

AGRICULTURAL SMALL WATER BODIES: CARBON SOURCE OR CARBON SINK?

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Abstract

The terrestrial landmass is thought to be a major greenhouse gas sink (GHG), and while freshwaters were thought to be neutral entities, they may in fact be potent producers and emitters of CO₂ and CH₄. Little is known about their contribution to the global carbon budget, especially in agricultural landscapes. This research aimed to determine the rate of organic carbon accumulation in agricultural ponds in relation to nutrients, and to estimate CO₂ and CH₄ production processes in, and emission routes from, ponds in relation to physico-chemical conditions. Sampling sites were three catchments in Leicestershire in a characteristic agricultural environment. Year 1 fieldwork (April–June 2013) covered 98 of an original 180 sampling sites, and encompassed a mixture of water bodies. Sites were accessed in stages according to specified access routes, and samples were analysed the same day. Year 2 fieldwork (August–September 2014) focused on ponds, those from year 1 and new established ponds. Analysis was done with a quadrupole mass spectrometer.

The data obtained from the air-flux time series samples, plotted as scatter graphs, showed a very varied scatter with, at times, negative sloping trendlines, resulting in very low r^2 values in all but one graph. It is thought that a lack of measurement precision, analysing the air-flux samples, is the main cause of unreliable data. The inconclusive data was initially thought to derive from a poor sampling regime, but was ultimately linked to the analytical method and instruments. Investigations suggested that an inter-technique comparison of the analyser would help to determine the validity of the data. Although data analysis could not be completed, descriptive analyse of the processes undertaken are provided.

Water samples, from ponds, streams, and ditches were collected for analysis of dissolved CO₂ and CH₄ in the three catchments (Eye, Stonton, and Barkby). Data analysis revealed significant differences between catchments in both, CO₂ and CH₄ concentrations amongst the three catchments.

To establish sediment organic and inorganic carbon content of ponds, sediment traps were installed, and the retrieved samples analysed using the loss on ignition method. The regression analysis showed that about 33% of CO₂ emission is dependent on organic carbon. Organic carbon and inorganic carbon were not significantly correlated with CH₄ emissions.

Data obtained from water samples analysed for dissolved CO₂ and CH₄, and the slope values of the air-flux samples were compared to the 2013 baseline data collected by Freshwater Habitats Trust using correlation half-matrix. The analysis showed correlation at $P=0.050$ $r=0.381$ between CO₂ and nitrogen, between CH₄ dissolved in water and suspended solids,

and between CO₂ slope air-flux and zinc. At $P=0.10$ $r=0.487$ correlation was between CO₂ water and CO₂ slope air-flux, between CH₄ slope air-flux and turbidity, and between CH₄ air-flux and zinc. Since an essential part of the data was deemed unfit for data analysis recommendations are given for ensuring reliability before commencing sampling, and for improving the methodology.

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1 General Introduction

1.1 Broad background and context

Carbon dioxide (CO₂) and methane (CH₄) are the two atmospheric air constituents most commonly referred to in the context of increased emissions of greenhouse gases (GHG) into the atmosphere.

The first sharp increase in what was a relatively stable concentration of CO₂ in the atmosphere was recorded in the 1920s, which suggested that anthropogenic activity had influenced the accelerated increase of GHG emitted into the atmosphere (Le Quéré *et al.*, 2015). In 1979, the concentration of CO₂ in the atmosphere was measured at c. 335 ppm (parts per million), while in 2016 the average was 402.59 ppm (Figure 1), and CH₄ was measured at c. 1560 ppb (parts per billion) in 1979 and 1850 ppb in 2016 (Figure 2) (National Oceanic and Atmospheric Administration (NOAA), 2017).

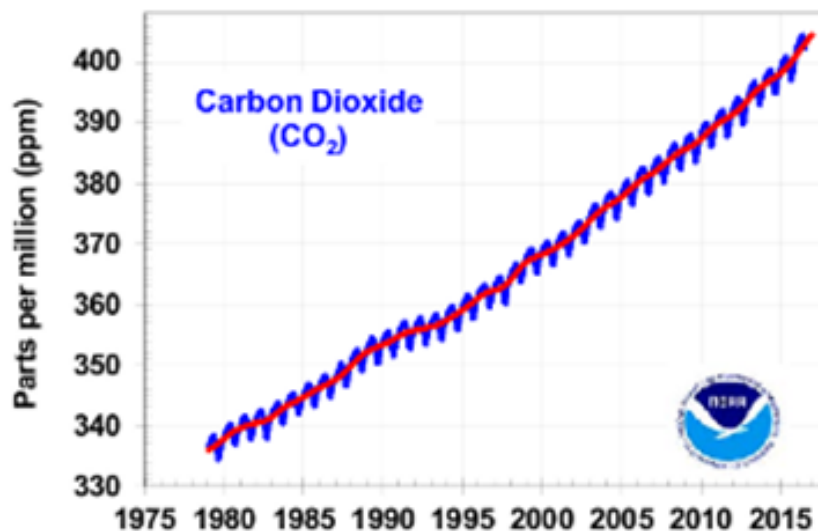


Figure 1: Measured concentration of CO₂ in the atmosphere plotted as a 'smoothed north-south latitude profile' since 1979 (National Oceanic and Atmospheric Administration (NOAA), 2017)

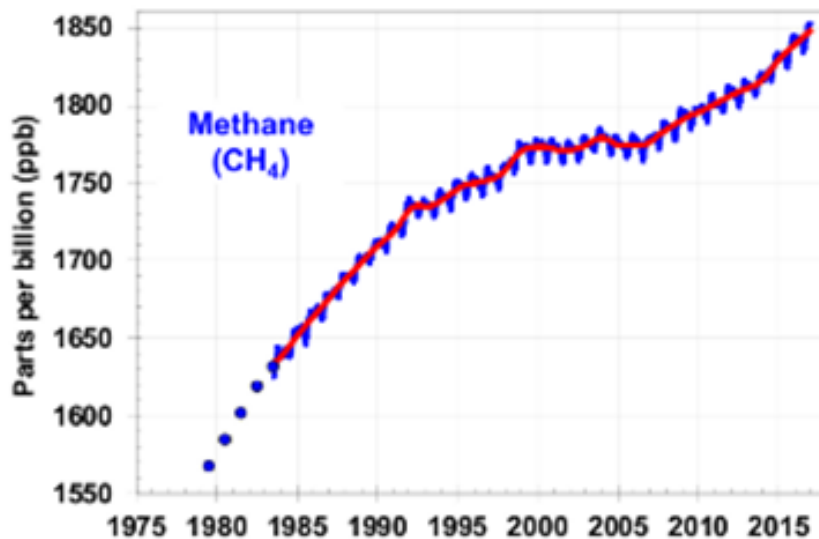


Figure 2: Measured concentration of CH₄ in the atmosphere plotted as a 'smoothed north-south latitude profile' since 1979 (National Oceanic and Atmospheric Administration (NOAA), 2017).

