE) or Tris Acetate (TAE). Fragment separation was run on

Agarose gel and this occurred based on molecular weight. When ran in Agarose gel, poor quality DNA produced a smear and high quality DNA produced tight bands of high molecular weight compound.

5.4.3.3 DNA Amplification and Pyrosequencing

This procedure was also performed by Northumbria University PCR/DNA Analysis Lab using Wet-Lab Miseq Standard Operating Procedure (SOP) and an Illumina Next Generation Sequencer. This involved preparation and sequencing of the sediment sample for 16S rRNA gene sequence libraries using the Illumina Miseq (Schloss Lab Indices). 16S rRNA wwasere first amplified by Polymerase Chain Reaction using primers containing small sample wells and unique barcodes. The 16S rRNA genes (250 bp paired ends of the V4 region) were amplified using the Schloss Lab universal primer set. Samples were arranged in a 96 wells format with two left to run the controls (a mock community and water for negative control). The sample wells were grouped and named, and using the Illumina Experiment Manager, a sample sheet was created. The sheet served as run parameters and indexing scheme for Miseq analyses. Thereafter, a subset of 12 samples from each plate underwent electrophoresis on a 1% agarose gel to confirm the certainty of the amplification process. Library clean up and data normalisation was performed using Invitrogen SequalPrep Plate Normalization Kit. Finally, samples from all plates were pooled and library quality control was performed. This included quantification using a KAPA Biosystems Q-PCR kit and obtaining a Bioanalyser trace using the Agilent Technologies HS DNA kit.

The PCR analysis of 16S rRNA genes allows for identification of existing, pre-existed and seed banks of microorganisms (Ansola *et al.,* 2014, Hahn *et al.,* 2003 and Zwart *et al.,* 2002). These identifications are not quantitative but do show relative abundance or prevalence of microbes by amplifying copies of identified DNA or segments of DNA. The 16S rRNA genes in pond sediment were amplified to target Bacteria. The results of the sequences were retrieved in a fastq file format and were further filtered using Mothur 1.29 software pipeline (bioinformatics analysis) removing DNA sequences shorter than 250 bp, removing sequences that aligned to the incorrect region of the 16S rDNA and chimeric sequences, as recommended by Scholss *et al.* (2009). During the analysis, Sequences affiliated to archaea were found, when bacteria were targeted as recommended by Cruaud *et al.* (2014), and they added to results for completeness as recommended by (Taylor, 2017 *unpublished*). Taxonomic assignments and assessment were referenced with the Silva database (Silva rRNA database project. No Date). Reads were classified based on phylogeny and operational taxonomic units (OTUs).

5.4.3.4 Operational Taxonomic Unit (OTU)

Unclassified phyla were also removed from the results for ease of data analysis as they were not covered in the Silva database when analysing the bacteria kingdom. Operational Taxonomic Units (OTU) of 16S RNA reads <80% of sequence identity were removed to improve the accuracy of bacterial identification as standard practice.

5.4.3.4 Experiment Control

A mock community of *Halomonas* and *Streptococcus* was used as positive quality control for the PCR reaction while deionised water was used as negative quality control. The negative quality control was set to check for potential contamination that would be represented in the OTU abundance and this contaminant was consequently removed from the analysis.

5.5 Results

5.5.1 Pond Sediment - OTU Abundance

5.5.1.1 Total Number of DNA Reads per Pond Core

Table 5.2 shows the depth of pond cores and the abundance of microbial DNA identified in the PCR analysis. The number of DNA reads per sediment core varied across pond types and pond sizes. DNA reads ranged from 295,580 in arable ponds in size range 100 – 1,000 sq. m to 786,001 DNA reads in size range 1,000 – 10,000 sq. m. The distribution of DNA abundance across all pond types and size ranges did not follow any clear pattern but there were more specific relationships. In the pasture ponds, DNA abundance increased with a decrease in water body size and the DNA abundance in all pasture ponds was higher than arable and sand dune ponds.

Table 5.2: Total number of DNA reads per sediment core

Pond depth **DNA reads per sediment core**	**Size Ranges (sq. m)**		
	10 -100	100 -1,000	1,000 – 10,000
Arable Pond	7 cm	5 cm	5 cm
	389,319	295,580	307,896
Natural pond		10 cm	12 cm (a), 12 cm (b)
		379,029	410,714 (a), 786,001(b)
Sand dune pond		8 cm (a), 8 cm (b)	8 cm
		380,465 (a), 465,815 (b)	454,917
Pasture Pond	10 cm	12 cm	12 cm
	524,442	510,586	496,588

5.5.1.2 Down core variation of DNA reads

The down core profile of DNA abundance did not follow any clear overall pattern. DNA read varied at different depths of each core (Table 5.3, Table 5.4 and Figure 5.2). In all ponds, the top 1 cm sediment recorded the lowest microbial DNA reads on average and these top sediments were associated with high carbon stock. In size range 10 - 100 sq. m, the top 3 cm recorded higher DNA reads compared to size ranges >100 sq. m but at lower depths, there was a drop in DNA reads. DNA abundance varied in each core with no distributive pattern in the other size ranges (Table 5.3).

In size range 10 - 100 sq. m, arable pond top sediment accounted for the highest DNA within the pond core reads and these were followed by depths of 3 cm and 5 cm. In pasture pond of size range 10 – 100 sq. m, the top sediment had lower DNA abundance compared arable pond of the same size range. Although, PCR DNA abundance is not a reflection of the microbial quantity within the sediment but a reflection of amplified microbes' diversity. More carbon was stocked in the top sediment of pasture ponds compared to arable ponds at size range 10 – 100 sq. m despite having a lower DNA read. DNA reads in pasture pond 10 – 100 sq. m were highest at depth 2 cm and varied at lower depths with an abundance range of from 63,000 – 76,000 DNA reads (Figure 5.2).

In size range 100 - 1,000 sq. m, arable pond showed a gradual increase in DNA reads from top to bottom of the sediment. In the two sand dune pond cores of similar depths of 8 cm, the top sediment varied in microbial DNA reads (77,169 and 42,216 DNA reads respectively). Also in this size range, natural and pasture ponds had low DNA reads in the top sediment compared to the arable pond and their DNA abundance varied down core profile (Figure 5.2).

In size range 1,000 - 10,000 sq. m, the top sediment ranged from 51,000 - 69,000 DNA reads in sand dune pond sediment and the two cores analysed in natural ponds showed widely varying DNA reads from the same pond (Figure 5.2).

In all, Pearson correlation affirmed the independent behaviour of individual ponds in response DNA abundance to pond depths. DNA abundances in each pond were no statistically significantly correlated with pond depth except for arable pond in size range 100 – 1,000 sq. m (Table 5.3).

Table 5.3: Correlation of Pond Depth with DNA Abundance

Correlation of Pond Depth with DNA Abundance (Pearson Correlation, P value, N)

pond type	Size Range 10 - 100 sq. m		
	Pearson Correlation	P value	N
sand dune	-		
arable pond	-0.328	0.526	6
natural pond			
pasture pond	-0.104	0.824	7
pond type	Size Range 100 - 1000 sq. m		
sand dune A	-0262	0.616	6
sand dune B	0.686	0.132	6
arable pond	0.979	0.004	5
natural pond	0.593	0.160	7
pasture pond	0.493	0.215	8
pond type	Size Range 1,000 - 10,000 sq. m		
sand dune	-0.476	0.340	6
arable pond	-0.391	0.515	5
natural pond 2A	0.200	0.962	8
natural pond 2B	0.048	0.909	8
pasture pond	0.380	0.354	8

Table 5.4: Down core DNA abundance across ponds

	10 - 100 sq. m	*10 - 100 sq. m*	*100 - 1,000 sq. m*	*100 - 1,000 sq. m*	*100 - 1,000 sq. m*	*100 - 1,000 sq. m*	*100 - 1,000 sq. m*	*1,000 - 10,000 sq. m*	*1,000 - 10,000 sq. m*	*1,000 - 10,000 sq. m*	*1,000 - 10,000 sq. m*	*1,000 - 10,000 sq. m*	
Depth (cm)	Arable Pond	Pasture Pond	Arable Pond	Sand Dune Pond (a)	Sand Dune Pond (b)	Natural Pond	Pasture Pond	Pasture Pond	Sand Dune Pond	Arable Pond	Natural Pond (2a)	Natural Pond (2b)	**Average DNA abundance**
1	80,163	43,827	50,491	77,169	42,216	35,827	34,505	64,921	69,277	55,517	51,103	53,504	**54,877 ± 4,387**
2	44,523	131,224	51,207	75,500	73,557	50,822	66,296	73,449	109,100	92,508	39,324	56,620	**72,011 ± 7,927**
3	74,278	75,280	60,107	52,147	89,798	34,733	75,839	42,184	62,665	54,080	43,394	49,393	**59,492 ± 4,784**
4	66,844	67,293	63,924	54,998	87,883	71,644	73,688	53,715	80,773	51,042	81,367	147,322	**75,041 ± 7,364**
5	73,992	63,569	69,851	46,428	86,667	64,557	49,703	56,143	91,019	54,749	40,041	206,043	**75,230 ± 12,696**
6 to 8	49,519	66,752		74,223	85,694	61,015	57,929	63,328	42,083		63,925	146,889	**71,136 ± 9,238**
8 to 10		76,497				60,431	78,008	70,618			38,272	76,662	**66,748 ± 6,287**
10 to 12							74,618	72,230			53,288	49,568	**62,426 ± 6,413**

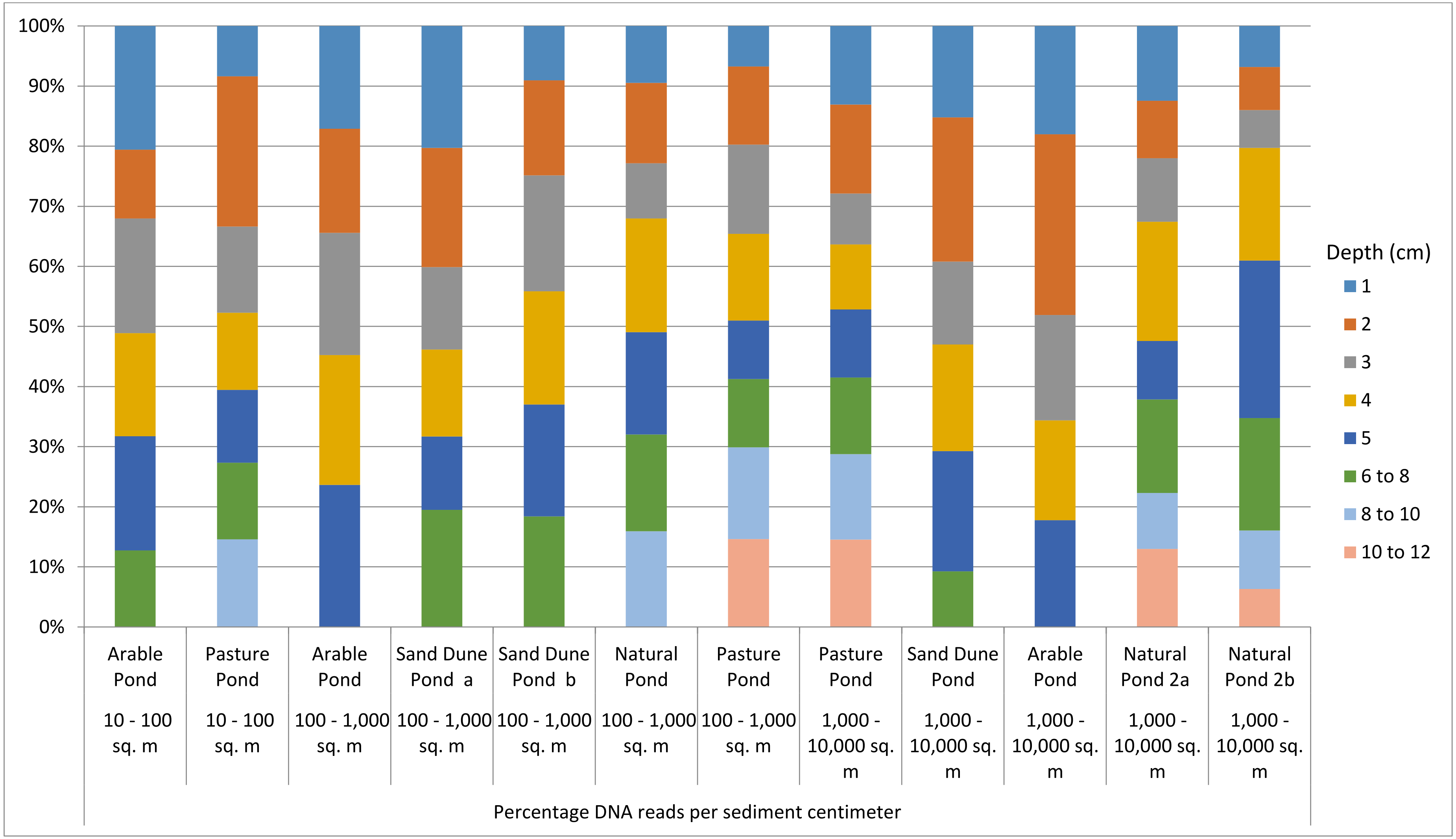

Figure 5.2: Percentage DNA Reads down core profile across ponds

5.5.2 Phylum Analysis

There were variations in the microbial communities' at all taxonomic levels in all ponds. The PCR pyrosequencing analysis was set up to identify Bacteria, but, two Archaea phyla of Crenarchaeota and Euryarchaeota were identified in the results. The Archaea were artefacts of the analysis but were included in the results for completeness and they also provided valuable information on methanogenesis, which was useful in analysing oxygenation in ponds.

5.5.3 Microbial community structure of Ponds

All ponds contained mainly phyla associated with metabolism in anoxic sediments at varying abundance compared to oxic metabolism. Although the PCR process does not provide quantitative abundance, the presence and interactions between the phyla obtained from all ponds suggest predominantly anoxic pond sediments.

Acidobacteria, which are ubiquitous in nature, in soil and pond environments (Quaiser *et al.,* 2003), were predominant. Acidobacteria and Proteobacteria were the most abundant phyla analysed in all ponds. Acidobacteria ranged from 17.67% in one of the natural ponds in size range 1,000 – 10,000 sq. m to 53.64% in arable ponds in size range 1,000 – 10,000 sq. m. They were more dominant in arable ponds compared to other pond types. In size range 10 – 100 sq. m, the abundance of Acidobacteria can be largely tied to their high proliferations in the pond dry phase, when small ponds mimic the soil environment (Dunbar *et al.,* 2002, Sait *et al.,* 2002 and Quaiser *et al.,* 2003). In natural pond size range 1,000 – 10,000 sq. m, where one core was sampled at the pond edge and the other core in the deeper ends of the pond, Acidobacteria abundance was higher on the pond edge which will have a more frequent and longer dry phase (Figure 5.3).

Verrucomicrobia are also highly diverse species predominant in oxic and anoxic processing of carbon (Bergmann *et al.,* 2011). Verrucomicrobia were less abundant than Acidobacteria, ranging from 4.73% of the total phyla abundance per pond in sand dune ponds of size range 1,000 – 10,000 sq. m to 12.43% in arable ponds of size range 100 – 1,000 sq. m (Figure 5.3). The overall distribution of Verrucomicrobia showed that their abundance increases with decreasing pond size and they were predominant in arable ponds and pond edges. Their abundances reflect Verrucomicrobia being associated with soil environments and they flourish in pond environments with the ability to mimic soil environments, as with Acidobacteria. In all ponds, Verrucomicrobia abundance increased or decreased with increase or decrease in Acidobacteria and Proteobacteria abundance.

The presence of Firmicutes in ponds gives clear signals of pond stratification processes at the chemocline and their abundance indicates potentially anoxic pond bottoms (Briée *et al.,* 2007). Firmicutes abundance was low in temporary ponds in size range 10 – 100 sq. m and 100 – 1,000 sq. m and arable ponds in size range 1,000 – 10,000 sq. m (Figure 5.3). There was a higher abundance of Firmicutes where pond cores were collected from deeper ends of ponds that were completely covered in water compared to pond edge.

The archaea (Crenarchaeota and Euryarchaeota) are found predominantly in methanogenesis-related environments (Abreu *et al.,* 2001, Barns *et al.,* 1996 and Lundgren *et al.,* 2008). They were more abundant than Firmicutes and their total phyla abundance per pond ranged from 1.58% in the arable pond of size range 100 – 1,000 sq. m to 11.65% in pasture pond in size range 1,000 – 10,000 sq. m (Figure 5.3). Higher methanogen abundances also relate to the nature, texture and composition of pond sediments as arable ponds with high clay composition showed low methanogens abundance compared to other ponds. Water cover also favoured abundance of methanogens in the permanent ponds compared to the temporary ponds. Thus, ponds of size range 1,000 – 10,000 sq. m contained higher Crenarchaeota and Euryarchaeota abundance compared to size range 10 – 100 sq. m.

Bacteroidetes are associated with heterotrophic feeding in anoxic environments. Bacteroidetes abundance was 5.50% in arable ponds of size range 1,000 – 10,000 sq. m but in all other pond sediments, the abundance exceeded 10% of the total phyla abundance per pond (Figure 5.3). Bacteroidetes abundance in ponds also influences the abundances of other phyla such as OD1, which are also predominantly heterotrophic feeders. Fibrobacteres were found in low abundances in some ponds and they were mainly associated with pasture ponds with nearby cattle grazing.

All ponds showed varying abundances of Fibrobacteres, Chlorobi and Bacteroidetes which suggests a potential superphylum relationship of Fibrobacteres-Chlorobi-Bacteroidetes (FCB) superphylum in association with WS3 (Youssef *et al.,* 2015), ecological interdependence or individual growth of each phylum in ponds of Druridge Bay, Northumberland. The phylum components within the superphylum have metabolic relationships that increase their abundance in the pond sediment environment by sharing nutrients, genes and substrates (Baker *et al.,* 2015). However, this research does not go into details of identifying superphyla. Another potential superphylum relationship in the ponds will be Planctomycetes-Verrucomicrobia-Chlamydiae (PVC) groups in association with Lentisphaerae (Wagner and Horn, 2006) which could exist in all ponds except arable pond in size range 1,000 – 10,000 sq. m (Figure 5.3).

Bacteria candidate phyla of OD1, TM7, WS3, SR11 and BRC1 were found in trace abundance in some ponds compared to the abundance of Acidobacteria, Proteobacteria and Bacteroidetes.

Chloroflexi, Chlorobi and Cyanobacteria were the main predominantly phototrophic microbes or autotrophs in all ponds analysed. They can perform their autotrophy in oxygen depleted environments (Elshahed *et al.*, 2003 and Madigan, 2003). Chloroflexi were most abundant in all ponds in comparison to Cyanobacteria and Chlorobi. They ranged from 2.00% in the arable pond in size range 1,000 – 10,000 sq. m to 14.76% in one of the natural ponds in size range 1,000 – 10,000 sq. m (Figure 5.3). Chloroflexi abundance was also higher in permanent ponds than temporary ponds. Cyanobacteria abundance was very low in all ponds in comparison to Chloroflexi, with 1.5% or less of the total phyla abundance in all ponds analysed. Chlorobi abundance in all ponds was much lower than Chloroflexi and Cyanobacteria in arable, pasture and natural ponds. But in sand dune ponds, their abundances were 5.32% in size range 1,000 – 10,000 sq. m, and 2.84 and 1.65% in size range 100 – 1,000 sq. m. As well as phototrophic processes, Chlorobi are associated with pond stratification and symbiosis with heterotrophs (Glaeser and Overmann, 2004) and their abundance varied in each pond analysed.

Proteobacteria are a very successful group of microbes in nature (Briée *et al.,* 2007) and their abundance varied in all ponds. They ranged from 17.31% in the arable pond to 40.24% in the sand dune ponds of size range 1,000 – 10,000 sq. m (Figure 5.). Their functionality widely varies as well as their diverse metabolic influence in the pond environments. Proteobacteria are examined at subphylum level in section 5.5.4 to identify the various metabolic processes occurring within the system and how they influence carbon processing in ponds.

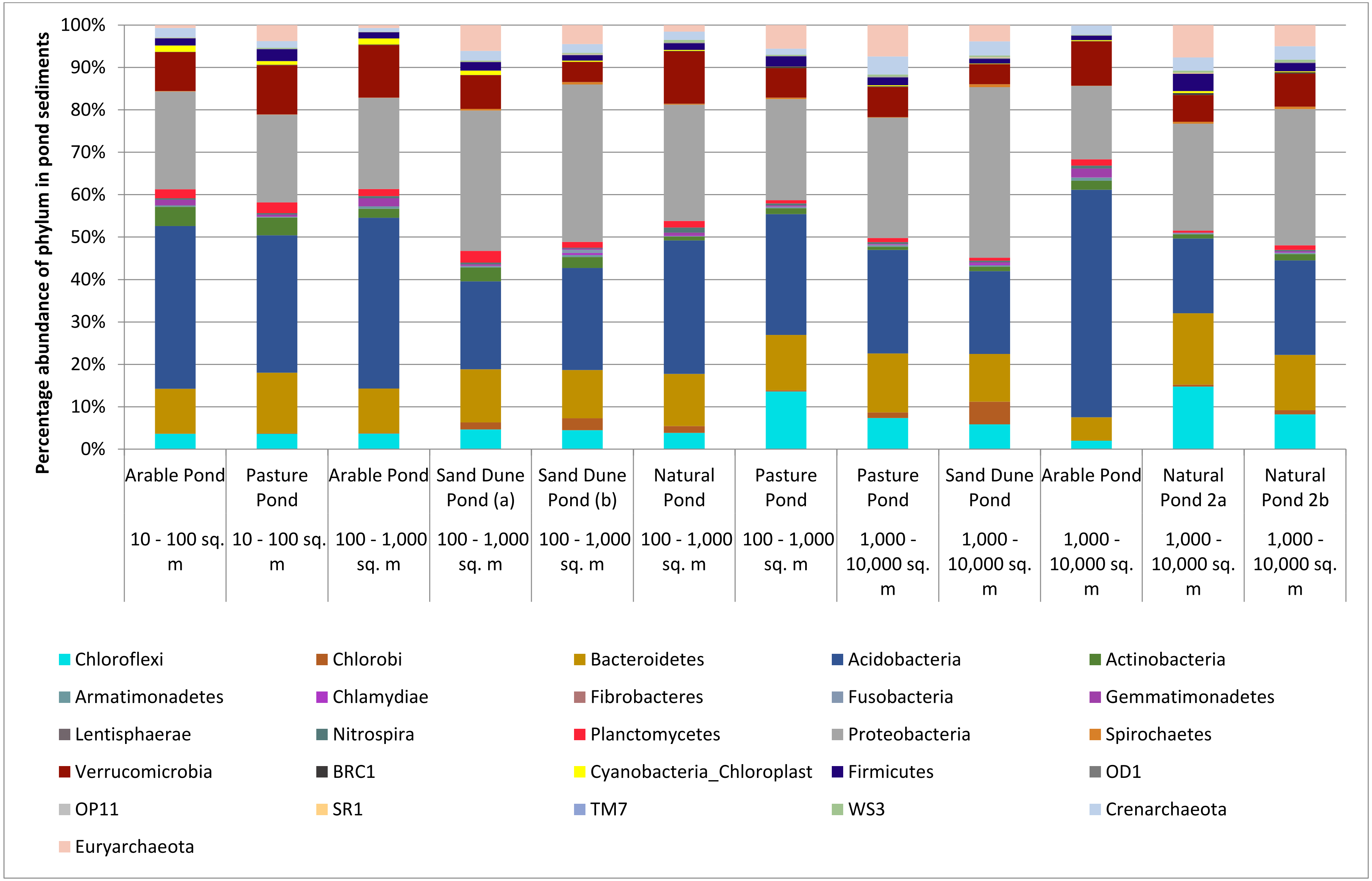

Figure 5.3: Phyla distribution in pond sediments

5.5.4 Proteobacteria Subphyla

The abundance of each Proteobacteria subphylum varied in all pond sediments (Table 5.5). There were more Betaproteobacteria and Deltaproteobacteria in comparison to Alphaproteobacteria and Gammaproteobacteria. As expected in the terrestrial environment, Epsilonproteobacteria abundance was low in comparison to other Proteobacterial subphyla because they are associated with sulphur springs and deep sea vents that are sulphur rich (Briée *et al.*, 2007). Betaproteobacteria have been known to be associated with various metabolic processes and relationships with other microorganisms (Briée *et al.*, 2007 and Glaeser and Overmann, 2004) and these potentially favoured their abundance in pond sediments.