In 2015 the NOAA recorded the highest yearly increase of 3.05 ppm CO₂ in the last 56 years; in the last four years the yearly increase was 2.0 ppm and above (National Oceanic and Atmospheric Administration (NOAA), 2017). These numbers stand in stark contrast to pre-industrial values estimated between 260-270 ppm CO₂ in the mid-18th Century (Wigley, 1983) and would suggest approximately an 85% increase of CO₂ concentration in the atmosphere within 266 years (1750-2016). This is compared to what was a relatively stable CO₂ concentration of between 180 and 300ppm during the previous 650,000 years, prior to the industrial period (Joos and Spahni, 2008).

Recent studies have shown that terrestrial landmasses are major GHG sinks and play a vital role in global terrestrial carbon burial, estimated at $2.6 \pm 1.7 \text{ Pg C year}^{-1}$ (excluding C emissions derived from deforestation) (Tranvik *et al.*, 2009; Bastviken *et al.*, 2011; Liu and Dreybrodt, 2015; Rasilo, Prairie and del Giorgio, 2015). Other studies suggest that freshwaters, constituents of the terrestrial landmass, are potent producers and emitters of CO₂ and CH₄ (Bastviken *et al.*, 2011). There has been very limited research investigating small freshwater bodies as significant contributors in this system (Downing *et al.*, 2008; Liu and Dreybrodt, 2015). In 2011 the estimated total emission of CH₄ from fresh water was 103 Tg CH₄ year⁻¹, which is equivalent to 0.65 Pg C as (CO₂ eq) year⁻¹ (Bastviken *et al.*, 2011). This amounts to approximately 25% of the terrestrial GHG sink, assuming that the warming potential of 1 kg of CH₄ equals 25 kg of CO₂ over a 100-year period (Tranvik *et al.*, 2009; Bastviken *et al.*, 2011). Rasilo, Prairie and del Giorgio (2015) points out that there are still too few studies investigating the source of CH₄ from inland freshwaters and that estimates vary widely, ranging from 8 to over 100 Tg CH₄ year⁻¹. This makes estimating future scenarios

problematic, as does the lack of proxy data accounting for the increase or decline of ponds and impoundments since the 1920s.

Inland waters were categorised as neutral entities which simply transport sedimentary carbon, yet Cole *et al.* (2007) questioned this 'neutral pipe' conceptualisation of freshwater bodies (Figure 3). Their work established that, contrary to the 0.9 Pg y^{-1} neutral pipe land-to-ocean transport previously estimated, terrestrial loading was likely to be 1.9 Pg y^{-1} with sediment burial of 0.23 Pg y^{-1} and 0.75 Pg y^{-1} CO_2 evasion (gaseous emission) during transport.

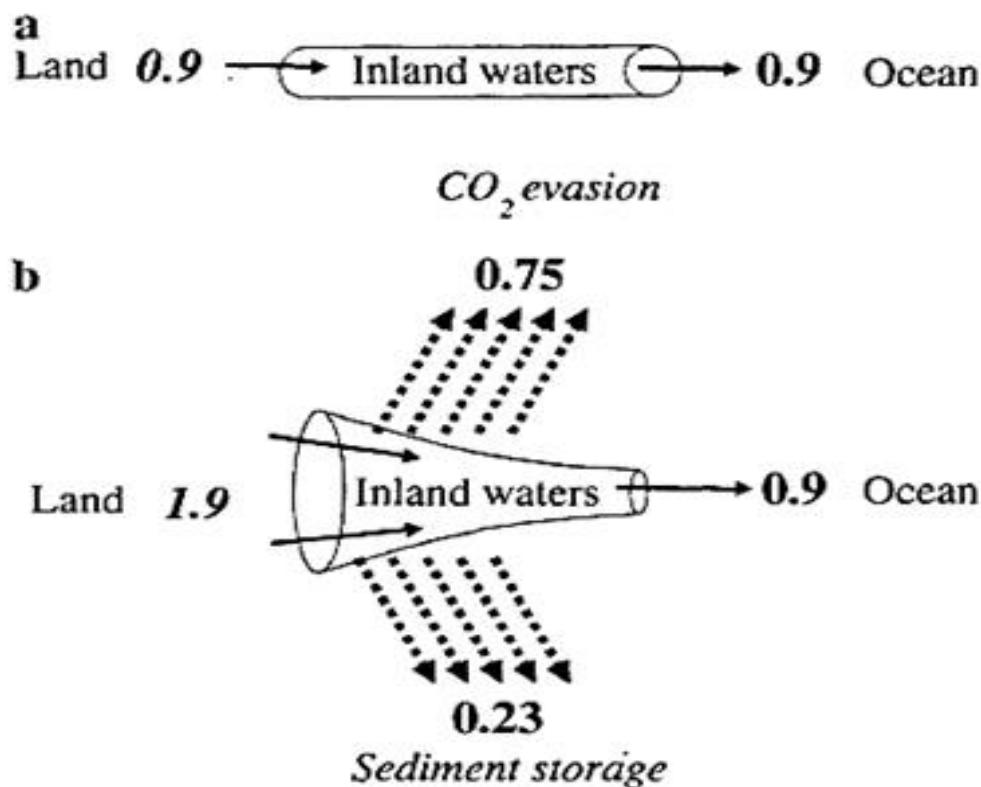


Figure 3: Diagram (Cole *et al.*, 2007) of the global carbon water balance from inland to sea. **a)** Dated model with inland waters as neutral pipe, transporting IC and OC from land to sea. Cole *et.al.* (2007) added to the 0.7 Pg C y^{-1} of riverine transport, a 0.2 Pg C y^{-1} of direct groundwater load to the sea not entering the riverine system. **b)** New estimated carbon cycle including CO_2 emissions into the atmosphere 0.75 Pg C y^{-1} and sediment store 0.25 Pg C y^{-1} which raise the initial transport load of carbon to 1.9 Pg C y^{-1} (Cole *et al.*, 2007).

Two years later, Tranvik *et al.* (2009) corrected these values further with a more than threefold increase from the initial neutral pipe model: the terrestrial loading is estimated to be 2.9 Pg y^{-1} , sediment burial is 0.6 Pg y^{-1} and 1.4 Pg y^{-1} . CO_2 evasion was twice as high as the 2007 estimates.