Table 5.5: Proteobacteria subphyla across all ponds in Northumberland

Proteobacteria Subphylum	*OTU Abundance*
Alphaproteobacteria	243,067
Betaproteobacteria	463,769
Gammaproteobacteria	316,504
Deltaproteobacteria	532,968
Epsilonproteobacteria	16,703
Unclassified Proteobacteria	190,722

Betaproteobacteria were the dominant Proteobacteria in ponds within the size range 10 – 100 sq. m, all sand dune ponds analysed and arable ponds in size range 1,000 – 10,000 sq. m (Figure 5.4a). In all ponds, the total abundance of Betaproteobacteria was closely followed by Deltaproteobacteria and in the natural and pasture ponds of size ranges 100 – 1,000 sq. m and 1,000 – 10,000 sq. m, Deltaproteobacteria were more abundant than Betaproteobacteria. Gammaproteobacteria showed the highest abundance amongst the Proteobacteria phylum in the arable pond of size range 100 – 1,000. Alphaproteobacteria abundances were low in all ponds compared to Betaproteobacteria, Deltaproteobacteria and Gammaproteobacteria. Epsilonproteobacteria were also identified in the ponds but their abundances were much lower than the other subphyla. Unclassified Proteobacteria were also added to the results for completeness (Figure 5.4a). The higher abundance of Betaproteobacteria and Deltaproteobacteria in most of the ponds analysed compared to Alphaproteobacteria and Gammaproteobacteria suggests anoxia within the pond sediment environment. Betaproteobacteria also interact with other microbes such as hydrogen oxidisers, methylotrophs, dechlorinated bacteria, nitrifying bacteria and anoxygenic green sulphur bacteria (Briée *et al.*, 2007) which enhances the anoxic nature of the ponds. Similarly, Deltaproteobacteria are known to be sulfate reducers (Briée *et al.*, 2007) and are only capable of existing in anoxia.

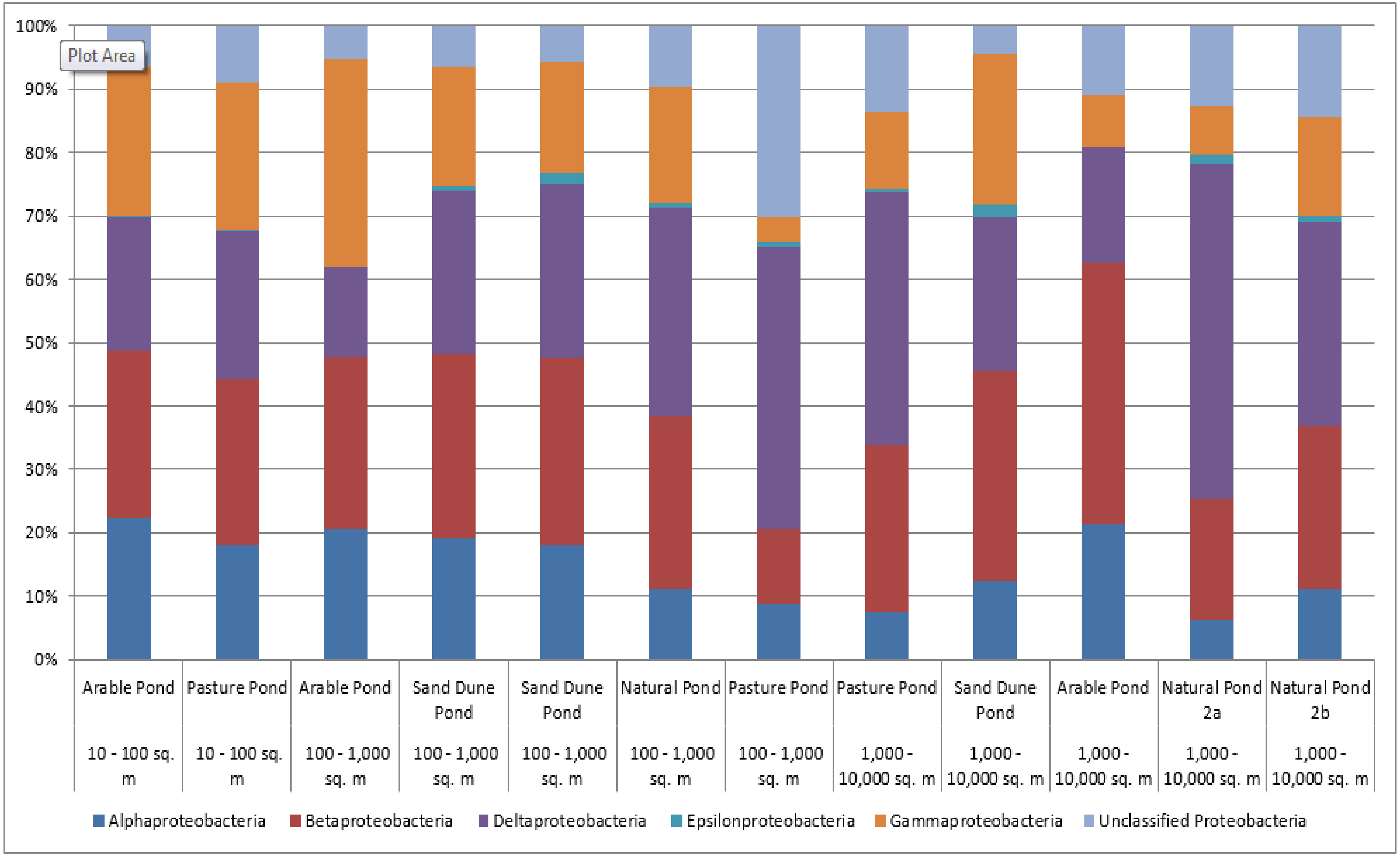

Figure 5.4a: Distribution of Proteobacteria subphyla in all ponds

The abundance of the Proteobacterial subphyla seems to have diverse effects on carbon stock in ponds. In a plot of Betaproteobacteria and Deltaproteobacteria (Figure 5.4b) to show a comparison of anoxia dependent microbes and sulfate reducing microbes, arable ponds had low carbon stocks in the size range 100 – 1,000 sq. m and 1,000 – 10,000 sq. m and this relates to low abundances of Betaproteobacteria and Deltaproteobacteria. In size range 10 – 100 sq. m, there was a higher carbon stock compared to size range 100 – 1,000 sq. m and 1,000 – 10,000 sq. m and this also was related to the higher abundance of Deltaproteobacteria in size range 10 – 100 sq. m. Natural pond cores in size range 1,000 – 10,000 sq. m had a varying abundance of Betaproteobacteria and Deltaproteobacteria. In one core there was high abundance in Betaproteobacteria and Deltaproteobacteria and this core contained a higher carbon stock compared to the second core. In the second core from the same natural pond, there was a reduction in Betaproteobacteria and this core had a lower carbon stock (Figure 5.4b). The two sand dune ponds in size range 100 – 1,000 sq. m contained a similar abundance of Betaproteobacteria and Deltaproteobacteria but the abundance of carbon stocks varied. Pasture ponds in all three size ranges contained varying abundances of Betaproteobacteria and Deltaproteobacteria. Pasture pond in size range 10 – 100 sq. m contained lower Betaproteobacteria and Deltaproteobacteria abundances compared to the other two size ranges but stocked more carbon (Figure 5.4b).

In a plot of Betaproteobacteria and Alphaproteobacteria (Figure 5.4c) to show a comparison between strictly anoxia dependent microbes and microbes that can exist in anoxia or oxia, all ponds aligned more towards Betaproteobacteria with more abundance compared Alphaproteobacteria. This indicates more anoxic microbe processes were occurring in the ponds than oxic.

In a plot of Alphaproteobacteria and Gammaproteobacteria, all ponds' carbon stocks were distributed towards the origin with ponds aligning more towards Gammaproteobacteria, which are mainly reduced sulfate oxidiser (Briée *et al.*, 2007) (Figure 5.4d). Also, there are no clear relationships of Alphaproteobacteria and Gammaproteobacteria, to carbon stocks, as the big and small bubbles (carbon stock), were mixed together.

A plot of Deltaproteobacteria and Gammaproteobacteria (Figure 5.4e) compares between sulfate reducing microbes and reduced sulfate oxidising microbes. Results showed the high carbon stock in the sand dune and one of the natural pond cores in size range 1,000 – 10,000 sq. m coincides with anoxia and sulfate reduction related microbes. Arable pond in size range 1,000 – 10,000 sq. m showed low abundance in sulfate reduction and reduced sulfate oxidation and these potentially were also contributory to the low carbon stocks in this pond's sediments. However, the overall distribution showed a wide variety of responses from each pond (Figure 5.4e).

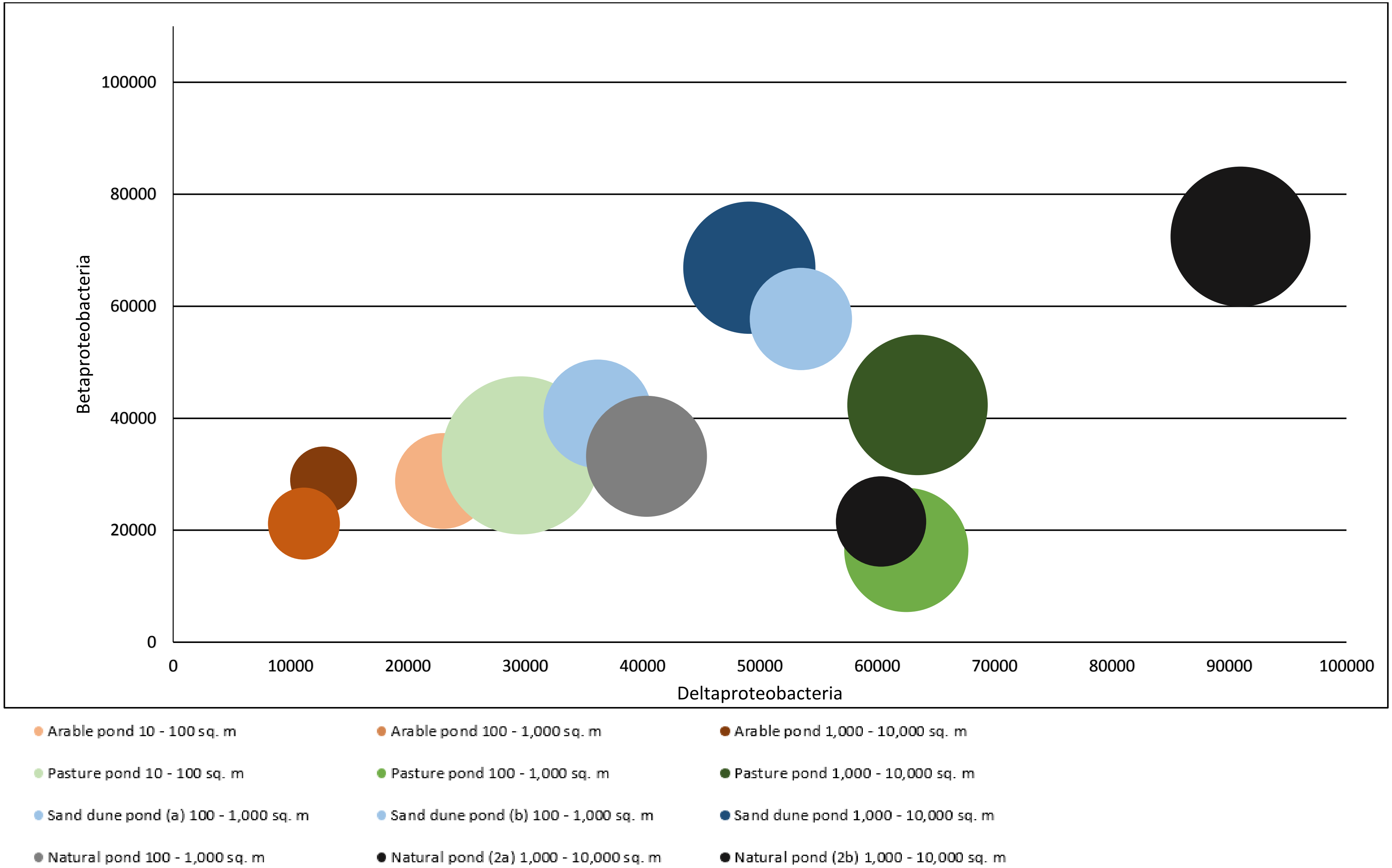

Figure 5.4b: Betaproteobacteria – Deltaproteobacteria relationship with carbon stocks in pond sediments

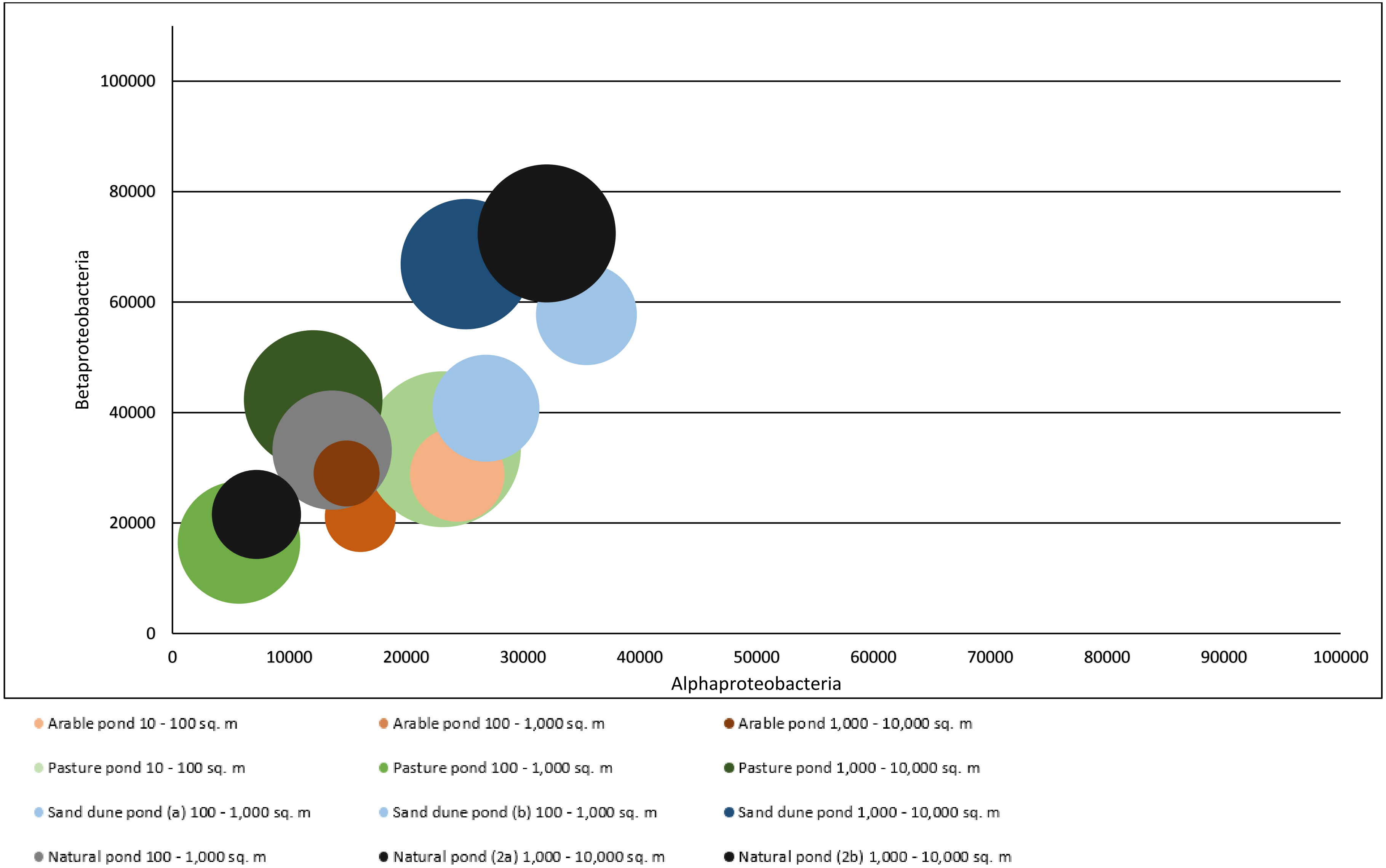

Figure 5.4c: Betaproteobacteria – Alphaproteobacteria relationship with carbon stocks in pond sediments

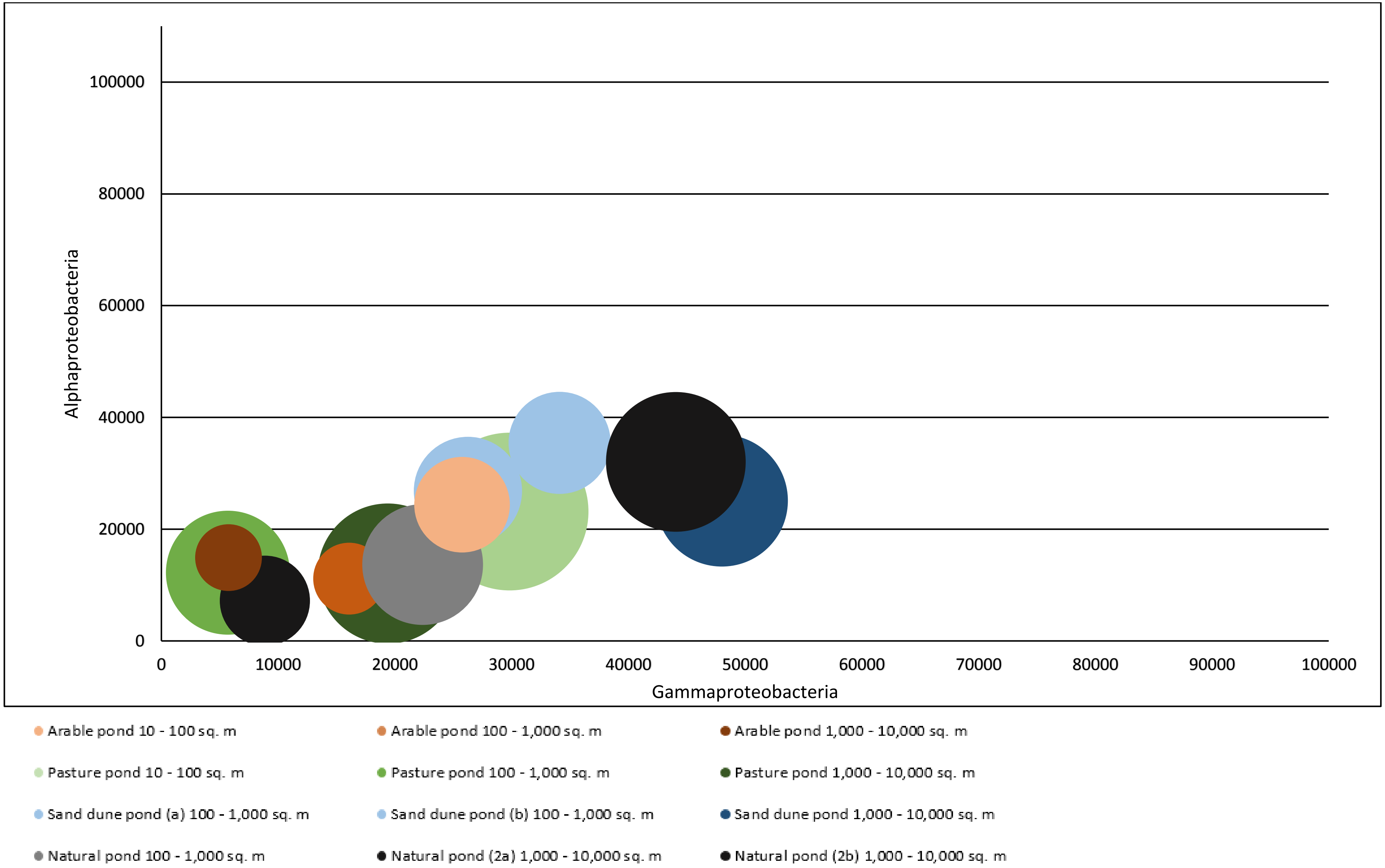

Figure 5.4d: Alphaproteobacteria - Gammaproteobacteria relationship with carbon stocks in pond sediments

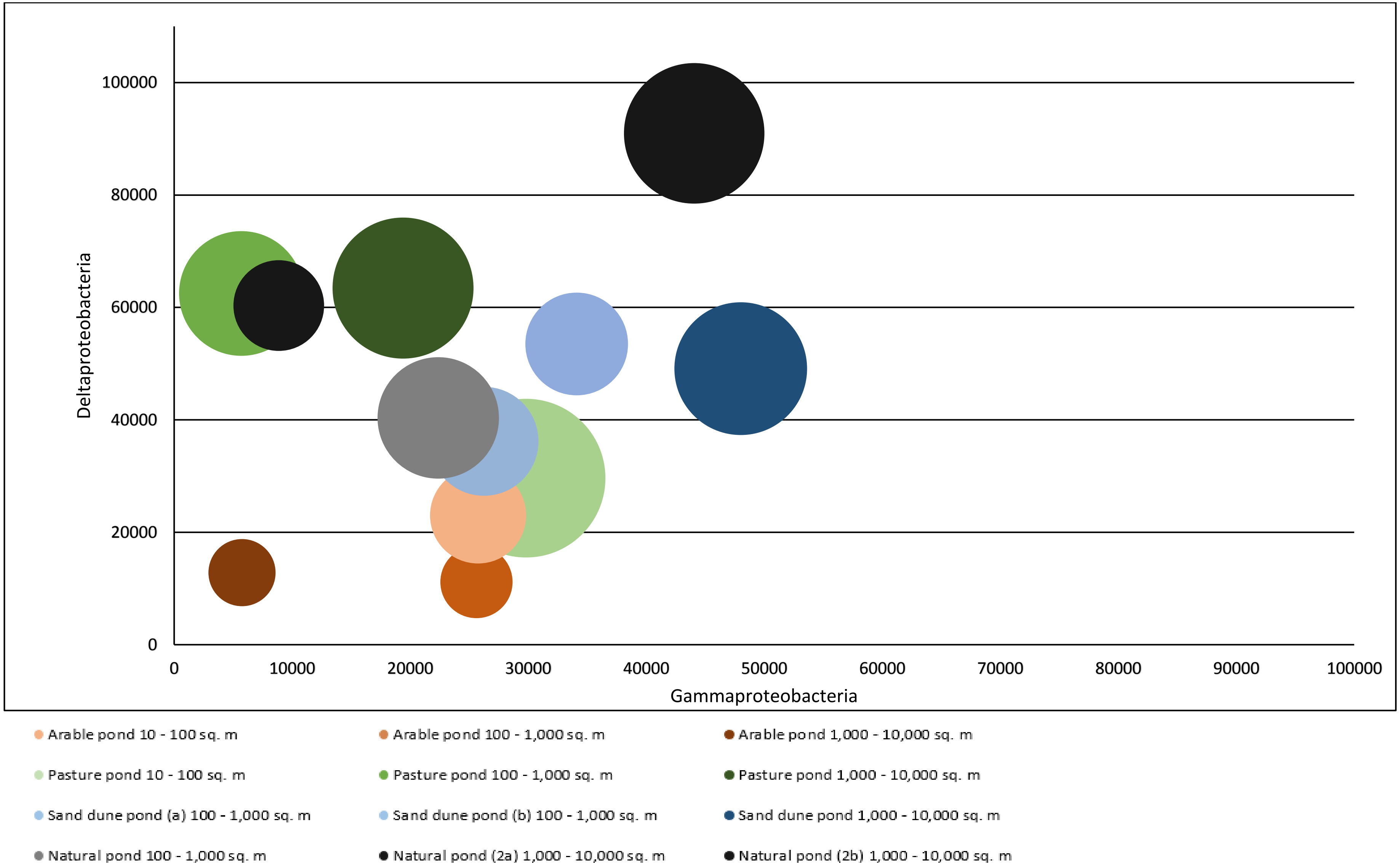

Figure 5.4e: Deltaproteobacteria - Gammaproteobacteria relationship with carbon stocks in pond sediments

5.5.5 Carbon Source in Ponds

A comparison between the main allochthonous and autochthonous microbe groups' abundance provides insight into the variation in carbon source in pond sediment. Bacteroidetes, Chloroflexi, Chlorobi and Cyanobacteria (Chloroplast) accounted for 20.59% of microbial DNA abundance or total phyla DNA abundance in all ponds analysed and these are useful for distinguishing between allochthonous and autochthonous carbon source.

Table 5.6 shows the abundance of microbes with respect to the carbon source. Overall, the average allochthonous and autochthonous microbes were 62.47 ± 2.86 % and 37.53 ± 2.86 % respectively based on averaging from all ponds analysed in Northumberland.

There was a greater abundance of allochthonous carbon utilising microbes than autochthonous carbon producing microbes in most ponds except in pasture ponds and sand dune pond of size ranges 100 – 1,000 sq. m and 1,000 – 10,000 sq. m respectively. In the ponds with two cores, the natural pond 1,000 – 10,000 sq. m had a wide variation in the main allochthonous and autochthonous microbes, while the two sand dune pond cores in size range 100 – 1,000 sq. m had similar levels of allochthonous and autochthonous microbes' abundance compared to natural pond 1,000 – 10,000 sq. m. This showed that carbon stock variation is a result of the non-uniform terrestrial or aquatic carbon influence and these variations also occurred within a pond. The arable ponds and pasture pond 10 – 100 sq. m were dominated by allochthonous carbon utilisers and this could relate to the small size, material inflow from surrounding environment and shallow depth of the ponds. And with increasing size and pond permanence, the autochthonous carbon influence increased.

Table 5.6: Proportion of allochthonous and autochthonous carbon producing microbes in each pond

		Microbial Group			
		Allochthonous		Autochthonous	
		DNA Abundance	%	DNA Abundance	%
10 - 100 sq. m	Arable Pond	50,088	67.31	24,326	32.69
10 - 100 sq. m	Pasture Pond	87,984	75.65	28,319	24.35
100 - 1,000 sq. m	Arable Pond	38,254	66.82	18,994	33.18
100 - 1,000 sq. m	Sand Dune Pond (a)	52,984	63.01	31,103	36.99
100 - 1,000 sq. m	Sand Dune Pond (b)	60,152	60.19	39,784	39.81
100 - 1,000 sq. m	Natural Pond	54,863	68.55	25,170	31.45
100 - 1,000 sq. m	Pasture Pond	76,588	48.26	82,121	51.74
1,000 - 10,000 sq. m	Pasture Pond	78,782	61.13	50,092	38.87
1,000 - 10,000 sq. m	Sand Dune Pond	56,672	49.89	56,930	50.11
1,000 - 10,000 sq. m	Arable Pond	22,289	70.56	9,300	29.44
1,000 - 10,000 sq. m	Natural Pond (a)	75,943	51.75	70,805	48.25
1,000 - 10,000 sq. m	Natural Pond (b)	115,138	58.02	83,318	41.98

5.6 Discussion

5.6.1 Total Microbial Abundance

The pond environment and microbial abundance determine the success of carbon stocking; microbes interact with carbon as a nutrient source while prevailing condition in the pond environment determines carbon dynamics. This research clearly shows that on pasture and arable land, smaller ponds (10 – 100 sq. m) contain greater microbial DNA abundance than larger ponds. This relates to the ability of microorganisms in the smaller ponds to proliferate more efficiently in the presence of higher nutrient concentrations and favourable environmental conditions created by the small size of the ponds (Battin *et al.,* 2009, Cole *et al.,* 2007 and Shirokova *et al.,* 2013). In reduced carbon stock environments, such as the arable ponds, low carbon stocks can be related to presence or absence of living microbes in compact clayey sediment that can limit nutrient migration for microbial proliferation (Avnimelech *et al.,* 2001, Boyd *et al.,* 2010 and Grabowski *et al.,* 2011).

Quick drying and wetting of ponds alters nutrient concentrations that leads to development of successional microbial groups. Thus, carbon processing by microbes remains continuous and ponds can sustain microbial growth irrespectively of the stages of the smaller ponds' life cycle (Rao, 2011 and Kobayashi *et al.*, 2015). These seasonal changes in ponds were also reflected in the changes in microbial species or phyla abundance as seen in the down core profile analysis of ponds in Northumberland.

The abundance of microbes, their growth dynamics and ecological interactions also tie to microbes' ability to utilise or produce allochthonous and autochthonous carbon respectively (Battin *et al.,* 2009, Biggs *et al.,* 1994, Gilbert *et al.*, 2014 and Jeffries, 2008). The top sediment of pasture pond in size range 10 – 100 sq. m contained lower microbial

DNA abundance compared to bottom sediments of the same pond. This could be because of an environmental limiting factor that stunts microbial abundance at that successional stage, such as loss of labile organic carbon, for example short chain alkanes, alkenes and light aromatics (Inegbedion, 2013) upon atmospheric exposure during the dry phase. But bottom sediments could have been deposited in more environmentally favourable conditions such as water cover and high nutrient availability.

Change in surrounding vegetation and seasonal weather flux also influence carbon stock (Jeffries, 2008 and Gilbert *et al.,* 2014) and microbial abundance in pond sediments. This was also observed in this research. Depositional environments differ in arable, pasture, sand dune and natural ponds causing variation in carbon stocks and microbial abundance. Physical/environmental, and chemical/biochemical factors/influences, and response of the microbial community to carbon source either favours or limits different phyla. Although the temporal changes in microbial communities were not defined to species level, the down core profile of DNA abundance across pond sizes reflected variations in microbial phyla. This potentially means changes in surrounding depositional environments can be reflected in nearby pond sediments.

Furthermore, these changes in the depositional environment are not uniform across a pond. Natural pond in size range 1,000 – 10,000 sq. m showed different carbon stock and microbial DNA abundance within the same pond. The dynamics clearly show the non-uniformity of lateral carbon deposition and microbial abundance in pond sediments. This non-uniformity also means that a pond’s microbial dynamics potentially may not accurately reflect successional changes or change in depositional environment as result of non-uniformity of water and aeolian material transport, sedimentation and variation in carbon oxidation in different parts of the ponds. These influences alongside quick wetting and drying in the temporal small ponds will further contribute to limiting the uniformity of carbon or material deposition in pond sediments (Baker *et al.*, 2015, Beazley *et al.,* 2012, Downing, 2010, Kortelainen *et al.,* 2004, Lloyd *et al.,* 2014 and Shirokova *et al.,* 2013). This further explains the variation in carbon stocks in the same ponds. Also, the pond edge is more efficient at reflecting the changes in depositional environments because of its proximity to the surrounding environment compared to central parts of the pond because of material dispersal and flux in sedimentary transport (Demaison and Moore, 1980).

PCR is not very effective at distinguishing living from non-living cells but it gives a snapshot of the existing phylogenetic diversity of living, dead and hibernating cells (Sheridan *et al.,* 1998 and Soejima *et al.,* 2008). Relating DNA abundance to carbon stock in pond sediment is skewed by the non-living microbes that are obtained using PCR analysis. Also in a nutrient-rich environment, microbial proliferation increases with the selective growth of successful microbes and their symbionts or any other ecological relationships. Thus, nutrient-rich environments are likely to show less diversity in PCR microbial identifications because of the large replication of similar microbes that have the same genes. This can be clearly distinguished in the top sediments of pasture and arable ponds in size range 10 – 100 sq. m, where arable ponds contained higher phyla diversity in microbial DNA reads, compared to pasture ponds. It is probable that PCR is identifying numerous 16s rRNA genes in arable pond top sediments that are living, dead or hibernating as a result of the growth limitations of compact clayey sediment. The wider microbial diversity could also reflect the absence of a quantitatively dominant microbial group in the arable pond’s top sediments. Conversely, the pasture ponds in size range 10 – 100 sq. m showed less DNA abundance compared to arable ponds. This could be because low PCR abundance reflects less diversity in the microbial community structure that potentially led to replication of similar microbes and microbes that share similar ecological relationships. Although quantitative abundance of microbes in ponds cannot be measured using PCR, pasture ponds stocking of more carbon potentially contributes quantitatively more ecologically successful and predominant microbes in the pond environment than microbes in stasis (Ansola *et al.,* 2014, Battin *et al.,* 2009, DeLong, 2005 and Nyström, 2003).

5.6.2 Microbial community structure and dynamics

Baker *et al.* (2015) showed in a 16s rRNA gene survey that estuary sediments of White Oak River, North Carolina contained Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Chloroflexi, Planctomycetes, Bacteroidetes, Gemmatimonadetes, Nitrospira, Chlamydiae and Spirochaetes and these were similar with other estuary sediments of Pearl River and Yangtze estuary in South China (Beazley *et al.,* 2012, Jiang *et al.,* 2011 and Zhu *et al.,* 2013). Although these environments were an estuary, the same microbial groups were found in Northumberland pond sediments. Northumberland pond sediments also shared similarities with the estuaries in having Acidobacteria and Verrucomicrobia amongst the more abundant phyla (Beazley *et al.,* 2012, Jiang *et al.,* 2011, Madigan, 2003, Quaiser *et al.,* 2003 and Zhu *et al.,* 2013). Therefore, there is evidence of some metabolic pathways to be similar in Northumberland pond sediments and other natural environments.

The varying abundance of 24 bacterial phyla in all pond sediments relates to pond microbes' interdependence in metabolising organic carbon and preferential ecological conditions. Baker *et al.* (2015) also identified variations and complexities in microbial metabolism in the estuary of White Oak River, North Carolina, USA. They showed that mapping metabolic pathway processes within sediments involved mapping complex and multiple enzymatic processes from different microbes, and it is limited by identification of uncultured microbial groups. Metabolism in Northumberland ponds showed a general overview of anoxic processes that can mediate carbon, sulphur, and nitrogen derived pathways from large substrates. Defining the entire microbial processes in pond sediments requires clear knowledge of processes within the known (taxonomically classified) microbial groups, unclassified/uncultured taxa and candidate phyla (Ansola *et al.,* 2014, Baker *et al.,* 2015 and Briée *et al.,* 2007), but PCR analysis was limited in analysing unclassified microbes. Each Northumberland pond had variation in microbial abundance, phyla and potentially metabolisms. These variations in the known phyla abundance suggest that microbial metabolic processes are environment specific and are not perfectly replicable in a different environment.

Close taxonomic similarities in Northumberland pond sediments suggest that microbes interact at varying levels to produce carbon source or sinks through the exchange of substrates, metabolites and genes (Baker *et al.,* 2015, Cole *et al.,* 2007, Tranvik *et al.,* 2009 and Youssef *et al.,* 2015). The resulting relationship creates an environment for microbes to co-exist efficiently. Temporary arable ponds showed a low abundance of methanogens while permanent ponds had higher abundance. Thus, methane-rich sediment in permanent ponds potentially performed more efficient sulfate-methane transition (SMTZ), which require interaction amongst methanogens, Deltaproteobacteria and Chloroflexi (Baker *et al.,* 2015 and Briée *et al.,* 2007) than in temporary pond, amongst other metabolic processes.

Other potential enzymatic processes within Northumberland pond sediments include carbohydrate hydrolytic genes found in Chloroflexi, Bacteroidetes, Gemmatimonadetes, and Planctomycetes amongst other phyla. These microbes have been found to degrade cellulose, hemicellulose and polysaccharide degrading genes (Berlemont and Martiny, 2013). Degradation of chitin involves multiple genes and requires interdependence of the microbes within pond environment to hydrolyse and degrade the intermediary substrates. N-acetyl-glucosaminidase genes are found in Bacteroidetes and Deltaproteobacteria and endo-acting chitinase genes are found in some Chloroflexi and Planctomycetes. Protein degradation is usually accessed from extracellular peptidases which vary depending on pond environmental conditions. Bacteroidetes and Gemmatimonadetes are involved in making carbon and nitrogen bioavailable (Lloyd *et al.,* 2014). Fatty acids or organic acid β-oxidation pathways for creating acetyl-coA involves various species of Gammaproteobacteria, Betaproteobacteria, Chloroflexi and Gemmatimonadetes which were found in all ponds. These organisms provide partial or total metabolism that forms acetyl coA (Beazley *et al.*, 2012 and Lloyd *et al.,* 2014). Bacteroidetes and Spirochaetes are mainly associated with fermentation using aldehyde dehydrogenase and alcohol dehydrogenase (Baker *et al.*, 2015). Most of the phyla groups within the ponds have a wide diversity of species that can perform these processes at varying levels in different environments and this further creates complications of distinguishing metabolic processes.

The environmental conditions can make specific microbial phyla perform certain metabolic functions, such as the Bacteroidetes behave as carbon utilisers or heterotrophs of cellulose and chitin in anoxia (Baker *et al.*, 2015 and Briée *et al.*, 2007). This further creates a Bacteroidetes relationship with other microbes to be predominantly heterotrophic feeding related in anoxia, such as the Bacteroidetes relationship with OD1 (Chouari *et al.,* 2005). In anoxia, the environment triggers Chloroflexi, Cyanobacteria and Chlorobi to become the main autotrophic microbes in sediments using H_2S amongst other molecules for the primary producing role (Elshahed *et al.*, 2003 and Madigan, 2003). Also, microbial abundances vary depending on other factors within the environment, such as a favourable growth environment for Chlorobi in sand dune ponds or growth limitation of Cyanobacteria in all ponds could be due to pond turbidity, metabolites and/or predation.

Furthermore, superphylum Fibrobacteres-Chlorobi-Bacteroidetes (FCB) groups in association with WS3 had previously been obtained from anoxic layers of Sakinaw Lake in British Columbia, Canada and the anoxic sediments of a coastal lagoon (Etoliko Lagoon, Greece) (Youssef *et al.,* 2015). The phyla that make up this superphylum were also present in some of the Northumberland pond sediments. Each phylum has metabolic relationships that enhance their abundance in each pond sediment environment by sharing nutrient, genes and substrates (Baker *et al.,* 2015). However, this research is not furnished with information to check the details leading to forming of a superphylum but it is clear that some Northumberland pond sediments show the prerequisites to establish such potentials. The ponds also had the potentials of superphylum Planctomycetes-Verrucomicrobia-Chlamydiae (PVC) groups (Wagner and Horn, 2006) in most of the ponds except in arable pond 1,000 – 10,000 sq. m because of the absence of Lentisphaerae.

5.6.3 Oxygenation in Ponds

Most phyla in the Northumberland ponds analysed were associated with anoxic sediments. Proteobacteria, Acidobacteria and Verrucomicrobia were more abundant than other phyla in the ponds and are both oxia and anoxia dwelling microbes (Briée *et al.,* 2007, Hahn *et al.,* 2003, Hahn, 2006 and Madigan, 2003). However, the other phyla in the ponds exist in anoxic ecological conditions, suggesting the dominance of anoxic dwelling species of Proteobacteria, Acidobacteria and Verrucomicrobia.

In the analyses of the subphyla of Proteobacteria, sulphate reducing and anoxia dependent Deltaproteobacteria and Betaproteobacteria were more abundant than

Alphaproteobacteria and Gammaproteobacteria (Briée *et al.,* 2007 and Garrity *et al.,* 2005) in most Northumberland pond sediments. The abundance and variation of other microbial phyla such as Euryarchaeota, Crenarchaeota and Bacteroidetes which are predominantly associated with methanogenesis and fermentative processes further supported the state of anoxia in the ponds (Boetius *et al.,* 2000, Elshahed *et al.,* 2003, Lundgren *et al.,* 2008 and Madigan, 2003). Betaproteobacteria have also been known to develop anoxic relationships with other microbes like methylotrophs, dechlorinated bacteria, anoxygenic green sulphur bacteria, nitrifying bacteria, hydrogen oxidisers, H_2S and sulfate oxidisers (Glaeser and Overmann, 2004) to share nutrients and metabolites while sustaining growth in an oxygen depleted environment. In Northumberland pond sediments, sulfate reducing Deltaproteobacteria were more abundant than Gammaproteobacteria that oxidise reduced sulfate. Their greater abundance meant that more reduced sulfate that is not oxidised by Gammaproteobacteria is made bioavailable for the furtherance of anoxia (Karr *et al.,* 2005). The reduced sulfate produced by Deltaproteobacteria could potentially be used by other anoxia dependent microbes such as myxobacteria, degraders, fermenters and methanogenic archaea in the Northumberland pond sediments (Briée *et al.,* 2007).

There were variations in the abundance of Firmicutes in different ponds which reflects varying levels of anoxia, suboxia and pond stratification at the chemocline (Briée *et al.,* 2007). In natural pond 1,000 – 10,000 sq. m, there were variations in Deltaproteobacteria abundance in the two cores analysed and this can be associated with the varying and unequal supply of allochthonous and autochthonous carbon in different parts of the pond. Also, Firmicutes abundance in the two cores varied and this confirmed varying levels of anoxia and pond stratification (Briée *et al.,* 2007). The core with high Firmicutes abundance showed high Deltaproteobacteria abundance and low Firmicutes abundance coincided with a lower abundance in Deltaproteobacteria. This suggests pond stratification improves anoxia and anoxia dependent metabolic processes in ponds, pond stratification is important for the effective carbon storage in bottom sediment, ponds with structured stratifications tend to accumulate carbon from the suboxic and oxic layers and store them in the anoxic layer. Also, large molecular weight of allochthonous materials is stored more efficiently due to reduced oxygenic mineralisation.

Methanogenesis by Archaea also contributes to anoxia. Methanogenic bacteria were more predominant in permanent ponds. During field sampling gas bubbles (potentially CH_4 and H_2S) were observed in some ponds. The temporary ponds (arable ponds and pasture pond of size range 10 – 100 sq. m) had low methanogenic bacteria abundance probably as a result of the dry environment which favoured outgassing. However, anoxia in temporary ponds could be created by the other microbial phyla such as Chlorobi (microbial mat formation). It is unclear if the quantitative microbial abundance related to anoxia was reduced by low methanogens abundance because it is possible for microbes to create other pathways that lead to anoxia.

In the Northumberland pond sediments analysed, anoxia further encourages the proliferation of fermenters, degraders and anoxygenic phototrophic microbes at varying levels. For the phyla with a spectrum of oxic and anoxic microbes such as Acidobacteria, Planctomycetes and Verrucomicrobia, anoxia, the pre-existing ecological relationships, metabolic pathways, metabolites and other physical/environmental and chemical/biochemical influences would determine their proliferation in pond sediments (Madigan, 2003 and Sangwan *et al.*, 2005). As with Proteobacteria, anoxic dwelling microbes of Acidobacteria, Planctomycetes and Verrucomicrobia phyla will be more predominant; with, for example, anoxygenic production of nitrate from ammonia by *Planctomyces* spp. (van Niftrik *et al.*, 2004). Other species that are unable to survive anoxia are forced to adapt, sporulate, die or form seed banks pending a change to more favourable environmental conditions or pond succession (Ansola *et al.,* 2014).

5.6.4 Microbial analyses of carbon source

The high abundance of more anoxic Proteobacteria, Acidobacteria, Verrucomicrobia, Bacteroidetes, Chloroflexi, Chlorobi and Cyanobacteria is possibly the main influence on allochthonous and autochthonous carbon processing within the ponds. Their abundance in the Northumberland ponds influences carbon stock level, carbon source and oxic/anoxic state of microbial processing. However, PCR analysis of microbial 16S rRNA should be used with caution as it gives no quantitative abundance of the individual microbial cells which carry out the carbon processing. But it is reasonable to infer that DNA abundances of the allochthonous utilising and autochthonous producing microbes provide an estimate of allochthonous and autochthonous carbon sources.

To meet their energy needs, microbes in Northumberland pond sediments utilise various metabolic pathways. Their feeding patterns and preferential carbon source differ based on microbial energy requirements and capacity to degrade or transform simple or complex organic matter. Acidobacteria and Verrucomicrobia species vary widely and are involved in both heterotrophism and phototropism. Proteobacteria have been known to have oxygen dependent, sulfate reducing, sulfate oxidising and other anoxic microbial metabolisms (Briée *et al.,* 2007 and Glaeser and Overmann, 2004). These variations make it difficult to differentiate between carbon source used by Proteobacteria, Acidobacteria and Verrucomicrobia and other phyla in the ponds. However, microbial abundance and environmental effects provide a strong level of selectivity in some aspects of microbial community growth, such as Chloroflexi being predominantly phototrophic in anoxia (Elshahed *et al.,* 2003), while in oxic environments they hydrolyse carbohydrate (Baker *et al.,* 2015). It is inferable that anoxia causes microbial species in a phylum to predominantly express specific carbon source preference.

Generally, Bacteroidetes are predominantly heterotrophic in nature and they degrade cellulose and chitin in anoxia (Briée *et al.*, 2007) and produce substrates that are useful to other microbes. They have also been known to have a phylogenetic relationship as a superphylum, where they share genes for growth with other microbes such as carbohydrate hydrolytic genes as Gemmatimonadetes, Planctomycetes and Chloroflexi (specifically *Anaerolineae*) (Baker *et al.,* 2015) could also be found in Bacteroidetes. Bacteroidetes are influential in the utilisation of allochthonous carbon and enhancers of other microbes that utilise allochthonous carbon in anoxia. Therefore, their abundance can provide a reasonable estimate of the level of allochthonous carbon utilisation by microbes in each pond. Similarly, Chloroflexi, Chlorobi and Cyanobacteria phyla are known to be predominantly phototrophic in anoxia and their abundances can be used to infer autochthonous carbon processes in pond sediments. In all Northumberland ponds analysed, the main allochthonous and autochthonous carbon utilising and producing microbes account for 20.59% of the total DNA reads or total classified phyla abundance in all ponds.

Most of the ponds analysed had more allochthonous than autochthonous influence and this had to do with their proximity to terrestrial habitats and material transports. This proximity and nature of organic carbon altered the microbial community and suggest varying levels of oxygenation in the different parts of a pond. Variation of microbial abundance and carbon stock within ponds also signified potential non-uniform microcosmic environmental conditions within the pond, which cause microbes to utilise allochthonous and create autochthonous carbon differently. This was previously identified in the dry bulk density, sediment wetness and material dispersal variations as environmental factors that influenced carbon stocks in Chapter 4. In the permanent natural, sand dune and pasture ponds in size range 1,000 – 10,000 sq. m, more allochthonous microbes were found in the pond edge and this decreased toward the pond centre as a result of non-uniform material dispersal. This encourages the proliferation of autochthonous microbes in areas further away from the pond edge although, on the other hand, autochthonous photosynthetic microbes can be limited by light penetration and pond depth (Bärlocher and Boddy, 2016, Boyd, 1995, Boyd and Tucker, 2012, Briée *et al.*, 2007 and Porter *et al.*, 1988).

Terrestrial inclusions in the sediment that are resistant to microbial influence (Shirakova *et al.*, 2013) can increase allochthonous carbon without a corresponding increase in allochthonous microbes. High terrestrial influence could be critical to the higher carbon stock in pasture pond in size range 10 – 100 sq. m which had low abundance of Betaproteobacteria and Deltaproteobacteria compared to natural ponds and pasture ponds in size range 100 – 1,000 sq. m and 1,000 – 10,000 sq. m (which had comparatively lower carbon stocks but higher abundance of Betaproteobacteria and Deltaproteobacteria). Therefore, small ponds stock more carbon because of their small size's ability to accumulate and process surrounding plant and animal waste and produce autochthonous sourced carbon in high nutrient environments. Larger water bodies process more aquatic derived carbon by utilising pond stratification and anoxia but allochthonous carbon is widely and non-uniformly dispersed.