Freshwaters accumulate soil carbon through lateral hydrological transport, where it is stored in sediments, emitted in the form of CO_2 and CH_4 into the atmosphere or transported

to the oceans (Bastviken *et al.*, 2011). Streams and rivers also play an active part in the freshwater accumulation of gaseous carbon, and ultimately the release of CO₂ and CH₄ into the atmosphere, and there is evidence that streams in peat landscapes are supersaturated with CO₂ and CH₄ (Billett and Harvey, 2013). The rate of emission of GHG from freshwater environments is dependent on different factors and varies between CO₂ and CH₄ (Bartosiewicz, Laurion and MacIntyre, 2015). Huttunen *et al.* (2003), Jacinthe *et al.* (2012) and Yang *et al.* (2008) indicate that CH₄ emissions from water bodies in catchments exposed to intensive farming are higher than in undisturbed landscapes, but the underlying mechanisms are not well understood.

While water bodies are carbon emitters, they are also potential carbon sinks, (Rasilo, Prairie and del Giorgio, 2015) but Cole *et al.* (2007) points out that the balance between the two is dynamic, with more than half of the carbon input into freshwater systems being emitted as gases. Whether carbon is sequestered or emitted from a water body will depend upon factors such as pH, depth, age, size, nutrients, temperature and vegetation, which influence the nature and activity of the microbial community (Euliss *et al.*, 2006; Tranvik *et al.*, 2009). Changes in the state of any of these variables are hypothesised to alter the balance between sequestration and emission and the relative rates of CO₂ and CH₄ production (Le Quéré *et al.*, 2013). In small water bodies these factors are dependent on agricultural practices in the catchments (Jacinthe *et al.*, 2012). Ponds and small reservoirs cover an estimated 77,000 km² of the global agricultural landmass (Downing, 2006). There is a predicted rate of increase in the formation of new ponds and impoundments in terrestrial farm land from the current 0.7% to 60% per year (Downing, 2010). Given these facts, there is scope to hypothesise the existence of a substantial, but so far underestimated, carbon sink. However, there is still uncertainty as to whether they are net emitters or sequesters and therefore further investigation is needed (Downing, 2010). In fact, the U.S. Department of the Interior and the U.S. Geological Survey published a 190-page report in 2010 on how to assess current carbon stocks and future scenarios in the U.S. (Bergamaschi *et al.*, 2010). This reiterates the need for a deeper understanding of the mechanisms of carbon storage, sequestration and fluxes (Myhre, Alterskjær and Lowe, 2009).

Over the last century, the main focus has been on the biosphere and ecosphere, such as oceans, rainforests and atmospheric circulation, whereas small freshwater biomes, such as small lakes and ponds have either not been on the research agenda or have received limited research funding, resulting in rather scattered research. This is especially true for terrestrial aquatic research, as shown by Oertli *et al.* (2009) and Downing (2010) in evaluating of aquatic research publications. Downing (2010), for example, estimates that only about a quarter of the aquatic studies, addressing a variety of issues, focus on ponds or small

reservoirs. In the rather philosophical words of Sir Arthur Conan Doyle from ‘A Case of Identity’ quoted by Downing (2010), “... little things are infinitely the most important.” Which underlines that understanding small things will help comprehend the big picture.

It is estimated that lentic freshwater bodies cover >3% of the earth’s terrestrial landmass (lakes and ponds about 4.2 million km², impoundments about 260,000 km², and agricultural ponds about 77,000 km²) which is twice previous research estimates (Downing *et al.*, 2006), the smallest of which, ponds (<2ha), have not been included in the global carbon budgets (Downing *et al.*, 2006; Cole *et al.*, 2007; Catalan *et al.*, 2014). However, there is evidence that the role of small water bodies in the carbon cycle is as important as that of oceans (Downing *et al.*, 2006; Cole *et al.*, 2007; Juutinen *et al.*, 2009; Catalan *et al.*, 2014) and Tranvik *et al.* (2009) estimates that the organic carbon (OC) burial in wetlands is three times of that in the world’s oceans. Over the last decade, an increase in the number of studies with a focus on small freshwater bodies suggests growing attention from the wider research community, which is now classifying these aquatic environments as possible significant contributors to global CO₂ and CH₄ budgets (Downing *et al.*, 2006; Downing *et al.*, 2008; Downing, 2010; Billett and Harvey, 2013; Catalan *et al.*, 2014). While there is debate over the total area of the terrestrial landscape that is covered by small bodies of freshwater, they undoubtedly play important roles in ecosystems processes (Catalan *et al.*, 2014). Small lentic systems are disproportionately more versatile in terms of ecology, macronutrients, turnover (Oertli *et al.*, 2009) and carbon deposition rates (Yang *et al.*, 2008; Holgerson and Raymond, 2016) than larger freshwater biomes. This can be ascribed to the following factors, (a) due to the smaller size and depth of ponds, mostly classified as polymictic, where water column turnover tends to be more fluid as it is not strictly bound to seasonal changes, and can therefore occur more frequently (Portielje & Van der Molen, 1999) compared to the stratified water column in dimictic freshwater bodies bound to seasonal changes. (b) The ratio of the circumference vs. surface area is larger in ponds than in lakes (Fairchild *et al.*, 2005). (c) Ponds usually have more light penetration as they tend to be shallower compared to lakes, which makes ponds and small freshwater bodies more dynamic and responsive in terms of nutrient abundance (Grzybowski, 2014), organic matter accumulation, and species diversity (Ruley & Rusch, 2004). This has implications for carbon budgets in small freshwater bodies as they are more active and responsive to changes due to intensified biogeochemical mechanisms (Müller *et al.*, 1998; Grzybowski, 2014; Holgerson and Raymond, 2016), which are less studied and understood than those operating in large lakes (Céréghino *et al.*, 2008; Downing, 2010).

1.1.1 Global carbon budgets

Due to growing concerns regarding the continuous rise of the concentration of greenhouse gases in the atmosphere derived from anthropogenic activities (Prather, Holmes and Hsu, 2012; Le Quéré *et al.*, 2018), the carbon cycle (Figure 4) gained notoriety over the last few decades.