5.6.5 Some Other Metabolic Processes in Ponds

Genus *Fibrobacter* of Fibrobacteres originates from the rumen of ruminant animals (Béra-Maillet *et al.*, 2004) and is found in environments associated with pasture grazing and ponds used as a drinking water source. This potentially explains why Fibrobacteres were more abundant in permanent ponds >100 sq. m. They support the degradation and processing of plant polysaccharides.

Epsilonproteobacteria are rare Proteobacteria that are found in deep sea environment and sulphur springs (Briée *et al.*, 2007 and Lindstrom *et al.,* 2005). However, they were found in some Northumberland pond sediments at low abundance compared to the other Proteobacteria subphyla. They were more abundant in ponds close to the North Sea shore. Thus, seawater and sea microbes potentially influence Northumberland ponds to some degree.

5.7 Limitation of Microbial Research

Ponds sampled for microbial analyses were the same as for carbon analyses. Thus, there were again no inland Northumberland analyses for microbial influence on carbon capture. Also, one sediment core was analysed per pond, except for the sand dune pond (100 - 1,000 sq. m) and natural pond (1,000 - 10,000 sq. m) where two cores were analysed. No pond was analysed in triplicates. This was a limitation of logistics and available financial resource to execute PCR analyses of pond cores. However, within the same research group, Taylor (2017) conducted investigations on microbes using PCR analyses with similar laboratory methodology and found variations in microbial abundance in triplicate sample analyses of arable, natural, pasture and sand dune pond sediments.

5.8 Conclusion

Microbial communities in ponds vary widely. Their influence on carbon stock is very important in determining carbon source or sink potentials of the ponds. Microbes contribute to the wide varieties of factors that control the carbon stock. Ponds analysed in Northumberland had varying levels of anoxia which seems to enhance carbon stocking. However, carbon stocking was not solely associated with predominant anoxia. Smaller water bodies of size range 10 – 100 sq. m showed less methanogenic activity but anoxia occurred through concerted efforts of the sulfate reducers, fermenters, anoxygenic microbes and other anoxia related microbes. Also, the predominant microbial phyla control the ecological relationships in pond sediment and their responses to different pond environments varied. Thus, anoxia prevalent Betaproteobacteria and Deltaproteobacteria and others were predominant in the ponds analysed.

Arable and pasture ponds confirmed postulations by Battin *et al.* (2009), Cole *et al.* (2007), Downing *et al.* (2006) and Gilbert *et al.* (2014) that decreasing sizes of water bodies leads to a corresponding increase in carbon stock in the pond sediments. Increased carbon stock in small ponds was predominantly as a result of more terrestrial inputs and high nutrient environments that favour autochthonous carbon production by microbes. Large ponds utilise microbial induced anoxia and stratification to stock carbon. The large size allows for nutrient dilution and non-uniform dispersal of terrestrial carbon with more autochthonous carbon production in the pond centre.

Chapter 6 – Northumberland regional carbon stock

6.1 Tying it all together

There are multiple factors/influences that control the abundance of water bodies and the amount of carbon stocked. However, the aim of this chapter is to quantify the importance of small water bodies in Northumberland for carbon capture based on:

- Number and surface area of water bodies
- Carbon stock per sq. m of ponds
- Main allochthonous and autochthonous microbes

Thus, this chapter:

- ties together the research from chapters 3, 4 and 5 to estimate regional carbon stocks and their sources
- discusses the importance of small water bodies to carbon capture
- identifies the research limitations and proffer recommendations
- and state research conclusions

6.1.1 Estimating Northumberland water body distribution (NWBD)

In Chapter 3, Northumberland water body distribution was assessed using multiple images of varying resolutions at two scales of study. Water bodies were categorised into size ranges 10 – 100 sq. m, 100 – 1,000 sq. m, 1,000 – 10,000 sq. m, 10,000 – 100,000 sq. m, 0.1 – 1 sq. km, 1 – 10 sq. km and 10 – 100 sq. km. There were a varying number and surface area of water bodies in the images with more variations in water bodies <10 sq. km than >10 sq. km. These variations were also observed in the temporal analysis using Landsat-8 imagery.

Although the research was designed to identify all water bodies in Northumberland, satellite imagery data was not effective at identifying water bodies within size range 1 – 10 sq. m due to the quality of imagery, cloud cover and the inability to abundantly see water bodies through vegetation at scales of 1:2,000 and 1:20,000. Jeffries (2016) showed that field survey provided another approach to identifying water bodies in size range 1 – 10 sq. m. In estimating NWBD, size range 1 – 10 sq. m has been omitted here as the majority of these small ponds is highly similar to the surrounding environment and soil with quick wetting and drying. This makes them difficult to distinguish in imagery. The number and surface area of water bodies are continuously in seasonal flux (Seekell and Pace, 2011) and this was observed in the multi-temporal Landsat-8 study. Jeffries (2016) data from Druridge Bay, Northumberland showed the high and low abundance of water bodies are as a result of the change in precipitation. Chapter 3's Northumberland water body distribution was inferred to be the median of high and low abundance of Northumberland water body distribution (NWBD) based on Jeffries (2016) data.

Northumberland water body distribution (NWBD) was derived from World Imagery, and Landsat-8 data collected from July 2013, September 2013, July 2014, December 2014 and April 2015. It involved combining the averages of number and surface area of water bodies in each size range obtained from each image that was analysed at 1:2,000 and 1:20,000 scales. Standard error was obtained in size ranges 10 – 100 sq. m, 100 – 1,000 sq. m, 1,000 – 10,000 sq. m, 10,000 – 100, 000 sq. m and 0.1 – 1 sq. km because the two scales of analysis and image types showed varying number and surface area of water bodies. At size range 1 sq. km – 10 sq. km and 10 – 100 sq. km, only World Imagery data at scale 1:20,000 was used because these large water bodies were equally well identified in all image types and scales. They contained 2 and 1 water bodies respectively, thus, no error bars (Figure 6.1a and b). At 1:20,000 scale, an average of 776 water bodies covering

65.83×10^6 sq. m ± 20.12×10^6 sq. m were identified on Landsat-8 in the images analysed. The highest number of water bodies counted was in July 2013 and the highest water body surface area covered in Northumberland was identified on Landsat-8 images of July 2015. Also at 1:20,000 scale, 1,395 water bodies covering a surface area of 26.24×10^6 sq. m were identified on World Imagery. At 1:2,000, water bodies were identified in selected areas of Northumberland and this was upscaled using three different approaches to establishing different versions of Northumberland water body distributions. And this was extensively discussed in Chapter 3.

In creating Northumberland Water Body Distribution (NWBD) (Figure 6.1 a and b), number and surface area of water bodies obtained from World Imagery and all dates on Landsat-8 at 1:20,000 scale, and the three upscaled approaches in determining number and surface area of water bodies using World Imagery at 1:2,000 scale were combined. Generally, water bodies increased in surface area from small to large and decreased in number from 10 sq. m to 1 sq. km (Figure 6.1a and b). Given images of Landsat-8 were obtained from different dates, World Imagery was obtained as a stitch of best quality high resolution images obtained from different dates and water body distribution is influenced by precipitation and evaporation (Jeffries, 2016). Images analysed suggest varying levels of water accumulation or pond wetting and pond drying or disappearance. Therefore, the error bars are also depicting lower and upper limits of number and surface area of water bodies between July 2013 and April 2015 and dates of data collection for World Imagery stitched images. As discussed in chapter 3, the extrapolated estimates must be considered with caution, because of limitations in the quality of imagery and the fluctuations in number and size of water bodies. Also, the estimates did not involve taking into account of land use or topography. However, these are the best estimates that have ever been produced for the Northumberland region, including water bodies < 0.1 sq. m.

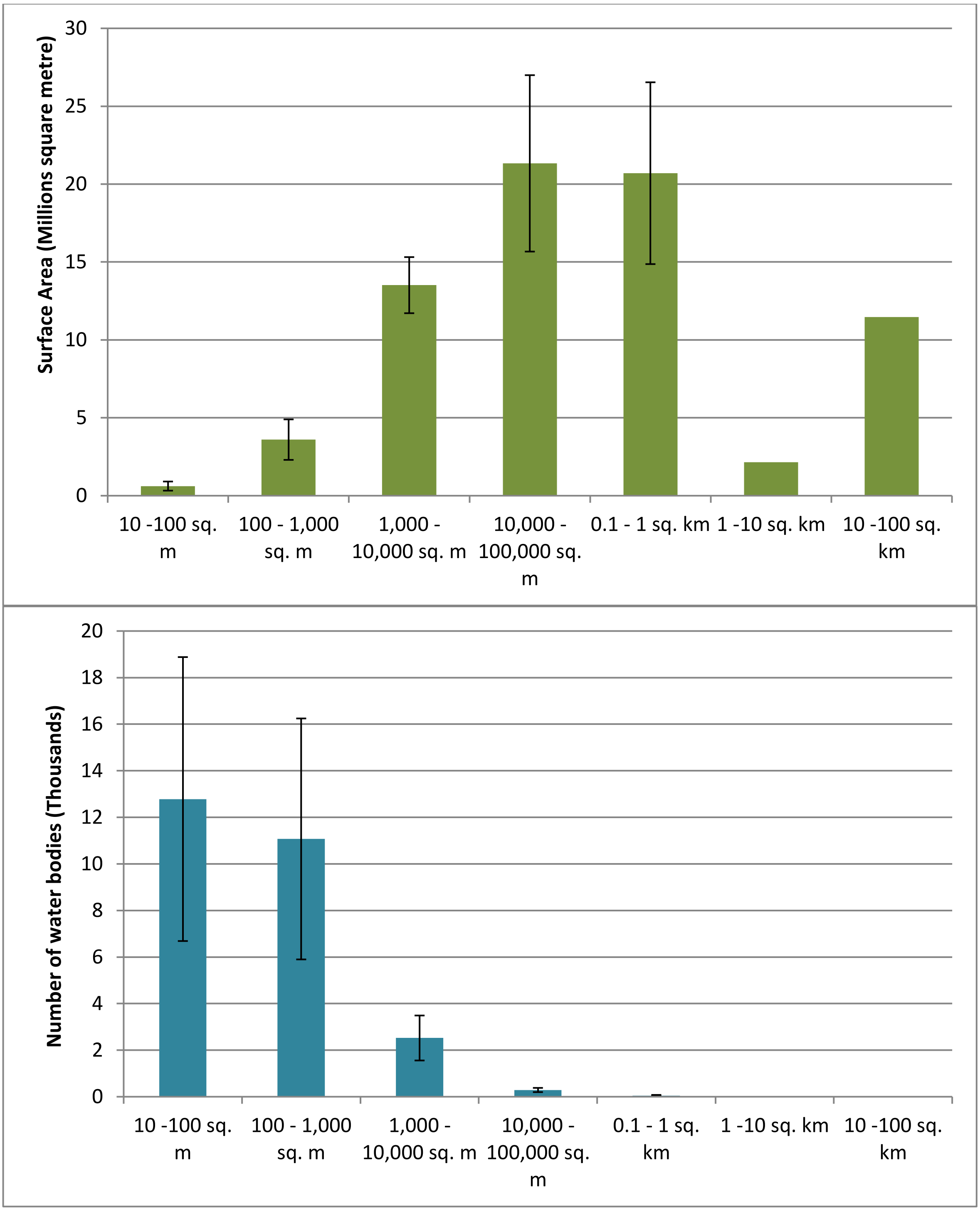

Figure 6.1: Estimate of Northumberland Water Bodies Distributions (a) surface area (b) number

6.1.2 Estimating carbon stock in Northumberland's large water bodies and regional carbon stock in all Northumberland water bodies

In the chapter 4 research, carbon stock has been found to vary across, within and down core sediment of water bodies. This is the consequence of the prevailing physical/environmental, biological and chemical/biochemical factors. These variations meant that there is no fixed estimate of carbon stock values for all the water bodies in Northumberland. Based on the pond types, arable, pasture, natural and sand dune ponds in Druridge Bay, Northumberland average carbon stock ranges from 1.65 ± 0.38 to 5.26 ± 0.73 Kg C/sq. m. Based on pond size, average carbon stock in Northumberland range from 3.04 ± 0.61 to 4.48 ± 2.07 Kg C/sq. m for ponds with surface area range of 10 sq. m - 10,000 sq. m.

To determine carbon stock per square metre in size range 10,000 – 100,000 sq. m, 0.1 – 1 sq. km, 1 – 10 sq. km and 10 – 100 sq. km, a proposed relationship between carbon stock and pond size was tested with the expectation of a linear relationship that could be extrapolated to large water bodies. However, the relationship was very weak ($R^2 = 0.01$). Figure 6.2 shows the relationship between carbon stocked and water body size.

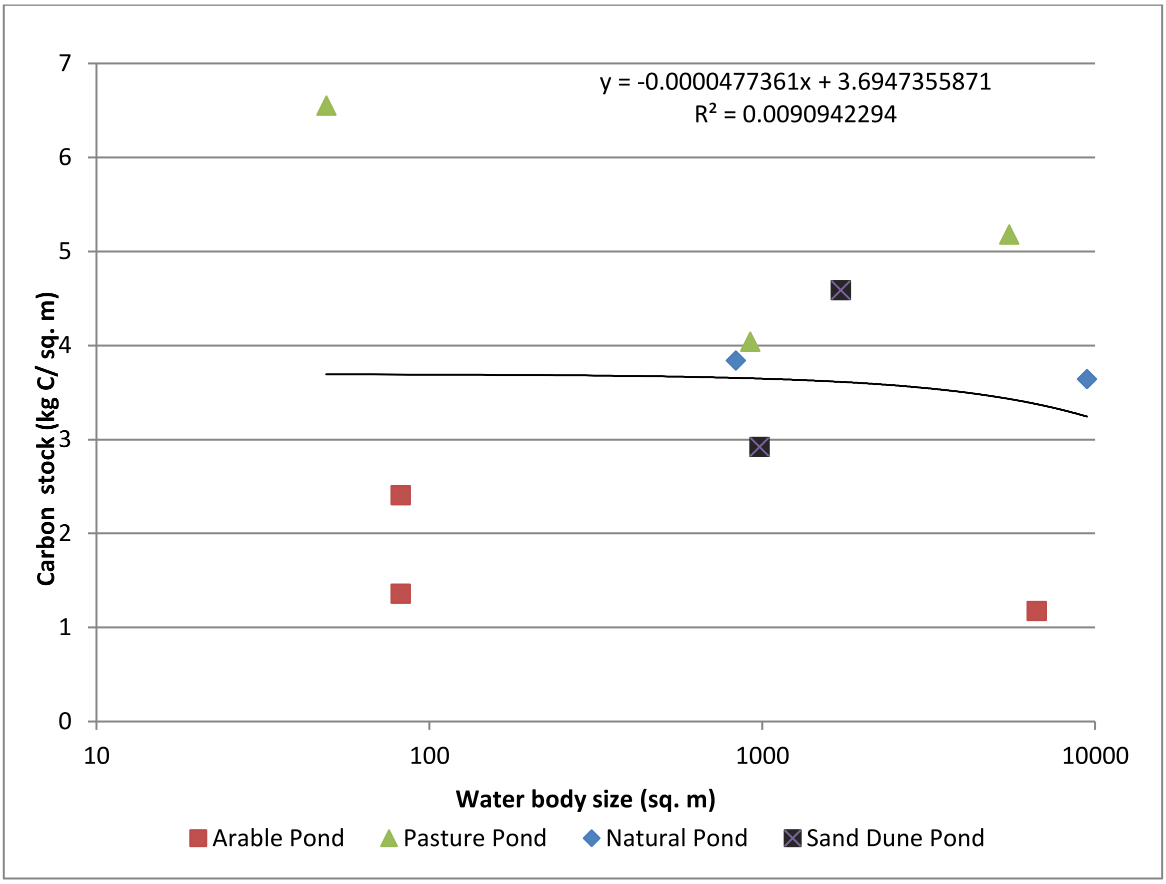

Figure 6.2: Relationship between carbon stock and pond size area

Water bodies provide physical structures that retain carbon stock and carbon cycling processes. Although, SWBs are known to be more efficient at carbon stocking (Battin *et al.*, 2009, Downing *et al.*, 2006, Premke *et al.*, 2016, Seekell and Pace, 2001, Seekell *et al.*, 2013 and Tranvik *et al.*, 2009), pond size as an individual physical factor seems to have little to no significant influence on the abundance or nature of carbon stocked. Carbon stock in ponds is potentially more influenced by extent of terrestrial deposition and primary productivity coupled with other environmental, biological and chemical factors (Baker *et al.*, 2015, Downing *et al.*, 2006, Shirokova *et al.*, 2013, Sobek *et al.*, 2005, Sobek *et al.*, 2009 and Sobek *et al.*, 2011).

Given the lack of a direct relationship between water body size and carbon stock, the average carbon stock in all pond sediments <10,000 sq. m was taken and multiplied by the total surface area of water bodies in Northumberland to determine total carbon stock in Northumberland water bodies. The average carbon stock in size ranges 10 – 100 sq. m, 100 – 1,000 sq. m and 1,000 – 10,000 sq. m was 3.57 kg C/sq. m from the ponds assessed in chapter 4 and the total carbon stock in ponds within these size ranges in Northumberland are 2.21 million kg, 12.85 million kg and 48.25 million kg of carbon respectively (Table 6.1). The three size ranges account for 63.31 million kg of carbon stored in pond sediments in Northumberland. Between size range 10 sq. m to 0.1 sq. km, SWBs contained an extrapolated carbon stock estimated at 139.46 million kg. Between 0.1 sq. km to Kielder Dam (~11 sq. km and the largest water body in Northumberland), they contained an extrapolated carbon stock estimate of 122.54 million kg, making a total estimate of 262 million kg of carbon in inland water bodies of Northumberland with the exclusion of lotic systems of rivers and streams. These results confirm underestimation of carbon stock potentials in SWBs especially within surface area <0.1 sq. km and there are more water bodies with sizes <0.1 sq. km in Northumberland. The extrapolation to create the regional estimates of carbon stock must be interpreted with caution, partly because of the limit of the accuracy of the estimates for number and size of water bodies, referred to in the previous section. Also, the carbon stock analyses were only conducted for a small number of ponds in the South East Northumberland coastal plain area. However, the results do fall within the bounds of estimates produced by other research.

Table 6.1: Estimate carbon stock in Northumberland water bodies

Ranges	*Water Bodies*	*Average size area of water bodies (x 10^6 sq. m)*	*Carbon stock in Northumberland water bodies (million kg)*
10 - 100 sq. m	Small water bodies	0.62 ± 0.29	2.21 ± 1.04
100 - 1,000 sq. m		3.59 ± 1.29	12.85 ± 4.64
1,000 - 10,000 sq. m		13.5 ± 1.80	48.25 ± 6.43
10,000 - 100,000 sq. m		21.33 ± 5.66	76.16 ± 20.21
0.1 - 1 sq. km		20.70 ± 5.83	73.91 ± 20.83

1 -10 sq. km	Large water bodies	2.15	7.68
10 -100 sq. km		11.47	40.95

6.1.3 Source of carbon in Northumberland water body sediments

Distinguishing between allochthonous and autochthonous carbon was difficult using C:N ratio due to complications of the biochemical restructuring of organic carbon by microbial oxidation and physicochemical processes in ponds (Bärlocher and Boddy, 2016, Briée *et al.*, 2007 and Emerson and Hedges, 2003). Checking the microbes' preferential carbon source was a more effective tool for identifying allochthonous and autochthonous carbon sources in pond sediments. Given that there were no linear relationships between carbon stocks and water body size, the percentage of microbes utilising allochthonous and producing autochthonous carbon were extrapolated to determine carbon stock from allochthonous and autochthonous sources across all pond size ranges. In Chapter 5, the average allochthonous and autochthonous microbes that utilise/produce carbon were 62.47 ± 2.86 % and 37.53 ± 2.86 % respectively. Therefore, in the ~262 million kg of carbon stock in all Northumberland ponds, 163.66 million kg of carbon stock were associated with terrestrially-derived carbon while 98.34 million kg were from aquatic source as described in Figure 6.3.

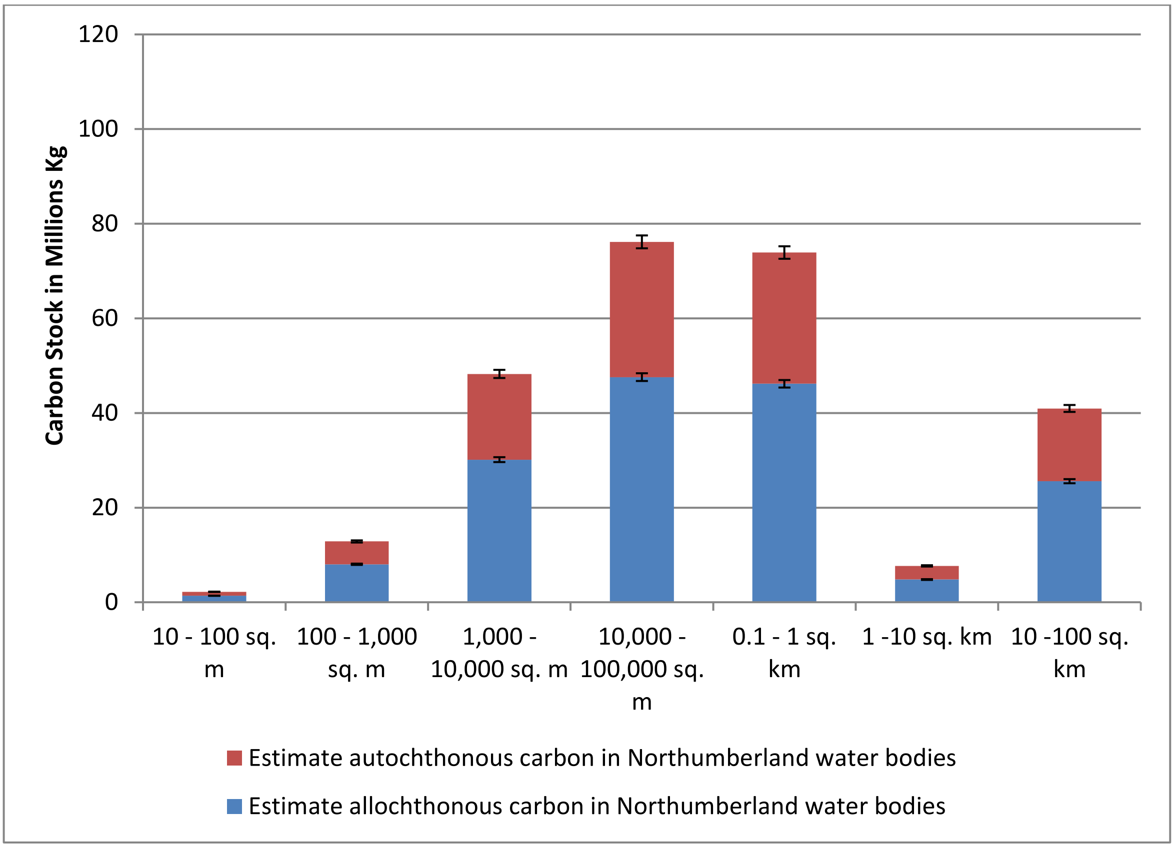

Figure 6.3: Estimate allochthonous and autochthonous carbon stock in Northumberland

However, these are only rough estimates of carbon source in pond sediment because:

- not all particulate organic carbon (POC) or colloidal DOC are utilised by microbes prior to burial
- recalcitrant carbon and high molecular weight are difficult to degrade by microbes
- material transports and burial efficiency affect the nature of carbon source
- photo-oxidation of organic carbon leads to atmospheric loss without microbial action
- environmental factors such as turbidity and light penetrations control phototropism in water bodies
- PCR analyses do not provide quantitative estimates of microbe abundances
- not all microbial phyla that utilise and produced carbon which are identified using this PCR method

The extrapolation is also limited by uneven material dispersal and mixing in water bodies, especially from allochthonous transports. Terrestrial materials tend to settle at pond edge and the rest of the pond potentially will produce more carbon from aquatic source. Depending on depths, the larger ponds will also show pond stratifications which favour carbon storage (Dunalska *et al.*, 2003). Most allochthonous carbon that enters SWBs is particulate with complex organic structure and requires more energy to degrade. Thus, it experiences less microbial utilisation compared to the dissolved organic carbon (DOC) prior to storage and is more abundant in sediments (Shirakova *et al.*, 2013).