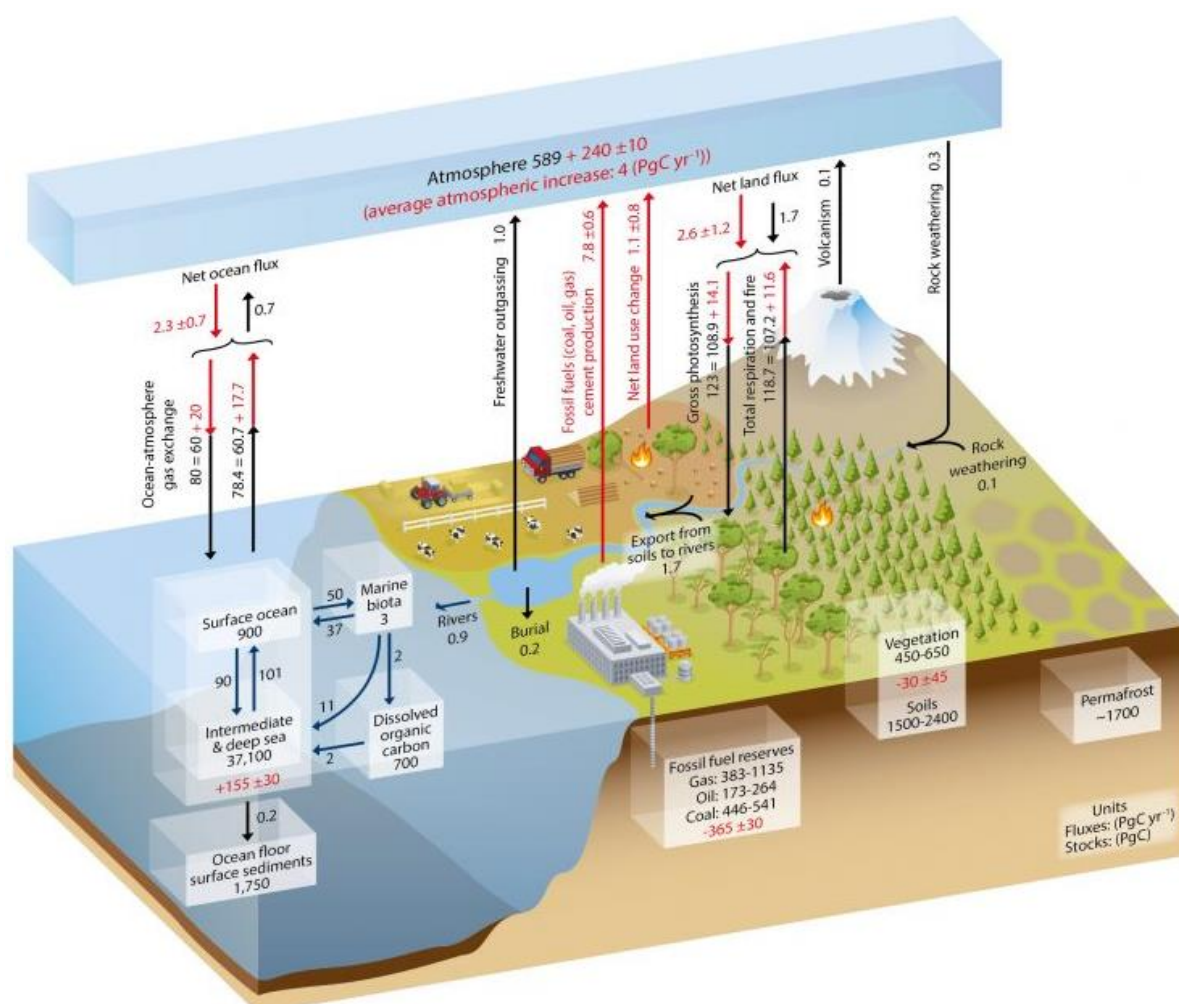


Figure 4: The Carbon Cycle. Numbers in boxes denote carbon inventories in PgC (1 PgC = 10^{15} gC = 1 GtC). Atmospheric CO₂ conversion is used at 2.1 PgC = 1 ppm. Numbers next to arrows indicate fluxes in PgC/y⁻¹. Black numbers and arrows represent estimates of the natural (pre-industrial) carbon cycle. Red numbers and arrows indicate estimates of anthropogenic effects for 2000-2009 (Ciais *et al.*, 2013)

In the Intergovernmental Panel on Climate Change (IPCC) 5th Assessment Report of 2013, the global CH₄ emissions into the atmosphere from inland waters (wetlands, freshwater and rice cultivation), were estimated at 218-397 Tg CH₄ y⁻¹ (Ciais *et al.*, 2013). Comparing the median of the global CH₄ emissions of the IPCC report with the findings of Saarino *et al.*, (2009), which approximated an efflux of 5.2 Tg CH₄ y⁻¹ from European inland waters, shows a proportional congruence of emissions per landmass in Europe.