Nevertheless, it is clear from Chapter 5 that carbon source in small ponds across Northumberland shows high terrestrial influence compared to in-situ aquatic microbial carbon processing (Figure 6.3). Potentially, high total carbon stocks or total organic carbon (TOC) in the pond sediments directly relate to high particulate organic matter (POC) (Shirakova *et al.*, 2013). Also, the variations between carbon sources are more likely to be water body specific, and this is based on the variations found in the microbial community structure, carbon stock and surrounding depositional environment.

6.2 The importance of small water bodies to carbon capture in Northumberland

In addition to the anthropogenic values of lakes and wetlands in providing water resources and agriculture (Lehner and Doll, 2004), this research has shown they also have an important role in carbon capture from the atmosphere and terrestrial environment.

Downing *et al.* (2006), Lehner and Doll (2004), and Meybeck (1995) derived various estimates of the number and surface area of water bodies <0.1 sq. km. These estimates were developed without considering localised and regional factors that control water bodies <0.1 sq. km. Also, this research furthers previous research such as Lal (2004), Gilbert *et al.* (2014), Premke *et al.* (2016) and Tranvik *et al.* (2009) to show that SWBs stock more carbon than previously anticipated. In Northumberland, it is estimated that ~139.46 million kg of carbon is stocked in water body sizes <0.1 sq. km and this is more than the carbon estimated to be stocked in pond >0.1 sq. km (~122.54 million kg). This also suggests that the prevailing physical/environmental, biological and geochemical/chemical conditions favour more efficient autochthonous and allochthonous carbon stocking potentials in SWBs than in larger water bodies. Therefore, more attention should be accorded to small water bodies' biogeochemistry and carbon stock as they can no longer be classified as a miscellaneous element of the terrestrial carbon budget and SWBs represent a major player in the "missing links" of the carbon cycle.

Small water bodies should no longer be considered part of the terrestrial carbon budget, but should be accorded similar status as rivers, large lakes and oceans because they can:

- receive terrestrial carbon
- process terrestrial carbon
- produce autochthonous carbon
- and preserve carbon in sediment

Battin *et al.* (2009) suggested a rough estimate of 20% of the terrestrial carbon budget is buried in inland water bodies. This research confirms the presence of terrestrially-derived allochthonous carbon being utilised by microbes. A novel element of this research is identifying that approximately 37.53% of carbon in inland water bodies is autochthonous. Therefore, SWBs are not just processing terrestrial carbon but produce aquatic carbon as well. In Northumberland, an estimated 98.34 million kg of aquatic carbon has been derived, while 163.66 million kg were from the terrestrial source.

Tranvik *et al.* (2009) estimated 0.6 Pg/yr of carbon enters inland water bodies globally (and SWBs are a major component of inland water bodies). Using the Northumberland estimates, 0.6 Pg/yr of terrestrial-derived carbon equates to 62.47% of total inland water body carbon. Therefore, inland water bodies potentially produce another 0.36 Pg/yr of global carbon from autochthonous or aquatic carbon processes. These autochthonous or aquatic carbon processes involve photosynthesis, chemosynthesis, direct exchange of CO_2/CH_4 with atmosphere amongst other processes. A new global carbon estimate associated with inland water bodies potentially exist which expands Tranvik's *et al.* (2009) estimates to approximately 0.96 Pg/yr of carbon stock in SWBs. However, these estimates require constraining in accordance with the physical/environmental, biological and chemical/biochemical factors and influences that control carbon capture in different regions and sub-regions of the world.

Furthermore, ~20% of the global terrestrial carbon ends up in inland water bodies which cover ~3% of the earth's surface, 80% of terrestrial carbon is found in the terrestrial ecosystems, covering ~30% of earth land area (Battin *et al.,* 2009, Canadell and Schulze, 2014, Couwenberg *et al.,* 2011 and Premke *et al.,* 2016). In relation to their global coverage, SWBs are doing much more carbon capture and production than previously thought.

6.3 Research Method Limitations

In defining the importance of SWBs for carbon capture in Northumberland, there were limitations in achieving this goal. They were mainly around logistical issues, site access permission, equipment access, data cost, limited research funding and time. However, the available resources were maximised to provide the best achievable results without compromise. Details of the research methodologies' limitations were discussed in Chapter 3, Chapter 4 and Chapter 5.

6.4.1 Research Recommendations

The key recommendations to improve the methodologies used in this research are:

- analysing triplicate cores from all pond types and different parts of ponds for carbon and microbes' influences
- analyse more ponds to expand the dataset
- collecting samples from inland Northumberland
- analysing water bodies >10,000 sq. m for carbon and microbes' influences.

The purpose of this thesis is to contribute regional data in establishing regional and global carbon stock estimates in SWBs which is important for modelling the SWBs' global contributions to the global carbon budget and climate change. The research in Northumberland requires supporting information from other parts of the world at regional and sub-regional scales. This is in-line with Seekell and Pace's (2011) suggestion that regional estimates reduce the overestimations associated with extrapolation models for estimating water body abundance. The vegetation, landscape and environment of Northumberland differ widely from deserts in Africa or monsoon areas in India or permafrost regions of Russia. Therefore, to create effective global estimates of carbon stock in SWBs, it is important to analyse carbon stock in various depositional environments across the different regions/sub-regions of the world.

Furthermore, there is a need to better understand the variations in the environmental/physical, biological and chemical factors, and their influence on carbon stocking in water bodies.

6.4.2 Environmental/Physical Factors

Monitoring changes in number and surface area of water bodies are highly important. However, this research has found no direct statistical relationship between SWBs size and carbon stock. This needs further work because it is based on a relatively small number of water bodies in one particular area. The best approach to calculating the number and surface area of water bodies for a global estimate involves adopting multiple approaches using high resolution imagery from satellite, UAVs and field survey, where possible. This should be done with consideration of temporal changes, as number and surface area of SWBs are in continuously flux (Jeffries, 2016), due to precipitation and land use.

SWBs vary widely in terms of depositional environment. This research looked into dry bulk density and sediment wetness, and showed these had a strong influence on carbon stock. The level of sediment compaction, aeration, grain size and mineralogy are an untapped area of research. Knowledge on pond shape and water column will provide information on material transport, sediment wetness or moisture saturations, primary productivity and POC/DOC. Also, the climate components such as rainfall, snow, sunlight, temperature and wind have been associated with carbon capture in oceans, rivers, large lakes and the terrestrial ecosystems. There is need to research their dynamics in relation to carbon capture in SWBs.

In Northumberland, a key research area is to determine the annual burial rate in inland water bodies. This will provide a better understanding of the level and source of carbon that enters into the water body ecosystem. Given that depositional environments vary between ponds and go through successional stages in the pond’s life cycle (Biggs *et al.,* 1994, Gilbert *et al.,* 2014 and Jeffries, 2008), it should be expected that terrestrial transport and aquatic primary productivity will vary and this would affect burial rate in sediment. This should be researched alongside analysis of physical remobilisation, microbial utilisation of carbon and pond succession (Downing, 2010).

6.4.3 Biological factors

This research identified the biological variability across and within ponds using mainly bacterial phyla and these variations were related to carbon processing of allochthonous and autochthonous materials. Defining the activities in ponds at a more in-depth level of microbial species, their metabolic pathways and biological interaction would be an important and novel area of research. Similarly, the research into the microbiology of ponds in Northumberland used the PCR approach which provided bacterial abundance information, but these analyses showed no quantitative abundance of the bacteria being able to relate mass of microbial communities to carbon stock could also prove valuable in determining microbial diversity associated with carbon capture in SWBs. Also, it is important to research other Prokaryotes and Eukaryotes in SWBs and how they influence carbon capture, as well as, higher plant and animal forms in pond sediments.

6.4.4 Chemical factors

Carbon stock in ponds strongly ties to the dilution of bio-elements and microbial metabolites in sediments. Although, SWBs hold high concentrations of chemicals that foster carbon processing, their concentrations widely vary between ponds. Thus, there is the need for studies into the elemental and nutrient composition of pond sediments and how this relates to microbes, pond oxygenation and carbon stocking.

The potential for a water body to be a carbon source or sink is a result of autochthonous and allochthonous carbon processing. This research used the C:N ratios and the microbial phyla to define the abundance of autochthonous and allochthonous carbon. However, another effective tool for distinguishing carbon source is the actual component organic matter, its molecular composition and potential to be broken down into CO_2, CH_4 and other degradation by-products. A combination of multiple approaches further improves the estimation of allochthonous and autochthonous carbon in SWBs and in the distinguishing of oxidised carbon. Also, there is a need to study the metabolic processes that occur in ponds with respect to controlling oxygenation, temperature and salinity amongst other broad environmental processes in ponds.

Finally, there is a need for continuous monitoring of number and size of water bodies alongside their biogeochemical cycling of carbon. This relates to researching the potential for development of an extrapolation model that takes into account all control factors for carbon capture in SWBs.

6.5 Research Conclusion

The Earth is currently experiencing climate change events that can be associated with increased atmospheric carbon emission from various fossil carbon reserves. The planet seems to have an innate response or defence mechanism that can trap allochthonous and autochthonous carbon efficiently, that is, small water bodies (SWBs). But carbon capture in SWBs has previously been omitted from the components of global carbon cycle budgets and climate change analytics. This research shows that SWBs are to be classed as very valuable in carbon stocking and can be characterised as “a missing link” in estimating global carbon budget.

SWBs are regionally and globally more abundant than large lakes, rivers and oceans. SWBs comparatively stock more carbon per square metre in their sediments than larger water bodies. In Northumberland, many factors control carbon stocks but SWBs show an overarching value in creating anoxia by high concentration of bio-elements, material transport, primary productivity and microbial interactions. Also, their proximity to the terrestrial ecosystem allows ponds to collect, process and preserve allochthonous materials.

The carbon stock variation within and across ponds was related to variations in allochthonous and autochthonous processes, primary productivity and material aggregation in sediments. Sandy sediments of sand dune ponds allowed microbes to penetrate through porous material and this increased primary productivity. But sandy sediments are poor at preserving organic carbon due to low sediment compactions. Clay sediments of arable ponds have higher compaction that locks (preserves) carbon in sediments but limits microbial penetration and proliferation; and consequently, autochthonous carbon productivity. The ideal situation is a mix of porous and compacted sediment minerals and grain sizes for efficient production and preservation of carbon in SWB sediment.

Of the many potential influencing factors, this research considered water bodies' size, sediment wetness/precipitation and dry bulk density of pond sediment as physical/environmental factors and microbial variations as a biological factor that control carbon stocks in water bodies. The research shows that carbon stocks in water bodies respond to sediment wetness, dry bulk density and microbial variations, although these inter-relationships are complex. But no clear relationship was found in water bodies' size as an influential controller of the carbon stocking process.

Prior to this research, Battin *et al.* (2009), Cole *et al.* (2007) and others considered carbon in small water bodies to be from terrestrial entry. But there are also aquatic carbon activities by autotrophs, heterotrophs and decomposers that occur in small water bodies that contribute to autochthonous carbon capture. In Northumberland, an estimated 3.57 kg of carbon stock exist per square metre of water body sediment and its contents include carbon from allochthonous and autochthonous sources. Autochthonous carbon productivities are also enhanced by inherent high concentration of nutrients, microbes' availability and favourable environmental conditions that prevail in SWBs. The estimated ratio of allochthonous to autochthonous carbon in Northumberland was found to be approximately 62.47% to 37.53%.

The real value of SWBs is not just in the carbon stock per square metre but their regional abundance and total surface area. The Northumberland estimate of carbon stocks in water bodies with surface area <0.1 sq. km was more than the >0.1 sq. km counterparts. This is because more water bodies <0.1 sq. km exists compared to water bodies >0.1 sq. km.

Finally, more work is needed on the environmental/physical, biological and chemical factors to constrain estimates of carbon capture within small water bodies (SWBs) because these factors combined have strong influences on the carbon stock and their source or sink potentials. Also, development of a model and improved image analyses to efficiently account for temporal changes in number and surface area of water bodies are also important.

What is clear from this research is that small water bodies have massive importance for carbon capture. They hold untold potential in understanding the Earth’s climate and their global contribution to combating our changing climate remains largely unexplored.

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Zhu, D., Tanabe, S. H., Yang, C., Zhang, W., & Sun, J. 2013. Bacterial community composition of South China Sea sediments through pyrosequencing-based analysis of 16S rRNA genes. *PloS one*, *8*(10), e78501.

Appendices

Appendix A: Water Body Distribution

Appendix A.1 GLWD Global water body distribution using Lehner and Dolls (2004) dataset

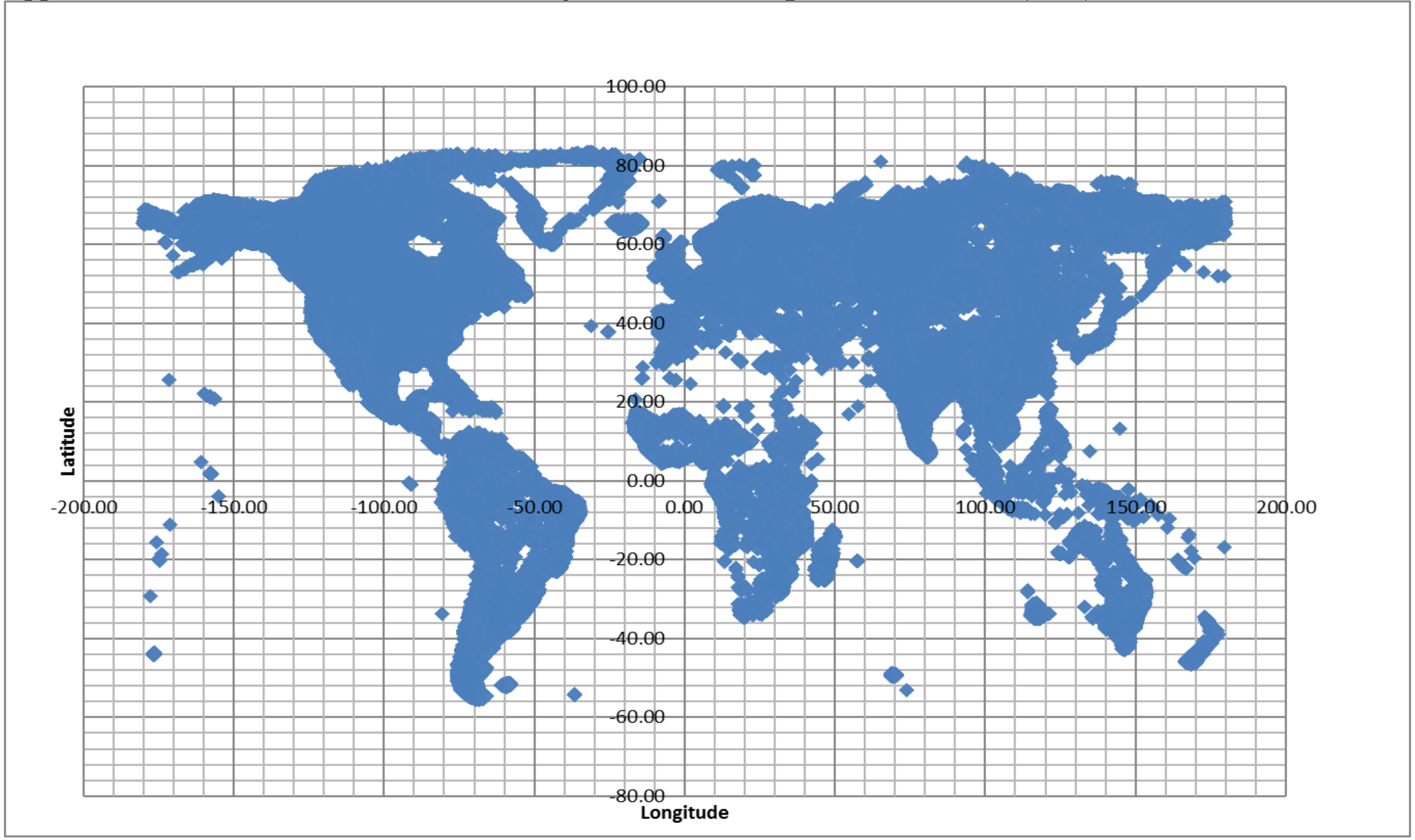

Figure A.1: Global Distribution of water bodies and rivers (surface area >0.1 sq. km)

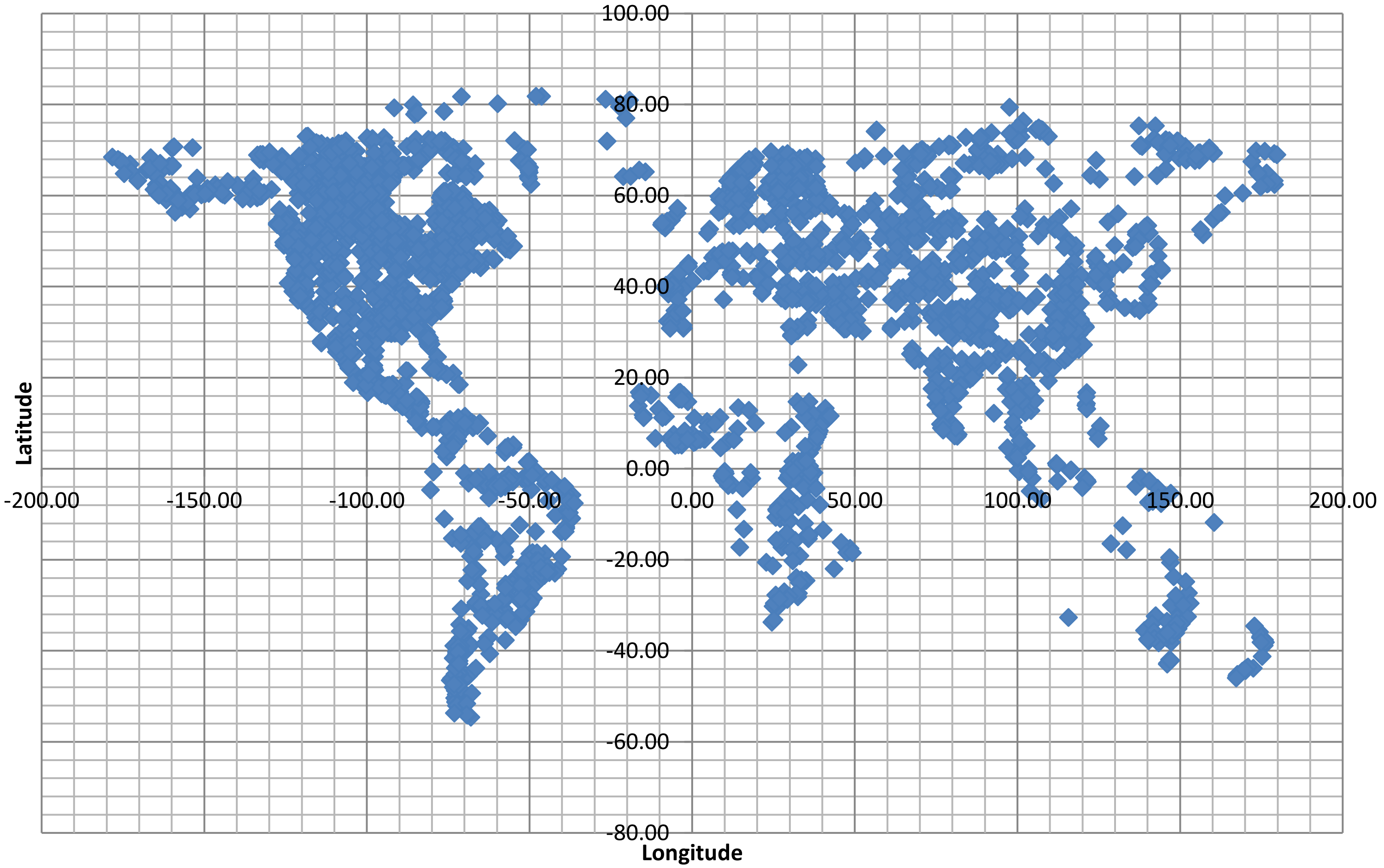

Figure A.2: Global Distribution of water bodies and rivers (surface area 10 sq. km)

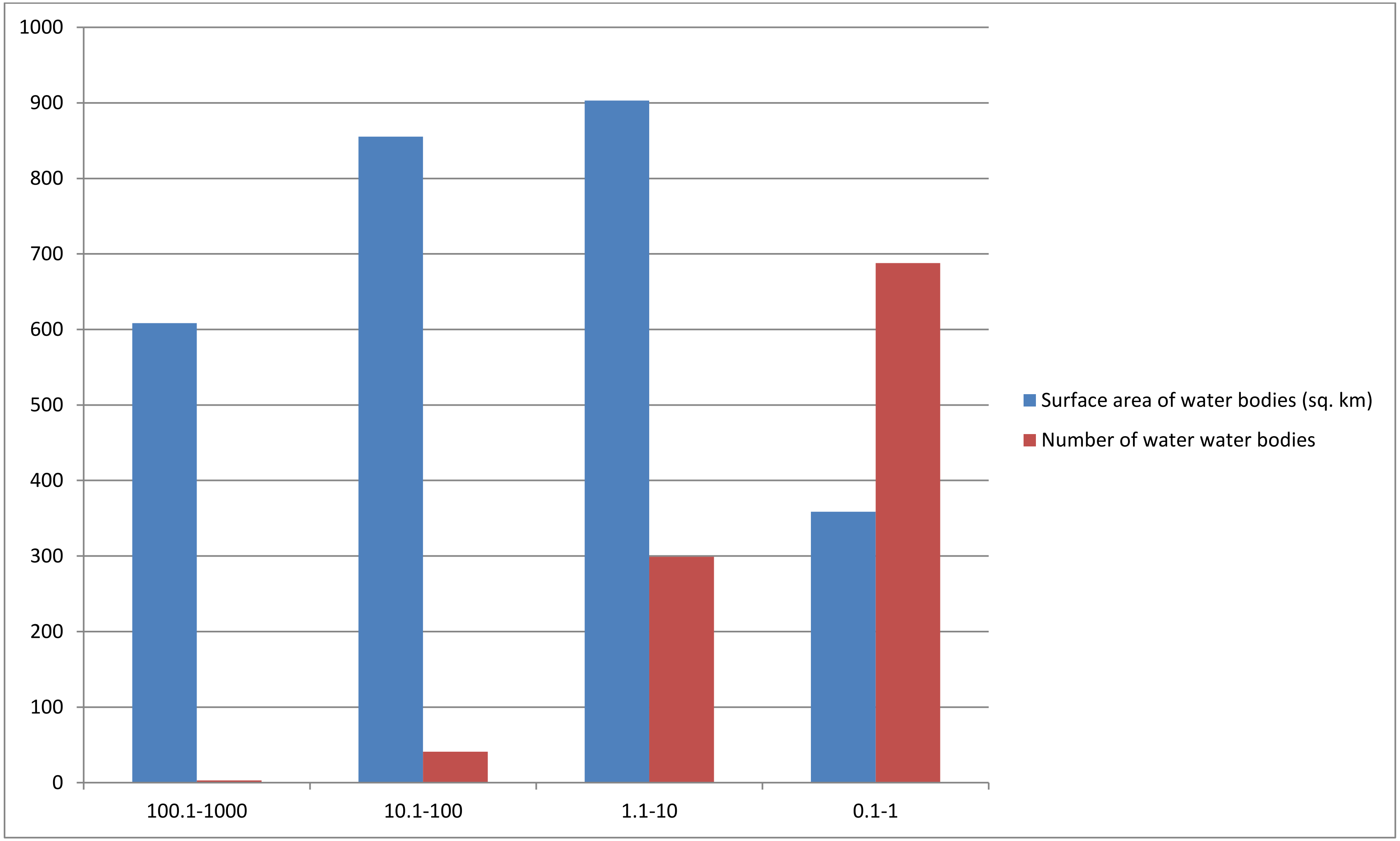

Figure A.3: Estimate of United Kingdom (UK) Distribution of water bodies and rivers (surface area 10 sq. km)

Appendix A.2 World Imagery on ArcGIS

“World Imagery contains images with 30cm to 15m resolution range. World Imagery provides 1 m or better satellite and aerial imagery in many parts of the world and lower resolution satellite imagery worldwide, which include 15m TerraColor imagery at small and mid-scales (~1:591 M - ~1:72 k) and a global 2.5m SPOT Imagery (~1:288 k - ~1:72 k). The Mainland United States and some parts of Western Europe data (earth features) are from DigitalGlobe viewed at 0.3m resolution imagery. World Imagery also contains DigitalGlobe sub-meter imagery featured in Eastern Europe, Japan, Middle East, Northern Africa, South America, Southern Africa, Australia, India and New Zealand. On a global scale, World Imagery contains sub-meter Pléiades imagery in selected urban areas and in 47 States of the USA, it features 1 m resolution NAIP 2013 and 2014 imagery (~1:36k - ~1:4k). While GeoEye IKONOS, Getmapping, AeroGRID, IGN Spain, and IGP Portugal provide 1 m resolution imagery in other parts of the Earth. Also, the GIS Users’ Community is known to provide different imagery at different resolutions for World Imagery” - ESRI.