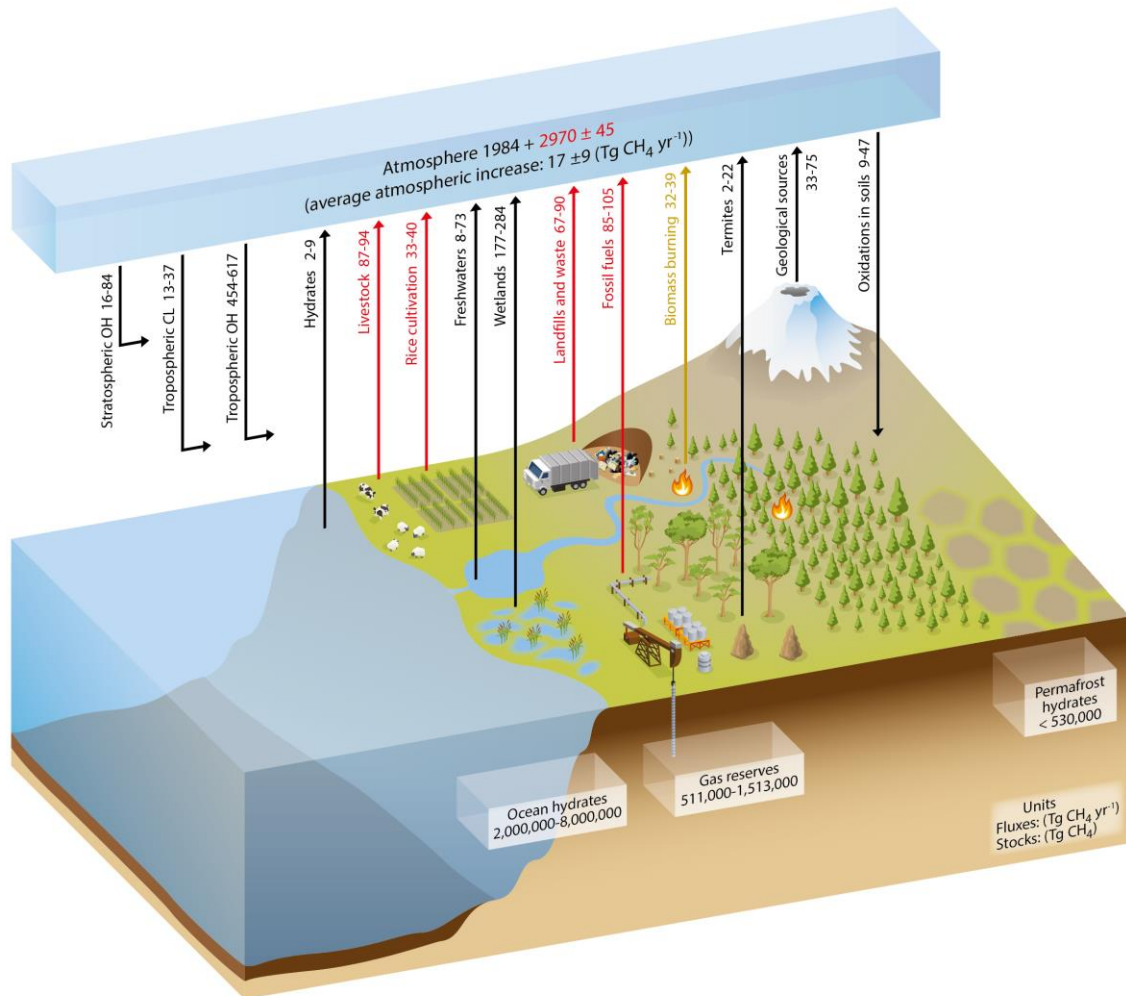


Figure 5: Diagram of the world's CH₄ cycle. Black arrows stand for flux of non-anthropogenic origin, red arrows for anthropogenic origin and yellow a mix of both (Ciais *et al.*, 2013).

1.2 Carbon fluxes from water bodies

The rate of emission of GHG from aquatic environments is dependent on different factors and the ratio of CO₂ to CH₄ is variable (Bartosiewicz, Laurion and MacIntyre, 2015; Holgerson and Raymond, 2016). Variation in CO₂ and CH₄ production is influenced by the water volume and the catchment and water surface areas (Holgerson and Raymond, 2016). In aquatic environments particulate organic carbon (POC hereafter) derives from dieback of either autochthonous particulate organic matter (POM; e.g. from algal biomass) or from allochthonous material deposited from the surrounding catchment. In oceans and large lakes, POM is mainly autochthonous, while in smaller water bodies POM, and therefore CO₂ and CH₄, derive mostly from allochthonous origins, such as plants, and soil material derived from runoff at the shore/water interface (Polis, Anderson and Holt, 1997; Eby, 2016; Roiha *et al.*, 2016). Primary production proportionally directs the rate of CO₂ accumulation in and

emission from freshwater environments by means of the rate of respiration and nutrient input (Kortelainen *et al.*, 2006; Holgerson and Raymond, 2016).

For about a decade, the carbon budget of freshwater bodies has constantly been adjusted as research has progressed. This suggests the need for a deeper understanding of the mechanics involved in the gaseous exchange process in freshwater bodies as there is concern that current land use changes may be increasing gaseous fluxes further (Vachon, Prairie and Cole, 2010). The increasing establishment of large reservoirs for hydroelectricity generation, flood control, drinking water, irrigation, recreation and aquaculture is also of great concern (Bergamaschi *et al.*, 2010). Large examples include the Three Gorges Dam in China, the Smallwood Reservoir in Canada, and the prospected Kalpasar project in the Gulf of Khambhat (Gujarat, India), where the world's largest reservoir will be built (St Louis *et al.*, 2000). Contrary to popular belief, reliable reservoir carbon flux data (available from around 1994) suggest that hydroelectric power stations are not producing carbon-free energy. On the contrary, these static freshwater bodies produce large amounts of CH₄ (St Louis *et al.*, 2000; Jacinthe *et al.*, 2012). The increase in agricultural impoundments for water treatment (Jacinthe *et al.*, 2012) may also have significantly increased aquatic carbon fluxes into the atmosphere.

1.2.1 Carbon fluxes from lakes and wetlands

Most wetlands (terrestrial landscapes which are wholly or partly submerged either year round or for part of the year) are located between 50° and 80°N (Semiletov, 1999; Kettunen, 2003; Whalen, 2005; Schrier-Uijl *et al.*, 2010). They are estimated to emit 60% of total CH₄ emissions globally and are the prime contributor to elevated CH₄ concentrations in the atmosphere (Moosavi *et al.*, 1996; Huttunen *et al.*, 2003; Laing, Shreeve and Pearce, 2008). Wetlands also account for the highest annual mean concentration of CO₂ in the atmosphere (Semiletov, 1999). In the past two decades increased research activity in the field of freshwater carbon emissions has undoubtedly contributed to an improved understanding of the mechanisms driving GHG emissions, but there is still scope to refine and extend this research topic (Repo *et al.*, 2007; Bridgham *et al.*, 2013).

Accumulated organic matter in freshwaters undergoes intricate decomposition by microbes, which degrade plant polymers such as cellulose through fermentation, hydrolysis, homoacetogenesis and syntrophysis. These processes ultimately produce H₂ and acetate, which are the critical components for the production of CH₄ by methanogens, which is generated during periods of oxygen depletion, establishing anoxic conditions that facilitate methanogenesis (Zehnder and Brock, 1979; Dolfig, 1988; Rothfuss and Conrad, 1993). If CH₄ is released from the anoxic zone and reaches, by diffusive transport, the oxygenated

zone, some proportion is oxidised (by methanotrophic bacteria in the water column) producing CO_2 (Jacinthe *et al.*, 2012). The un-oxidised CH_4 then reaches the atmosphere by diffuse flux (Figure 6). The scale and rate of these processes depend on the abundance of oxygen that is present (Bastviken *et al.*, 2004; Minkinen and Laine, 2006; Schrier-Uijl *et al.*, 2011).