Images updated in January and July 2015 were digitised on ArcMap 10 at scales of 1:2000 and 1:20,000 respectively.

Appendix A.3 Northumberland water body analysis

Table A.1: NCA Size Area for Northumberland

NCA	*NCA location*	*NCA Area (sq. m)*
South East Northumberland Coastal Plain	Coastal Plain	437092045.1
Mid Northumberland	Border Uplands	637262230.3
Tyne Gap and Hadrian's Wall	Border Uplands	434239162.6
Border Moors and Forests	Border Uplands	1271559266
Cheviots	Border Uplands	364878465.2
Cheviot Fringe	Border Uplands	515911460.4
Northumberland Sandstone Hills	Border Uplands	726946566.3

North Northumberland Coastal Plain	Coastal Plain	376696141.9
Northumberland total area cover		4764585338

Table A.2: Total surface area and number of water bodies identified in all NCA Test Area, which was used for estimation of Northumberland water bodies at 1:2,000 scale

RANGES (sq. m)	Total Surface Areas (sq. m)	Total number of water bodies
1-10	1023.492016	152
10-100	94102.77539	2016
100-1,000	517749.4185	1693
1,000-10,000	747921.5334	303
10,000.-100,000	888905.662	26
100,000-100,0000	2323224.207	12

Table A.3: Surface area and number of water bodies identified in NCA Test Area at 1:2,000 scale

NCA name	*Tyne Gap and Hadrian's Wall*	*Border Moors and Forests*	*Mid Northumberland / Northumberland Sandstone Hills*	*Cheviots / Cheviot Fringe*	*North Northumberland Coastal Plain*	*South East Northumberland Coastal Plain*
NCA test area	37 x 10^6 sq. m	33 x 10^6 sq. m	50 x 10^6 sq. m	45 x 10^6 sq. m	22 x 10^6 sq. m	40 x 10^6 sq. m
Surface area of water bodies						
1-10 sq. m	299.55	71.89	100.91	209.80	300.31	41.02
10-100 sq. m	20,708.70	6,250.89	9,361.71	22,898.54	20,370.53	14,512.39
100-1,000 sq. m	86,122.22	24,047.44	43,270.49	101,527.26	127,572.79	135,209.22
1,000-10,000 sq. m	58,224.56	24,840.65	79,099.65	120,115.23	220,685.04	244,956.41
10,000-100,000 sq. m			72,445.40	209.80	363,626.71	392,224.4418
100,000-1,000,000 sq. m			398,192.13			
Number of water bodies						
1-10 sq. m	43	10	14	32	47	6
10-100 sq. m	483	137	205	469	431	291
100-1,000 sq. m	311	75	142	361	411	393
1,000-10,000 sq. m	34	9	30	56	83	91
10,000-100,000 sq. m			4		11	9
100,000-1,000,000 sq. m			1			

Figure A.4: Water bodies identified in NCA Test Areas at 1:2,000 scale on World Imagery

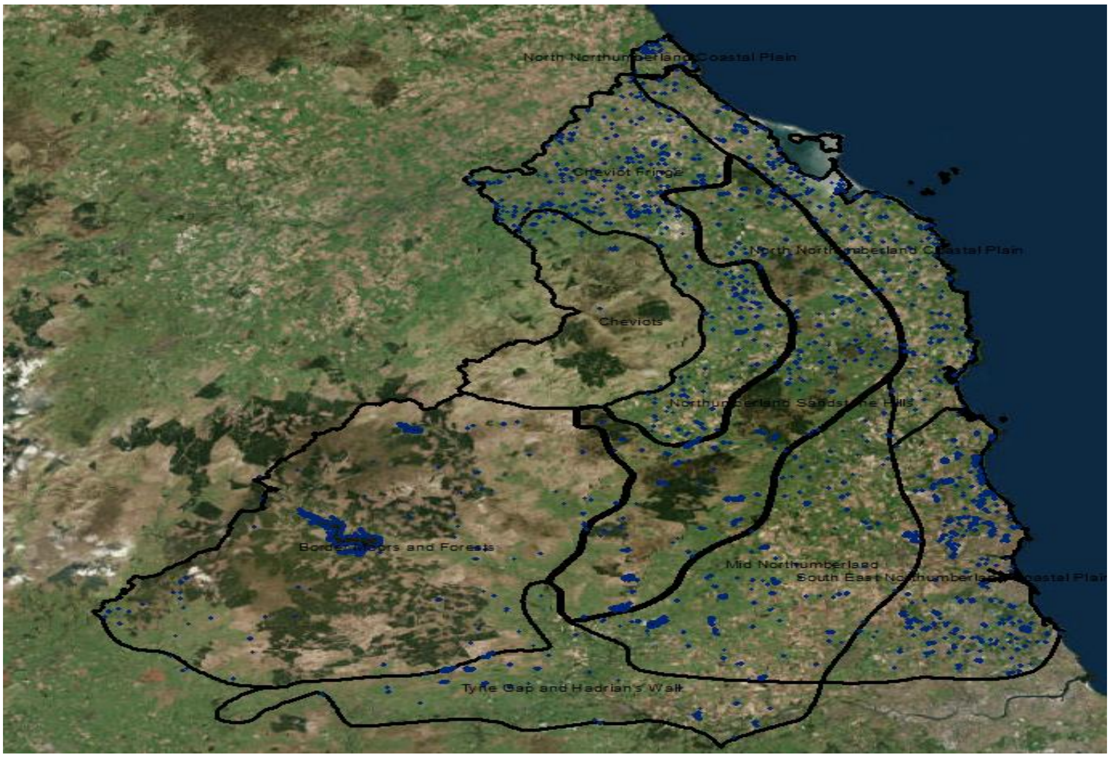

Figure A.5: Water bodies identified in Northumberland at 1:20,000 scale on World Imagery

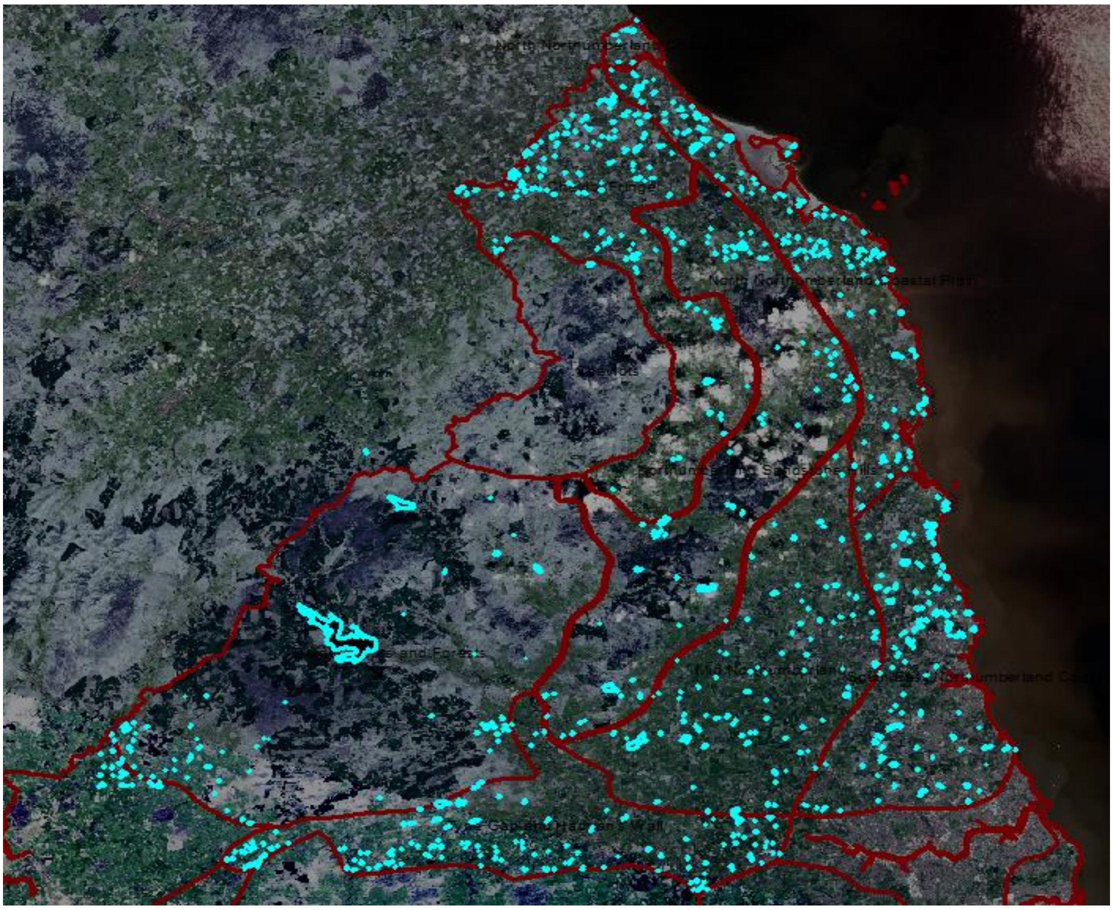

Figure A.6: Water bodies identified on Landsat-8 at 1:20,000 scale by land/water 5-6-4 band combination

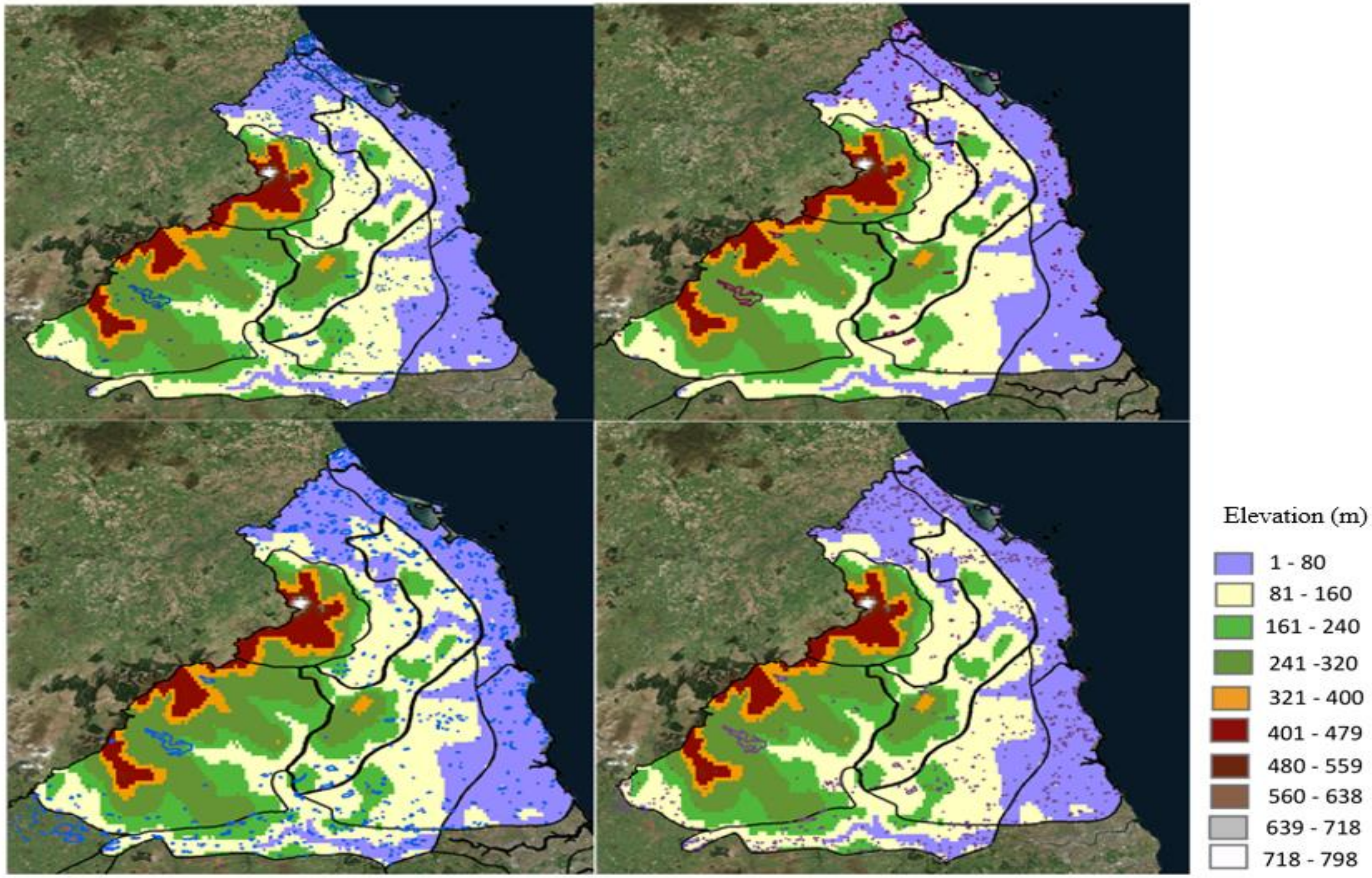

Figure A.7: Water bodies identified on World Imagery at 1:20,000 scale, land elevation in Northumberland and date of analysis (Top Left – July 2013, Top Right – September 2013, Bottom Left – December 2014 and Bottom Right – April 2015)

Appendix A.4 Sites analysed for Accuracy Assessment of water body digitisation on ArcGIS and Google ™ Map

Figure A.8: North Northumberland Coastal Plain site for Accuracy Assessment

Figure A.9: South East Northumberland Coastal Plain site for Accuracy Assessment

Figure A.10: Mid Northumberland / Northumberland Sandstone Hills site for Accuracy Assessment

Figure A.11: Tyne Gap and Hadrian's Wall site for Accuracy Assessment

Figure A.12: Border Moors and Forests site for Accuracy Assessment

Figure A.13: Cheviots / Cheviot Fringe site for Accuracy Assessment

Appendix B: Carbon Analysis

Appendix B.1 Pilot assessment of Druridge pools for carbon analysis

Appendix B.1.1 Sampling sediment cores

Pilot core samples were collected in April, 2014 for TEA analyses using a manual pole corer for background carbon and physical analysis of the Druridge pools (Figure B.1). Coring techniques, preparation of pilot core and amount of cores were discussed in Chapter 4.

Figure B.1: Photo of the pole corer used to down core sediment collection at Druridge pools and a sediment core collected from a pond

Physical observation of the first pilot core shows more compaction in the bottom sediment and the core sample becomes looser and wetter towards the top. The core profile showed there was gradual and distinct change in sediment colour and material content from more quartz-like grey black sediments (with small clay bricks) at the bottom (~30-35cm) to darker sediment at the mid core (17-29cm) and slurry sediments at the top (0-16cm) (Figure B.2). The pilot pond at Druridge Pools was observed to be oligotrophic (algal growth) within a predominantly grassland area with the pond mixing zone between 13-31cm.

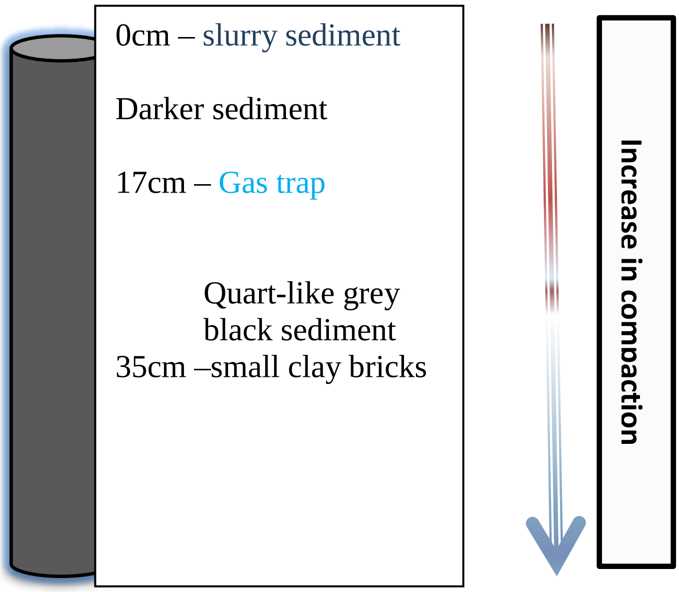

Figure B.2: Diagrammatic description of pilot core from Druridge pools

Appendix B.1.2 TEA Analysis of pilot core

The pilot core was sliced into 1cm thick discs, wrapped in thin foil and dried in a dry cabinet for 3 weeks for moisture removal. A weighed sample of sediment (4-5mg) from each disc was analysed in the TEA to estimate percentage carbon and nitrogen. Increased percentage carbon in the top pond is a result of a combination of biological, physical and chemical parameters interacting by mainly photosynthesis and carbon re-mobilisation. Interaction of algal distribution, macrophytes, plants roots, vertebrates and other microorganisms are enhanced by the low compaction in the top pond and top sediments (mixing zone); thus, higher carbon levels (Figure B.3a). However, upon calculation of the dry bulk density (DBD), there is a drop in the amount of carbon stock in the whole top sediment (Figure B.3b). Similarly, burial down core profile showed lower percentage carbon stored because of higher compaction and water reduction in deeper buried sediment.

Generally, results were closely similar to work by Gilbert *et al.* (2014) with Gilbert *et al.* (2014) recorded slightly higher carbon concentration potentially due to variation in the physical, chemical and biological influences in different ponds at the time of analyses. More study is needed to produce more confident data to affirm this hypothesis (Chapter 4). The water depth (and hydrologic processes), water body surface area; alongside pond age and biotic processes can be contributory factors to variations in carbon concentrations.

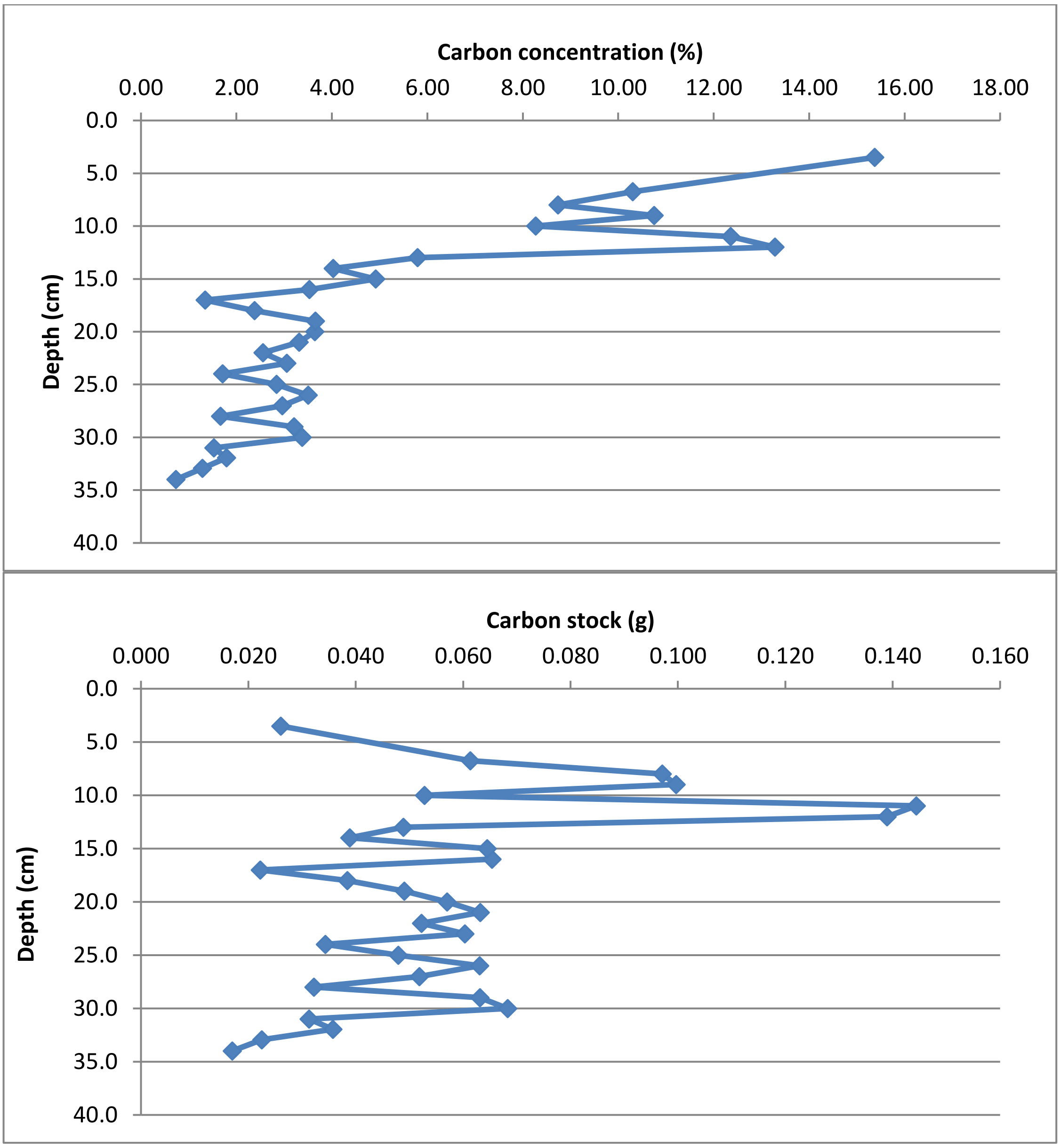

Figure B.3: (a) Organic carbon conc. (%) (Druridge pool) (b) Total carbon (g/cm3) (Druridge pool)

Appendix C: Photos of some small water bodies in Northumberland

Figure B.4a: A pond made for pond dipping (school kids)

Figure B.4b: Pond formed as part of old outfall from open cast coal mine

Figure B.4c: Pond type habitat in World War 2 (WW2) anti-tank ditch

Figure B.4d: Pond in dune slack

Figure B.4e: Temporary pond in dune slack

Figure B.4f: Pond in part of larger wetland

Figure B.4g: Spring fed pond in pasture

Figure B.4h: Subsidence pond flooded in middle of oil seed rape crop (arable field)