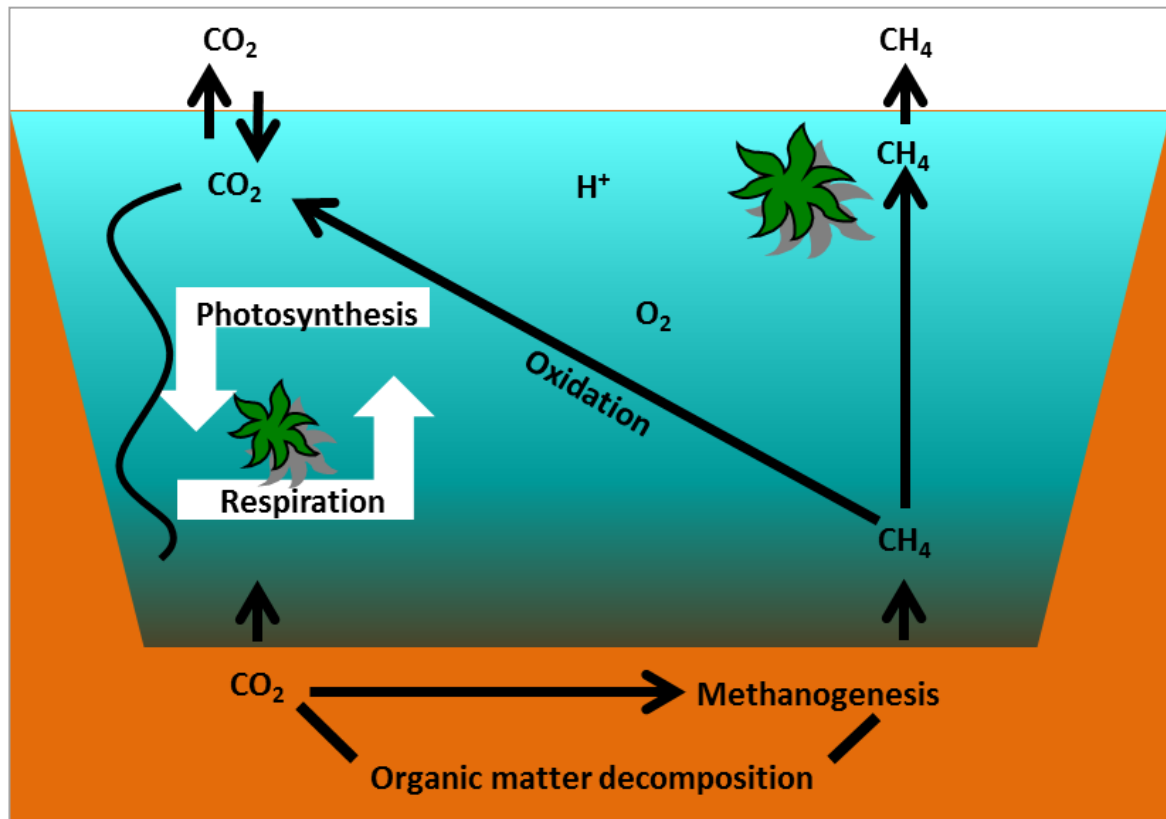


Figure 6: CO_2 and CH_4 production processes in an aquatic environment, adapted from Schrier-Uijl, Veraart *et al.* (2011).

1.2.2 Carbon fluxes from ponds

Biogeochemical processes in small freshwater bodies are influenced by many different factors, for example, the stimulation of CO_2 emissions under alkaline conditions (Potter, 1997; Prairie, Bird and Cole, 2002). The carbon flux in ponds is seasonal: for example, in spring CO_2 degassing is prevalent, and in the other seasons CO_2 uptake from the atmosphere dominates (Figure 7) (Trolle *et al.*, 2011; Pacheco, Roland and Downing, 2014).

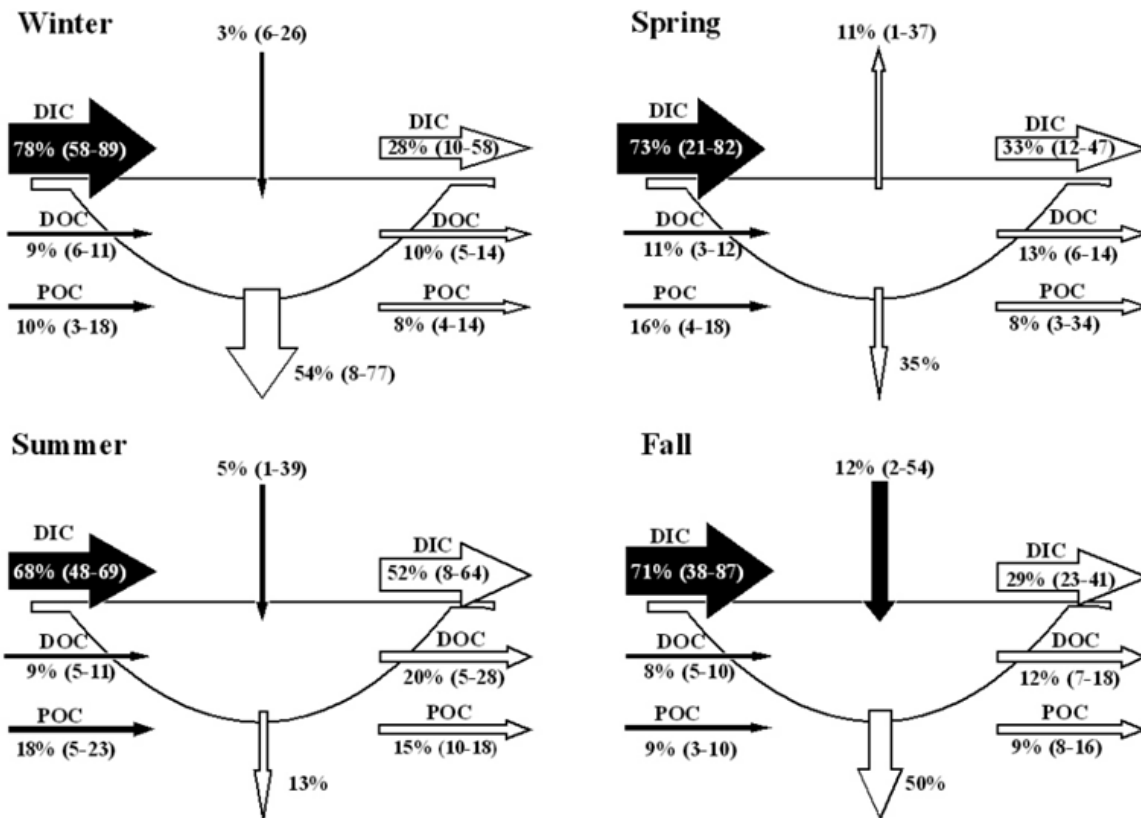


Figure 7: Seasonal comparison of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and particulate organic carbon (POC) of five lakes situated in agricultural environments and classified eutrophic. Black arrows pointing to water body indicate C input and white arrows output. Percentage values are the fraction mean of the values in brackets from (Pacheco, Roland and Downing, 2014).