Appendix D: Nitrogen, Carbon, Moisture and Dry Bulk Density Data

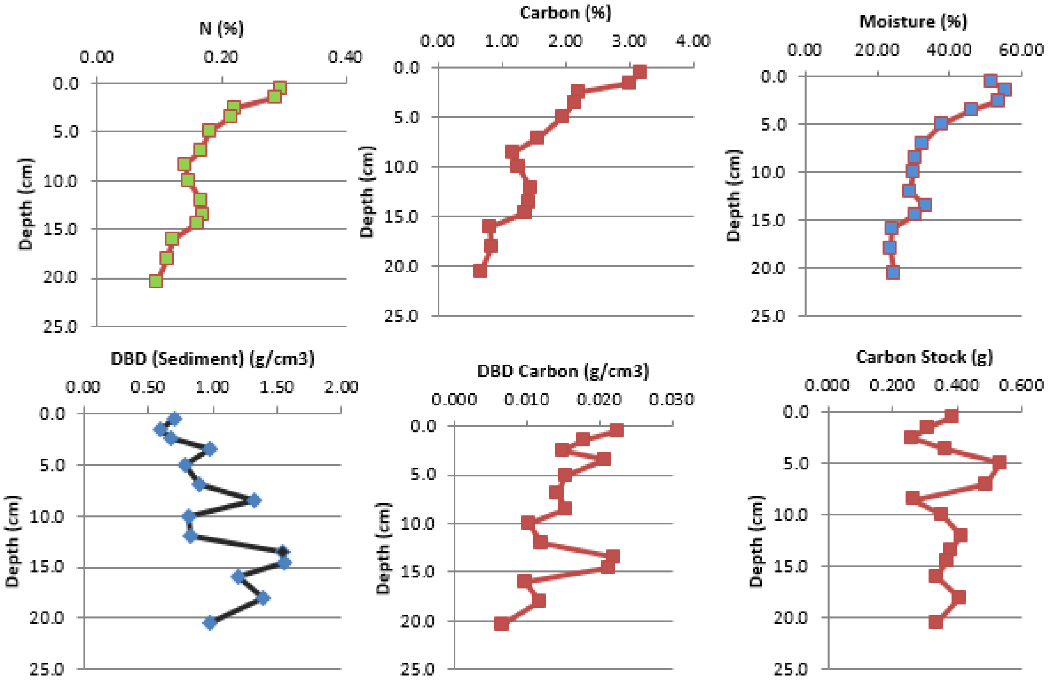

Figure B.5: Core 1 Natural Pond (Size range 1,000 – 10,000 sq. m)

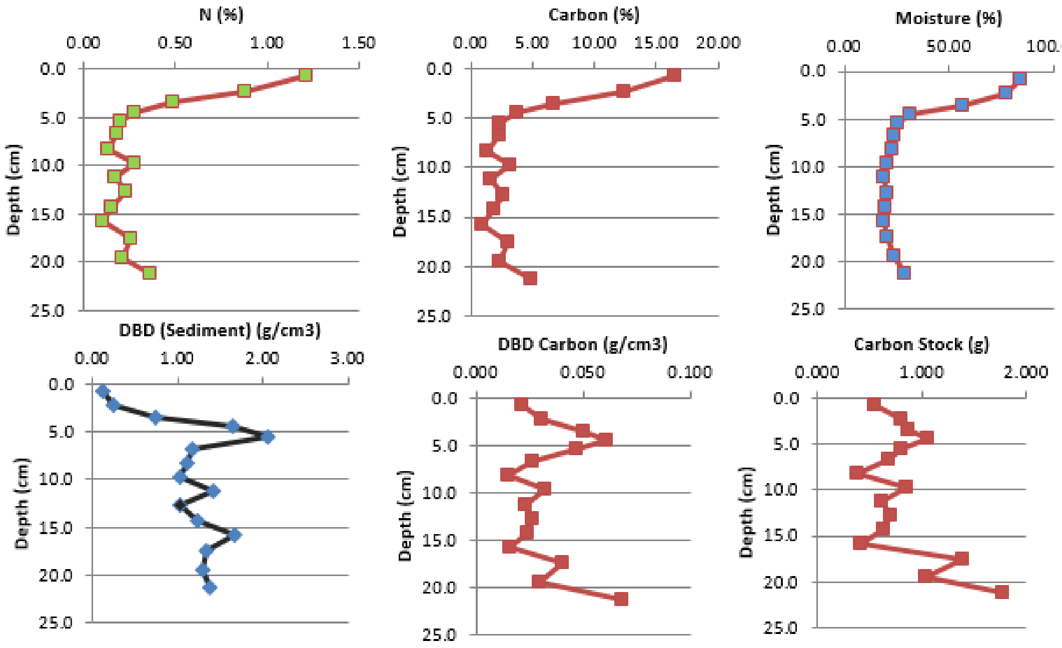

Figure B.6: Core 2 Natural Pond (Size range 1,000 – 10,000 sq. m)

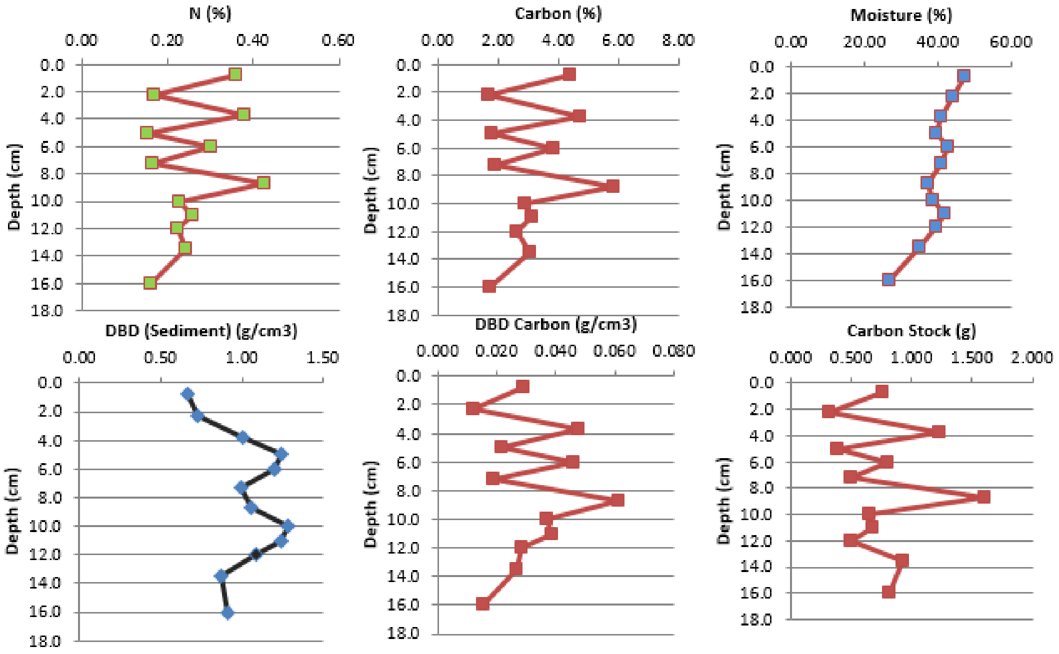

Figure B.7: Natural Pond (Size range 100 – 1,000 sq. m)

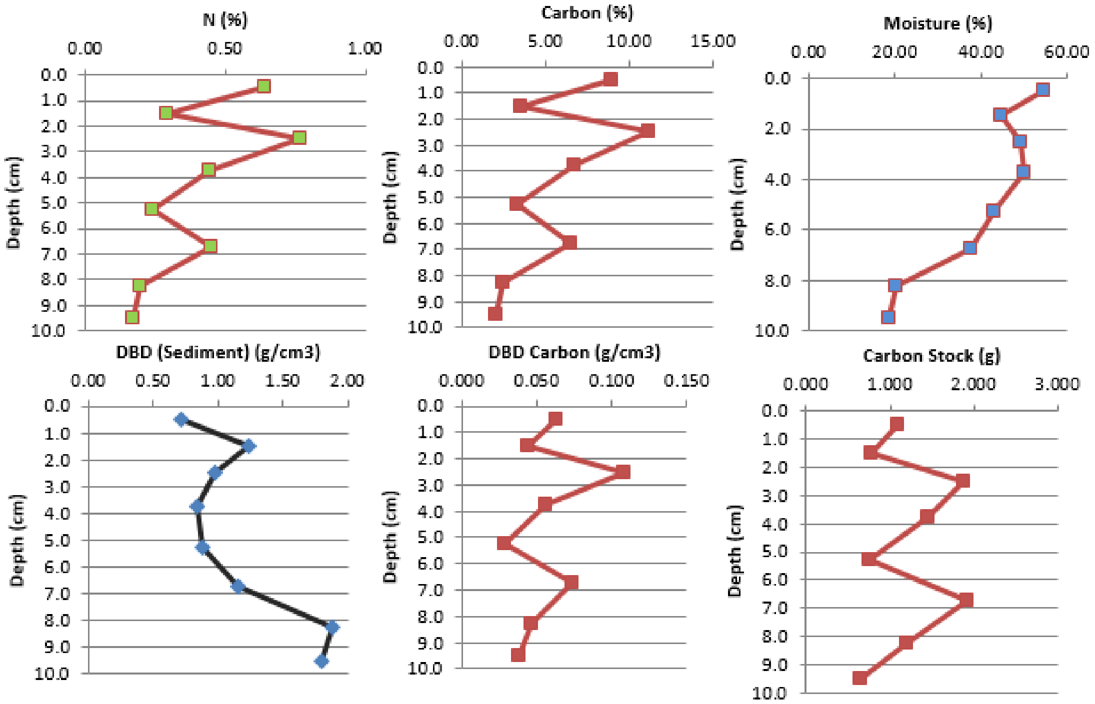

Figure B.8: Sand Dune Pond (Size range 1,000 – 10,000 sq. m)

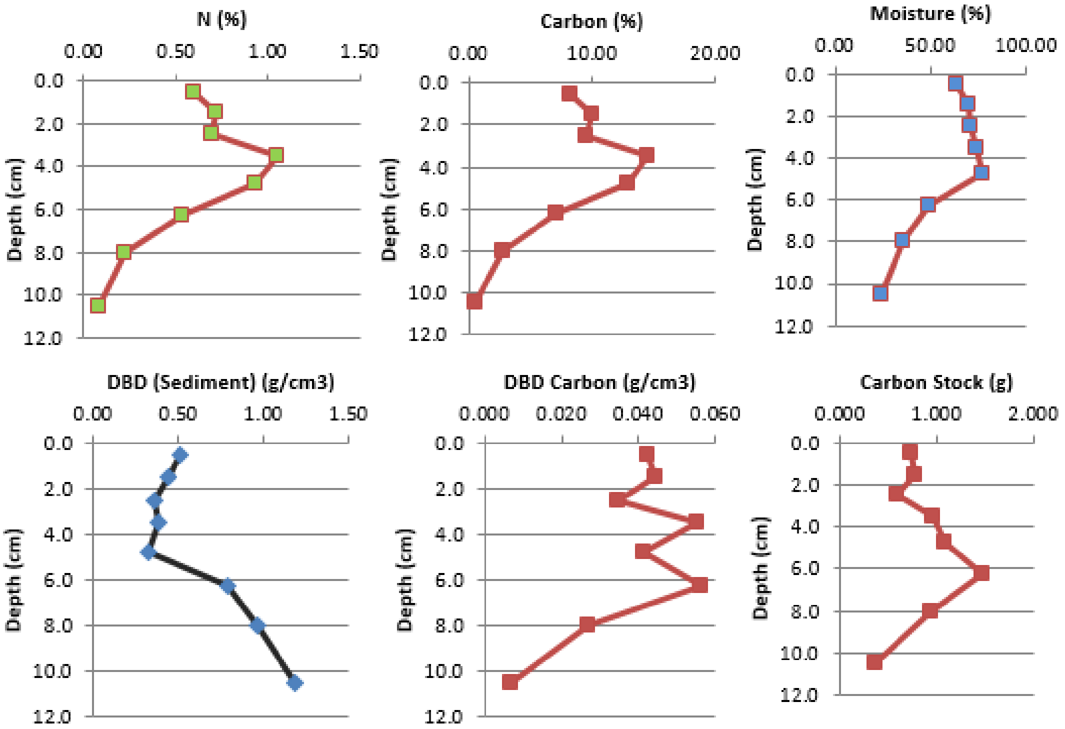

Figure B.9: Core 1 Sand Dune Pond (Size range 100 – 1,000 sq. m)

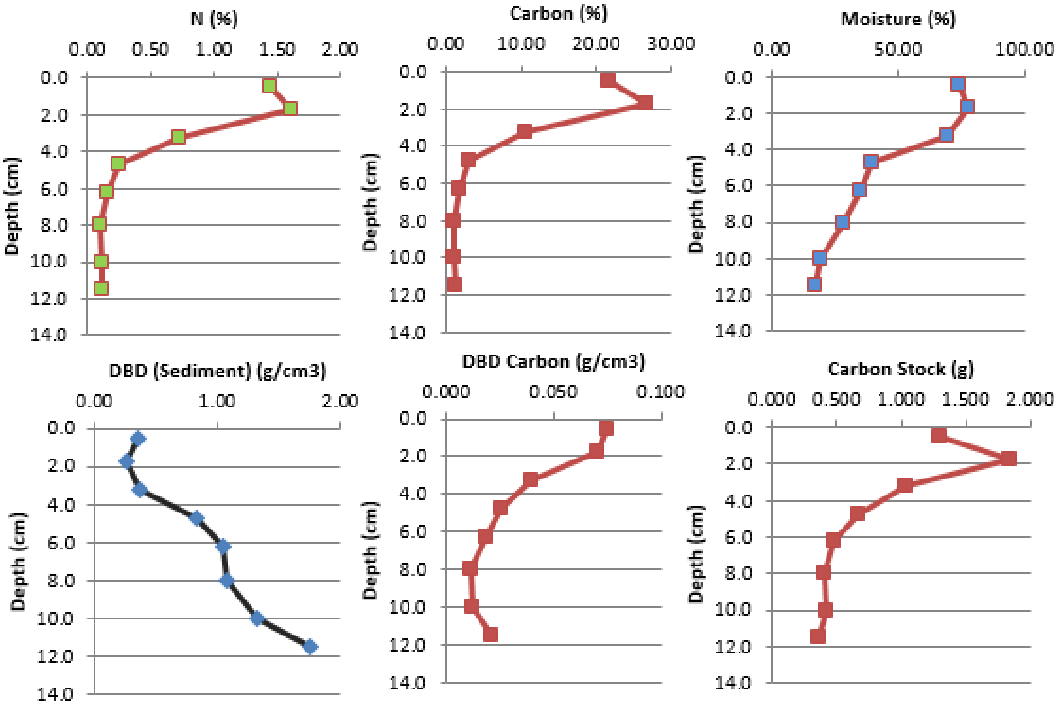

Figure B.10: Core 2 Sand Dune Pond (Size range 100 – 1,000 sq. m)

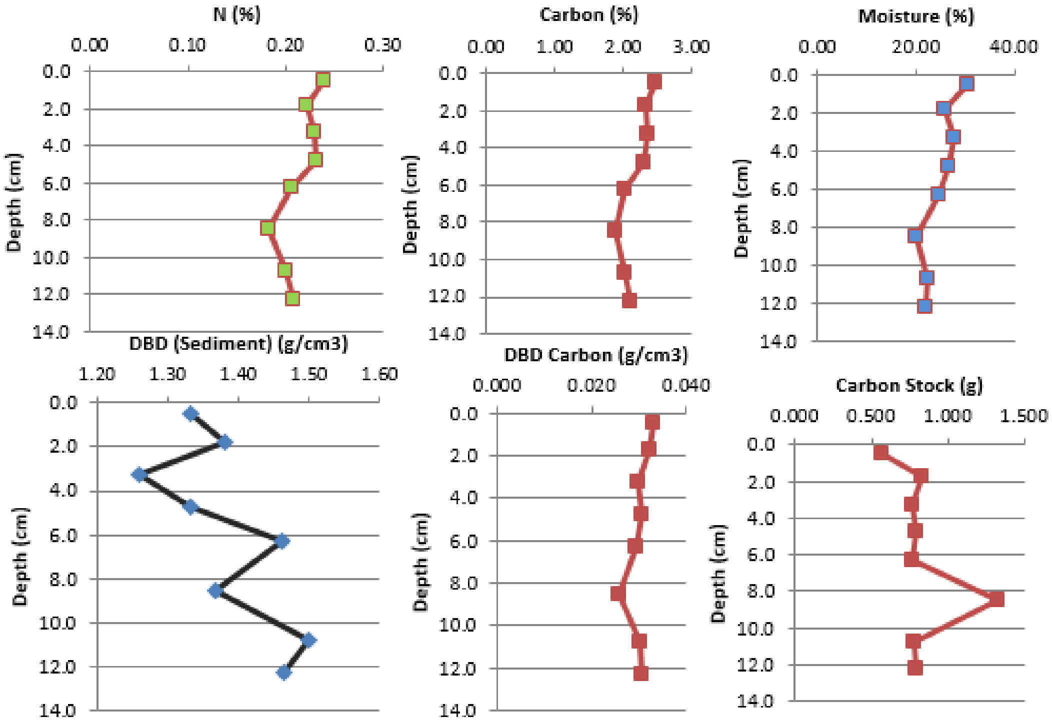

Figure B.11: Arable Pond (Size range 10 – 100 sq. m)

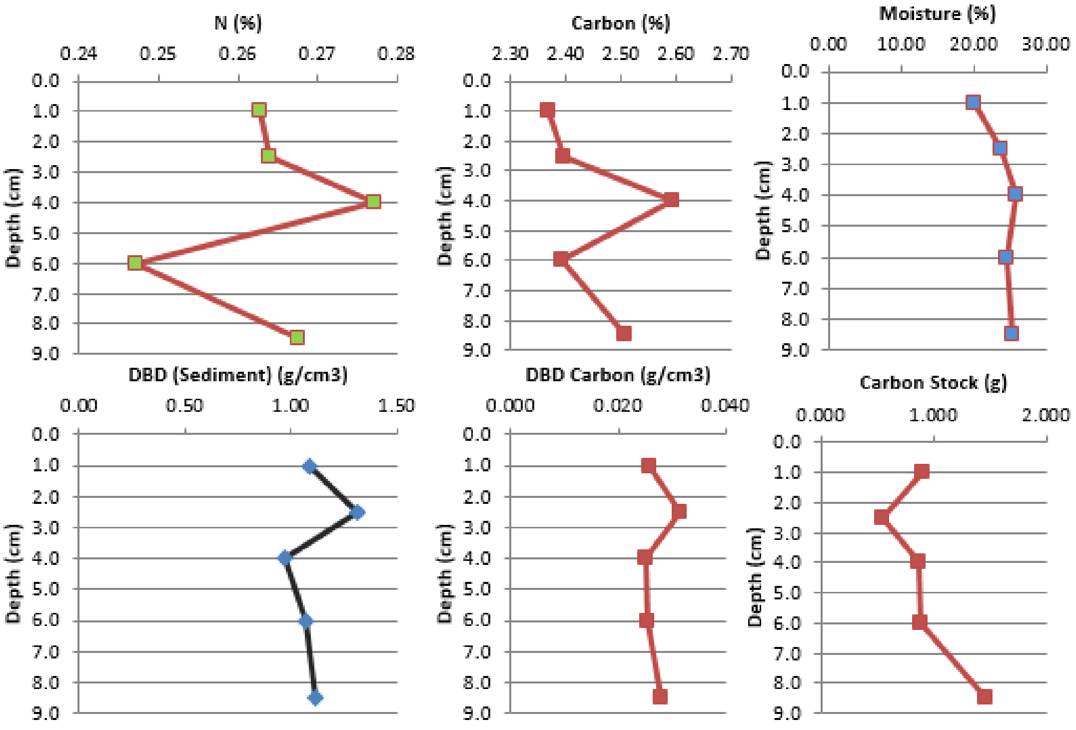

Figure B.12: Arable Pond (Size range 100 – 1,000 sq. m)

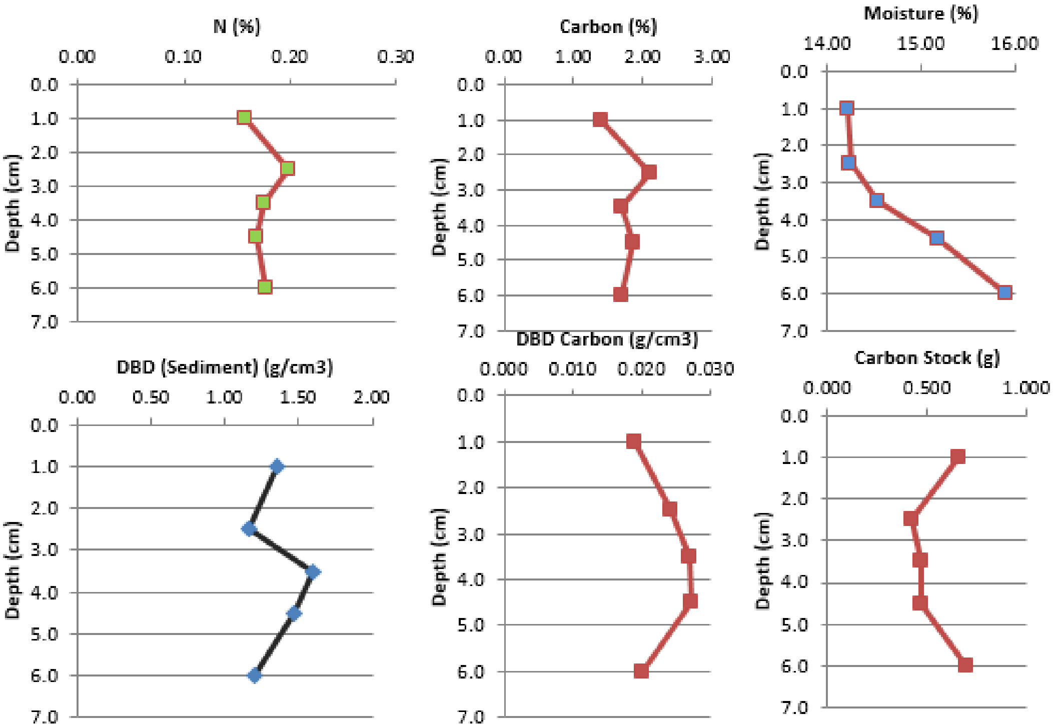

Figure B.13: Arable Pond (Size range 1,000 – 10,000 sq. m)

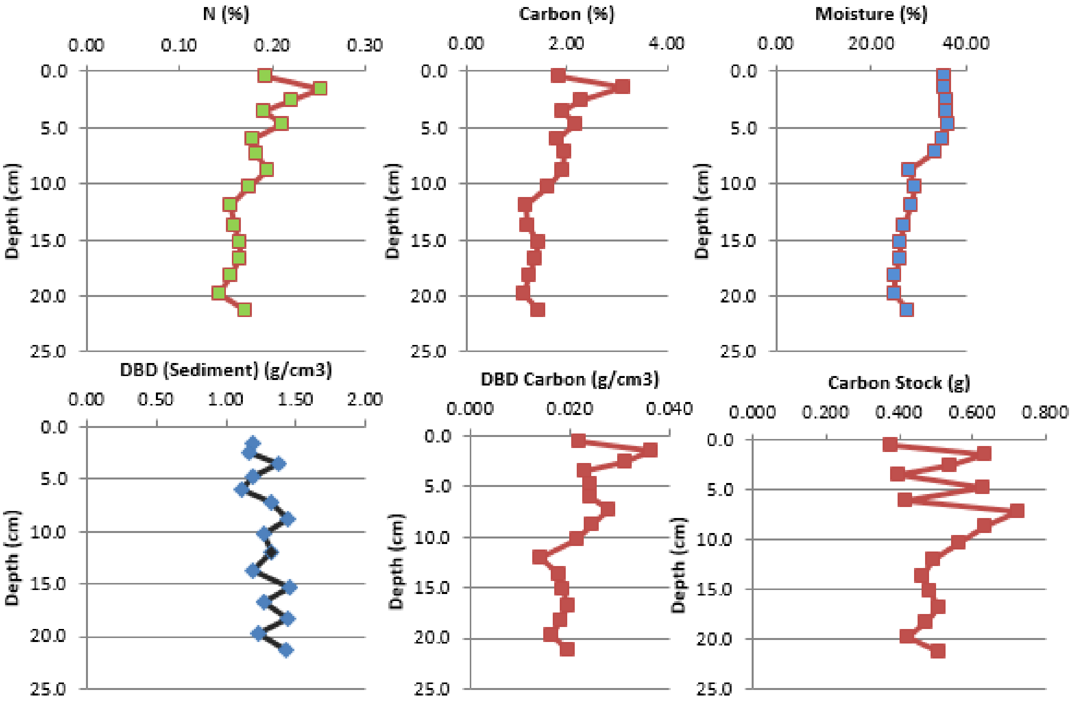

Figure B.14: Core 1 Pasture Pond (Size range 100 – 1,000 sq. m)