Although methane production in freshwaters has been thoroughly documented (Zehnder and Brock, 1979; Oremland, 1988) there is still a paucity of reliable CH_4 flux data (Rasilo, Prairie and del Giorgio, 2015). Carbon emissions from ponds situated in different types of landscape in the northern hemisphere (e.g. forest, tundra, bogs etc.) can vary greatly (Table 1), with values ranging from 0.00 to 3.00 g C m⁻² d⁻¹ (Abnizova *et al.*, 2012), and variations in emissions are also found in different climate zones (Table 2) (Wik *et al.*, 2016).

Table 1: Carbon flux of lakes and ponds in different landscape types: Forest (F), Forest Tundra (FT), Tundra (T), Peatland (P), Forest Peatland (FP) and Fens and Bogs (FB) (Abnizova *et al.*, 2012).

Study	Location	Latitude	Land Cover	Year/Period	Study Site	Emission	
						(g CO ₂ m ⁻² d ⁻¹)	(g C m ⁻² d ⁻¹)
<i>Åberg et al.</i> [2004]	Northern Sweden	Boreal	F	2001	Lake Skinnuddselet	1.06	0.29
<i>Blodau et al.</i> [2008]	Northern Siberia	Boreal	FT	2006	Lake Öträsket	0.94	0.26
<i>Casper et al.</i> [2000]	The English Lake District, U.K.	Temperate	FB	1997	Ponds near Igarka	0.07	0.02
					Priest Pot	1.76	0.48
<i>Cole and Caraco</i> [1998]	Central New Hampshire	Temperate	F	1991–95	Mirror Lake min	0.29	0.08
					Mirror Lake max	0.51	0.14
<i>Cole et al.</i> [2000]	Wisconsin	Temperate	F	1991–98	Four lakes min	–0.66	–0.18
					Four lakes max	2.20	0.60
<i>Duchemin et al.</i> [1999]	Taiga region of the Canadian shield	Boreal	F	1994	La Grande 2 and Laforge-1 reservoirs	2.50	0.68
					Lake de Voexut	0.16	0.04
<i>Hamilton et al.</i> [1994]	Hudson Bay Lowlands	Boreal	P	1990	10 ponds min	3.70	1.01
					10 ponds max	11.00	3.00
<i>Huttunen et al.</i> [2002a]	Northern Finland	Boreal	P	1994	Jänkäläinenlampi pond	0.53	0.14
			FP	1995	Kotsamolampi pond	0.02	0.00
<i>Huttunen et al.</i> [2002b]	Northern Finland	Boreal	FP	1994–95	Reservoir Lokka	1.52	0.41
			FP	1994	Reservoir Porttipahta	1.54	0.42
<i>Huttunen et al.</i> [2003]	Northern Finland	Boreal	F	1996–98	Lake Postilampi	0.79	0.21
			F	1996	Lake Heinälampi	0.57	0.16
			F	1997–98	Lake Kevätön	0.64	0.17
			F	1997–98	Lake Vehmasjärvi	0.86	0.23
			F	1997–98	Lake Mäkitjärvi	0.39	0.11
<i>Kling et al.</i> [1992]	Arctic Alaska	Arctic	T	1988, 1990	45 lakes	0.90	0.25
<i>Repo et al.</i> [2007]	Northern Russia	Boreal	F	2005	MT lake	0.50	0.14
					MT pond	1.60	0.44
					FT lake	1.50	0.41
<i>Riera et al.</i> [1999]	Northern Wisconsin	Temperate	F	1994	Crystal bog	1.34	0.37
					Trout bog	2.01	0.55
					Crystal lake	0.02	0.00
					Sparkling Lake	0.22	0.06
<i>Roulet et al.</i> [1997]	Thompson, Manitoba	Boreal	F	1996	Beaver pond	6.20	1.69
<i>Shirokova et al.</i> [2009]	Western Siberia	Boreal	FT-T	2008	Lakes and ponds	1.1	0.30
<i>Striegl and Michnerhuizen</i> [1998]	North-central Minnesota	Temperate	F	1992–93	Williams Lake	0.02	0.01
<i>St. Louis et al.</i> [2000] given by <i>Huttunen et al.</i> [2003]					Shingobee Lake	1.61	0.44
					Temperate and boreal reservoirs excluding Lokka and Porttipahta	0.22	0.06

Table 2: Large-scale estimates and future predictions of CH₄ emissions from lakes and ponds north of ~50° N (Wik *et al.*, 2016).

Type	Formation process	Sediment*	Area (km ²)†	Depth (m)†	Flux (mg CH ₄ m ⁻² d ⁻¹)‡	
					Diffusion	Ebullition
Beaver ponds	Beaver activity in rivers and streams in boreal and subarctic regions ⁴⁴	Peat/organic	0.01–0.05	0.5–3.0	116.5 (1–147)	83.8
Glacial/post-glacial lakes	Glacial and periglacial processes during the last deglaciation ⁴ . Common types are bedrock depressions, moraine dams and kettle lakes ⁷⁰	Organic/minerogenic	0.001–242	0.5–35	12.5 (3–18)	32.2 (9–58)
Peatland ponds	Slow (scale of millennia) peat degradation unrelated to permafrost thaw and thermokarst ⁷¹	Peat	0.0001–0.01	0.3–3.2	86.1 (26–122)	58.5
Thermokarst water bodies	Rapid (scale of decades to centuries) thaw of ice-rich permafrost followed by ground subsidence ⁵²	Organic/minerogenic/yedoma	0.003–5.3	1.0–17	33.2 (3–38)	87.5 (7–132)

This indicates how versatile freshwater bodies are and demonstrates the heterogeneity of carbon fluxes even amongst similar lentic systems, which in itself suggests that further and extended analyses are needed to understand the mechanisms influencing these systems.

1.3 Carbon sequestration

Globally, wetlands are considered a prime carbon sink, but at the same time are emitters (Clair *et al.*, 2002; Mitsch *et al.*, 2013). The major wetland carbon sink, by burial rate, are peatlands, which are classified as long-term sinks and, while they contribute as little as 3% to the wetland area, are estimated to hold about 20–30% of global carbon stock (Huth, Jurasinski and Glatzel, 2012). Studies have shown that carbon accumulation in the world's

wetlands is substantial, ranging from 0.003 to 0.305 kg C m⁻² yr⁻¹ (Table 3) (Kayranli *et al.*, 2010). Wetlands under agricultural regimes, particularly prairies, accumulate carbon at high rates (excluding constructed wetlands) (Kayranli *et al.*, 2010).