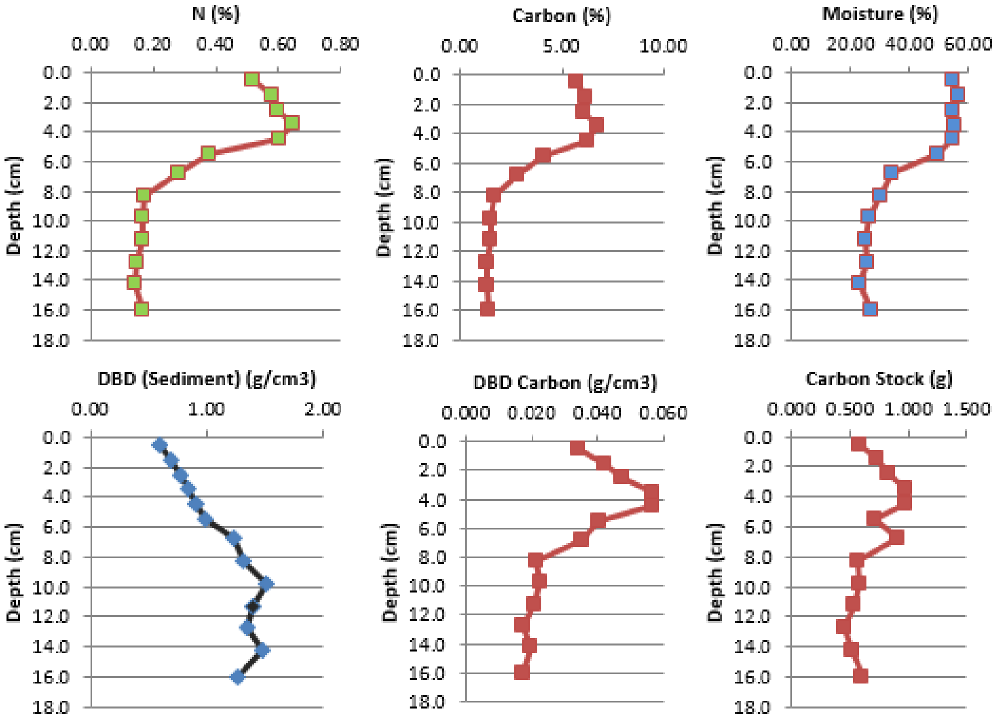

Figure B.15: Core 2 Pasture Pond (Size range 100 – 1,000 sq. m)

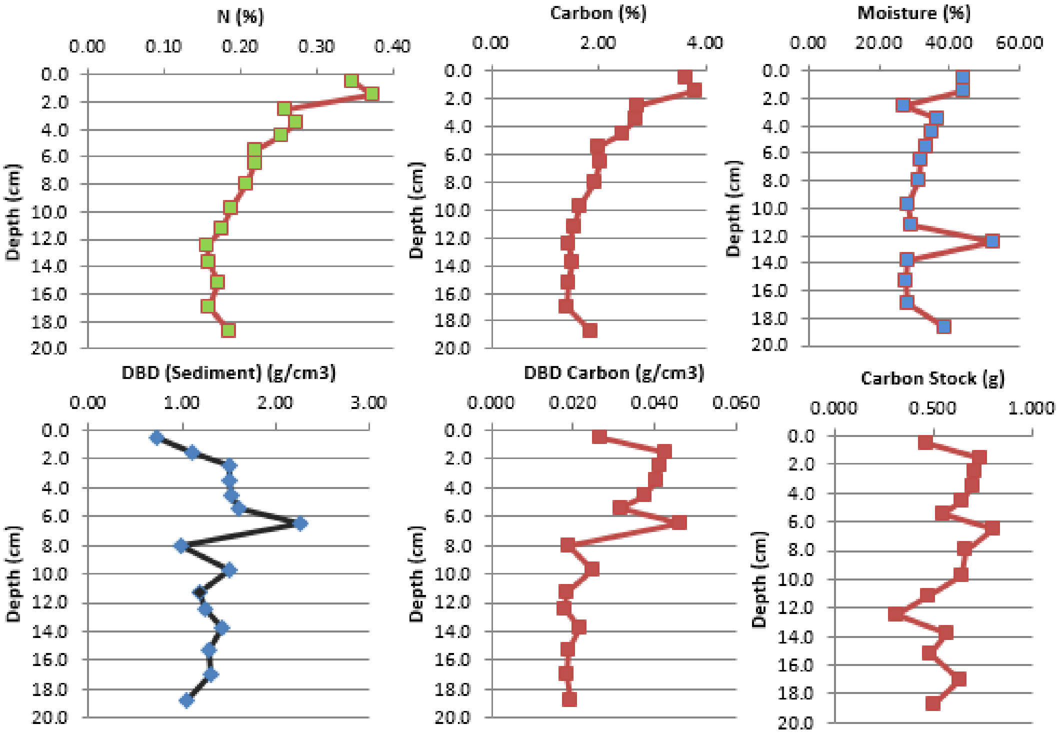

Figure B.16: Core 3 Pasture Pond (Size range 100 – 1,000 sq. m)

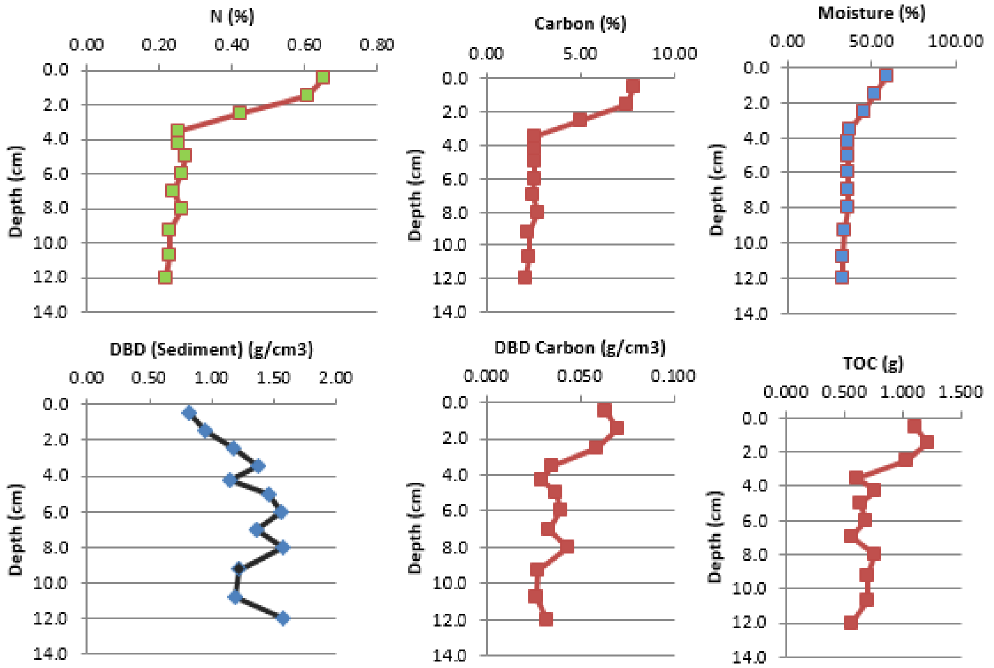

Figure B.17: Core 1 Pasture Pond (Size range 1,000 – 10,000 sq. m)

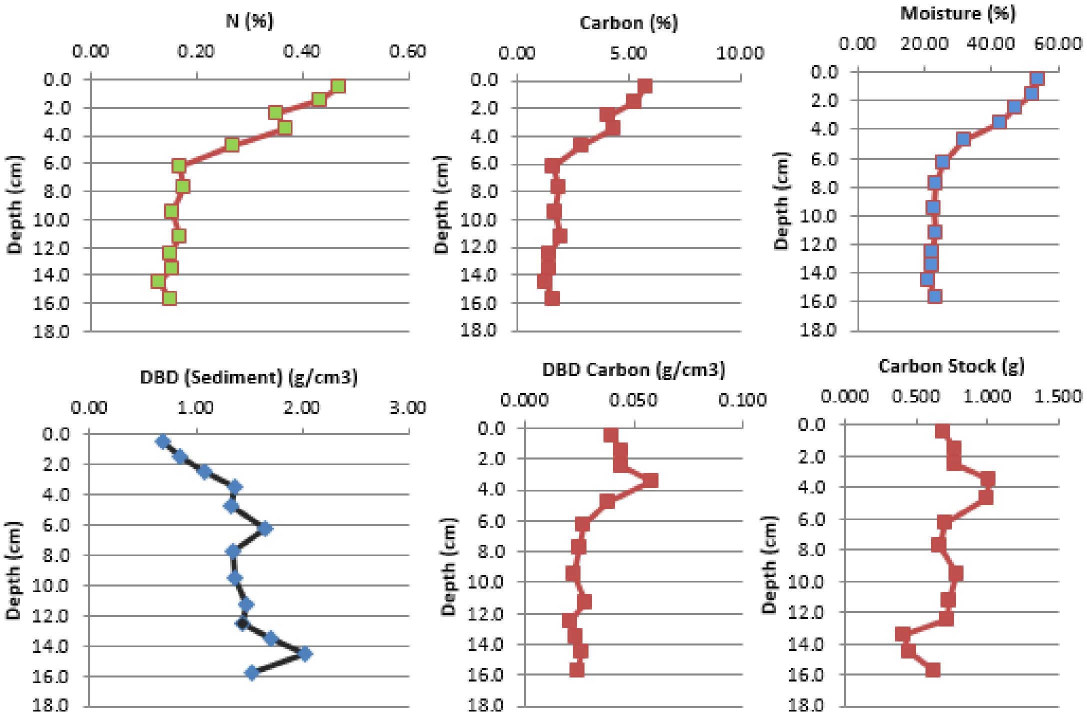

Figure B.18: Core 2 Pasture Pond (Size range 1,000 – 10,000 sq. m)

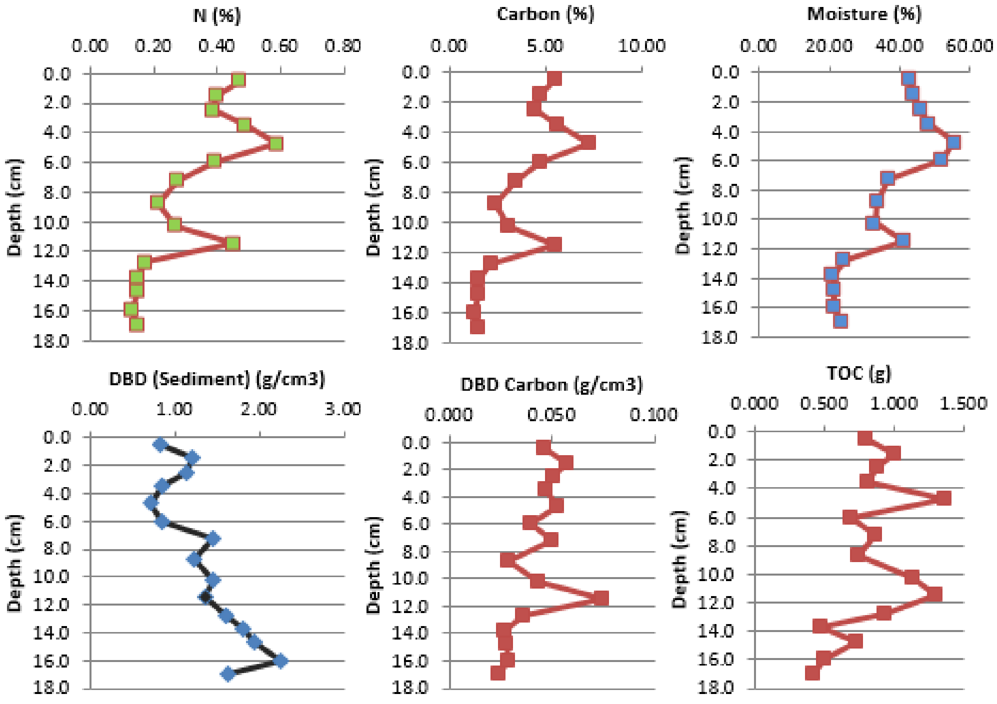

Figure B.19: Core 3 Pasture Pond (Size range 1,000 – 10,000 sq. m)

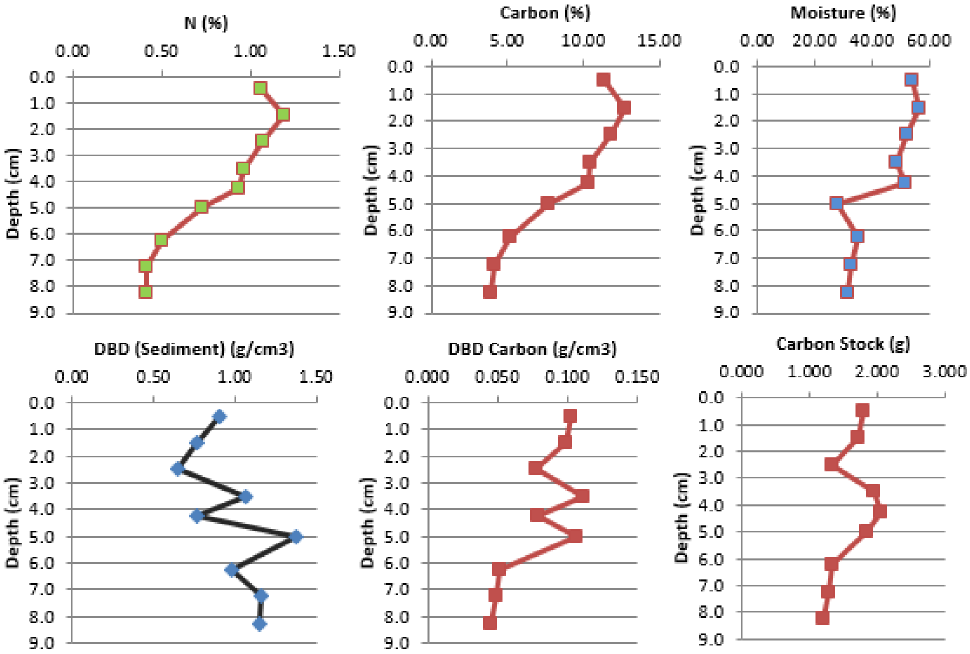

Figure B.20: Core 1 Pasture Pond (Size range 10 – 100 sq. m)

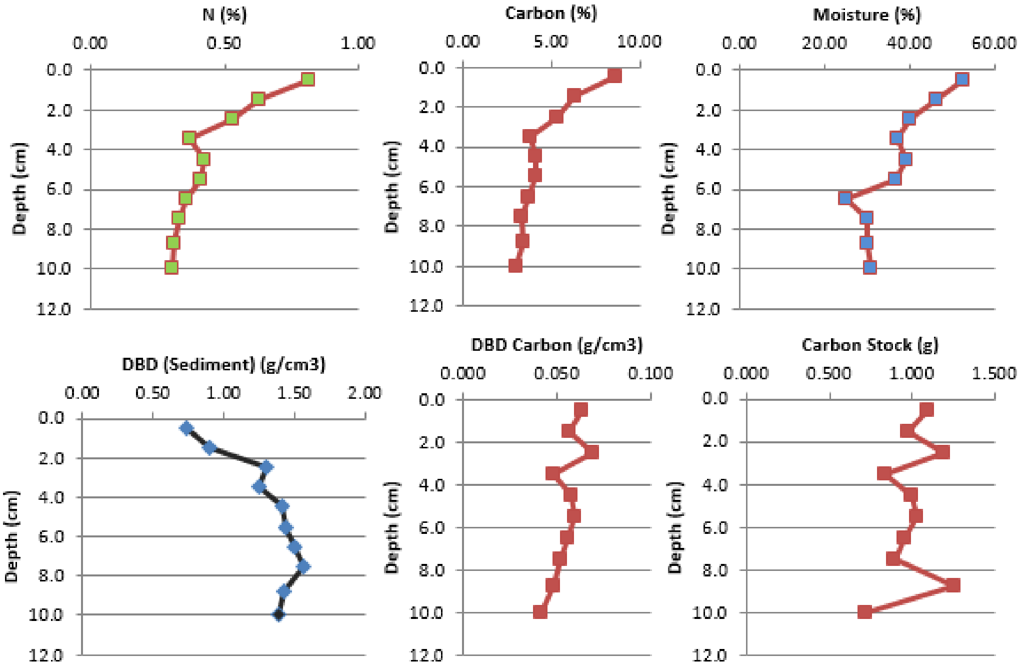

Figure B.21: Core 2 Pasture Pond (Size range 10 – 100 sq. m)

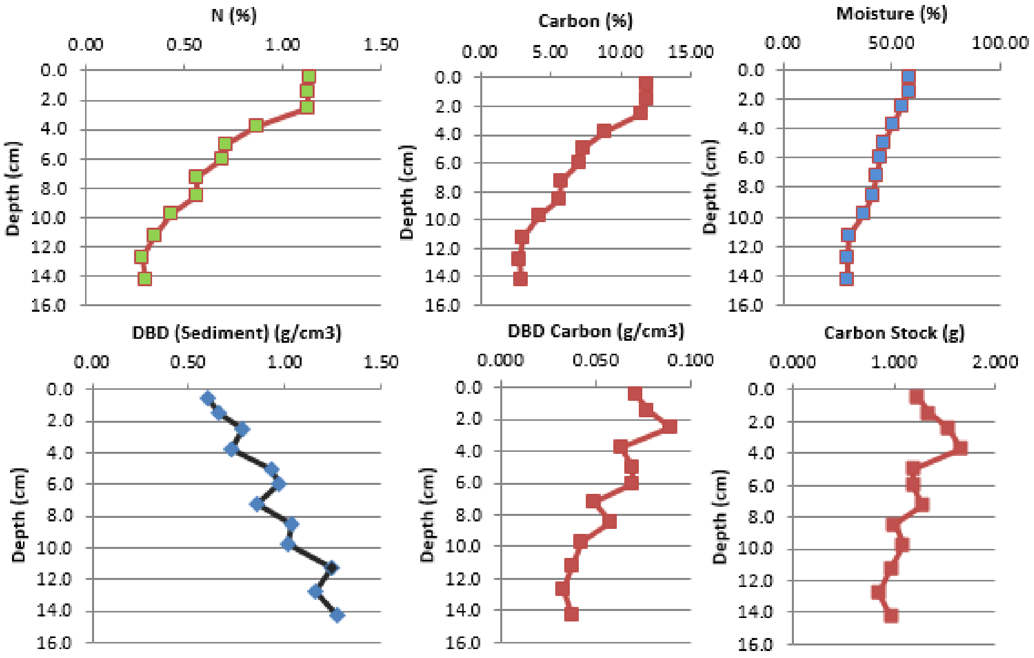

Figure B.22: Core 3 Pasture Pond (Size range 10 – 100 sq. m)

Appendix E: Data for Microbial Analysis

Table C.1: Phylum data

Phylum	*Number Genus per Phylum*	*Total Number of read per Phylum*
Acidobacteria	1,093	1,779,581
Proteobacteria	3,120	1,763,733
Bacteroidetes	1,268	769,739
Verrucomicrobia	833	517,815
Chloroflexi	735	411,315
Euryarchaeota	194	257,189
Crenarchaeota	56	153,539
Actinobacteria	362	130,409
Firmicutes	431	122,945
Planctomycetes	604	88,571
Chlorobi	67	76,522
Gemmatimonadetes	88	36,766
Cyanobacteria_Chloroplast	111	32,427
Nitrospira	14	25,454
WS3	77	21,206
Armatimonadetes	164	20,703
Spirochaetes	43	20,589
BRC1	106	9,783
Chlamydiae	131	8,754
OD1	95	6,118
Fusobacteria	8	5,806
OP11	46	2,771
SR1	20	2,400
Fibrobacteres	4	202
Lentisphaerae	1	122
TM7	1	40

Table C.2: Phylum DNA data in all ponds analysed in Northumberland

	10 - 100 sq. m	10 - 100 sq. m	100 - 1,000 sq. m	100 - 1,000 sq. m	100 - 1,000 sq. m	100 - 1,000 sq. m	100 - 1,000 sq. m	1,000 - 10,000 sq. m	1,000 - 10,000 sq. m	1,000 - 10,000 sq. m	1,000 - 10,000 sq. m	1,000 - 10,000 sq. m
	Arable Pond	Pasture Pond	Arable Pond	Sand Dune Pond	Sand Dune Pond	Natural Pond	Pasture Pond	Pasture Pond	Sand Dune Pond	Arable Pond	Natural Pond 2a	Natural Pond 2b
	Total number of DNA read per Phylum			Total number of DNA read per Phylum			Total number of DNA read per Phylum					
Acidobacteria	181,402	199,160	146,287	87,967	126,917	140,417	167,482	137,590	98,456	217,285	79,860	196,747
Actinobacteria	21,551	25,817	7,803	13,761	13,823	4,158	7,939	4,473	5,105	8,574	4,231	13,174
Armatimonadetes	1,590	1,059	2,086	1,204	2,343	535	1,590	1,876	1,852	3,010	880	2,678
Bacteroidetes	50,088	87,984	38,254	52,984	60,152	54,863	76,588	78,782	56,672	22,289	75,943	115,138
Chlamydiae	71	281	36	416	2,272	843	234	720	1,873	289	227	1,492
Chlorobi	70	638	331	6,984	15,005	7,086	1,731	7,259	26,812	37	2,123	8,446
Chloroflexi	17,148	22,297	13,389	19,752	23,423	17,043	79,860	41,516	29,417	8,127	66,727	72,614
Fibrobacteres	20	0	0	2	3	15	108	9	14	0	2	29
Fusobacteria	1	8	0	157	4,314	34	523	70	26	0	436	237
Gemmatimonadetes	6,157	2,873	6,911	1,468	1,481	2,700	1,680	1,091	1,749	8,314	256	2,086
Lentisphaerae	7	7	2	7	32	6	13	9	11	0	0	28
Nitrospira	1,616	1,827	1,824	1,638	1,006	5,330	2,586	2,420	1,737	2,954	15	2,501
Planctomycetes	9,944	15,596	6,027	11,677	7,319	6,817	4,699	5,494	3,372	6,035	2,262	9,328
Proteobacteria	109,102	127,325	78,131	140,311	195,754	122,192	139,968	160,060	202,473	70,127	113,967	283,560
Spirochaetes	473	422	83	1,671	3,218	1,082	2,075	706	3,367	22	2,126	5,344
Verrucomicrobia	43,320	71,111	45,183	33,606	25,067	54,997	40,697	40,209	23,807	42,476	28,385	68,954
BRC1	286	636	356	116	159	626	1,694	1,031	125	114	2,170	2,470
Cyanobacteria_Chloroplast	7,108	5,384	5,274	4,367	1,356	1,041	530	1,317	701	1,136	1,955	2,258
Firmicutes	8,163	17,192	5,349	8,574	6,846	6,845	14,272	10,229	5,588	4,350	18,522	17,012
OD1	144	561	26	188	520	845	258	1,059	871	2	187	1,457
OP11	3	62	9	85	640	68	218	253	636	8	160	629
SR1	59	48	1	42	505	14	68	210	744	0	598	111
TM7	0	7	0	0	18	0	0	0	15	0	0	0
WS3	545	1,456	321	1,138	1,228	2,657	1,520	2,514	1,708	640	2,352	5,127
Crenarchaeota	10,689	9,629	3,353	9,873	10,749	8,787	8,631	24,022	16,801	8,998	14,070	27,936
Euryarchaeota	3,200	23,267	2,391	25,659	23,724	6,765	32,549	41,772	19,224	267	34,502	43,869