Table 3: Example of carbon accumulation in sediments of different wetland types (Kayranli *et al.*, 2010).

Type of wetland	Location	Carbon accumulation (kg C m ⁻² yr ⁻¹)	Reference
Agricultural field and riparian ecosystem	USA (Maryland)	0.16–0.22	McCarty and Ritchie (2002)
Coastal wetlands	USA (California)	0.033±0.0029	Brevik and Homburg (2004)
Prairie wetland	Northcentral USA	0.305	Euliss et al. (2006)
Boreal forests	Canada (Ontario)	0.003–0.01	Trumbore and Harden (1997)
Horizontal subsurface flow constructed wetlands	Estonia	1.5–2.2	Mander et al. (2008)
Boreal peatlands	Finland	0.015–0.026	Turunen et al. (2002)
Temperate peatlands	Finland	0.01–0.046	Turunen et al. (2002)
Peatlands	North America	0.029	Gorham (1991)

There is evidence, therefore, that small water bodies are as influential in the carbon cycle as oceans (Downing *et al.*, 2006) and are proportionally more responsive to environmental impacts than larger aquatic systems (Müller *et al.*, 1998). Rates of carbon burial in impoundments are estimated to be in the range of 150–220 Tg C y⁻¹, compared to 120–240 Tg C y⁻¹ carbon burial in the oceans, whereas the annual C burial in natural lakes ranges from 30 to 70 Tg C y⁻¹ (Downing *et al.*, 2008). Carbon burial occurs in the majority of aquatic systems in a seasonal pattern, with decay rates peaking during the cold seasons (Pacheco, Roland and Downing, 2014). Where low mineralisation rates are predominant, for example in temperate climate zones, carbon burial is suggested to be higher than in boreal/tropical climates (Mendonça *et al.*, 2012). Downing and Duarte (2009) concluded that eutrophication accelerates carbon sequestration in lake sediments. However, decay of organic matter is driven by various factors: depending on the mineral matrix of a freshwater environment, it can be inhibited or slowed down (Laskov, Amelung and Peiffer, 2002).

1.4 Lentic and lotic systems in agricultural landscapes

Agricultural landscapes, where conventional farming practices (as opposed to organic farming) predominate, often undergo intensive fertiliser, herbicide and pesticide treatments. Biodiversity and ecosystems can be threatened by these intensive farm practices, and aquatic systems are especially vulnerable (Williams *et al.*, 2004; Scheffer *et al.*, 2006). Analysis of phosphorus data from 1965–2009 from the river Frome (Dorset), which is a landscape dominated by agriculture, shows that once phosphorus use was limited (from

2002 onwards), concentration of phosphorus in the watercourse dropped, resulting in the decline of algal blooms (Bowes *et al.*, 2011). Fertilisers, pesticides, and herbicides entering a water course, by run-off or leaking incidences, can cause long-term damage and wide-ranging problems in an entire catchment ecosystem: once nutrients enter aquatic environments, primary production increases, which in most cases results in the eutrophication of aquatic waterbodies and systems (Sager and Lachavanne, 2009).

Despite these ecological concerns, research shows that the mean rate of carbon burial of eutrophic lakes in agricultural environments is estimated to be in the range of $2122 \text{ g m}^{-2} \text{ y}^{-1}$ and projected to be proportionally higher compared with other global ecosystems (Mulholland and Elwood, 1982; Dean and Gorham, 1998; Gilbert *et al.*, 2014). Gilbert *et al.* (2014) compared carbon burial in four different pond types. They concluded that ponds with diverse characteristics bury carbon at different rates (Gilbert *et al.*, 2014). There is an understanding that eutrophic water bodies, due to the rapid conversion of CO_2 into particulate organic carbon (POC) by aquatic photosynthesising organisms, extract autochthonous atmospheric carbon (Heathcote and Downing, 2012). In this instance, and despite the bad press, eutrophication may just be the answer to reducing GHG emissions into the atmosphere. Moreover, with higher carbon burial in freshwater environments, especially in lentic systems, ponds in nutrient-rich environments become an interesting research feature.

Impoundments, ditches and streams in agricultural environments are prone to higher rates of carbon deposition due to the overall higher concentrations of carbon in their sediments, mainly caused by increased soil erosion through tillage (Downing *et al.*, 2008; Jacinthe *et al.*, 2012). Aquatic systems in agricultural landscapes are often exposed to high nitrogen concentrations through leaching. This leads to high vegetation growth, which generates high biomass, and ultimately increases organic carbon accumulation (Stadmark and Leonardson, 2005). When enhancing agricultural landscapes, it is important to consider that nitrogen removal and denitrification require an anaerobic environment (Johansson *et al.*, 2004; Stadmark and Leonardson, 2005; Stadmark, Seifert and Leonardson, 2009). However, this can result in higher concentrations of GHGs, particularly CO_2 , CH_4 and N_2O (Johansson *et al.*, 2004; Stadmark and Leonardson, 2005; Stadmark, Seifert and Leonardson, 2009).

1.5 Overall aims

Ponds and other freshwater bodies in the UK lowlands are highly polluted and/or degraded, with a large number of rivers and streams not meeting the EU Water Framework Directive minimum standard (Biggs *et al.*, 2014). The 'Water Friendly Farming' project, initiated by

the Freshwater Habitats Trust (FHT, formerly Pond Conservation) in collaboration with Game and Wildlife Conservation Trust (GWCT), University of York, the Environment Agency (EA), Chemicals Regulation Directorate (CRD-HSE), Syngenta, Oxford Brookes University, University of Sheffield, Welland Rivers Trust, Anglian Water and farmers and landowners in the three study catchments, was set to investigate whether landscape alterations would improve the state of lentic and lotic systems in the study catchments (Biggs *et al.*, 2014). This initiative was derived from the EU Water Framework Directive and establishes aims and targets for environmentally sustainable farming practice to enhance river basin management (Biggs *et al.*, 2014). A quantitative understanding of the contribution of factors such as pH, depth, age, size, nutrients, temperature and vegetation to small water body carbon budgets has the potential to influence pond management and agricultural practices to promote ponds as carbon sinks rather than carbon sources. This research aimed to determine the rate of organic carbon accumulation in agricultural ponds in relation to nutrients, CO₂ and CH₄ production processes, and emission routes from ponds in relation to physico-chemical conditions.

2 General materials and methods

2.1 Site descriptions

The sampling sites were located in Leicestershire, east of Leicester bordering Rutland, and contained three catchments: Eye Brook (Eye), Stonton Brook (Stonton) and Barkby Brook (Barkby) (Figure 8).

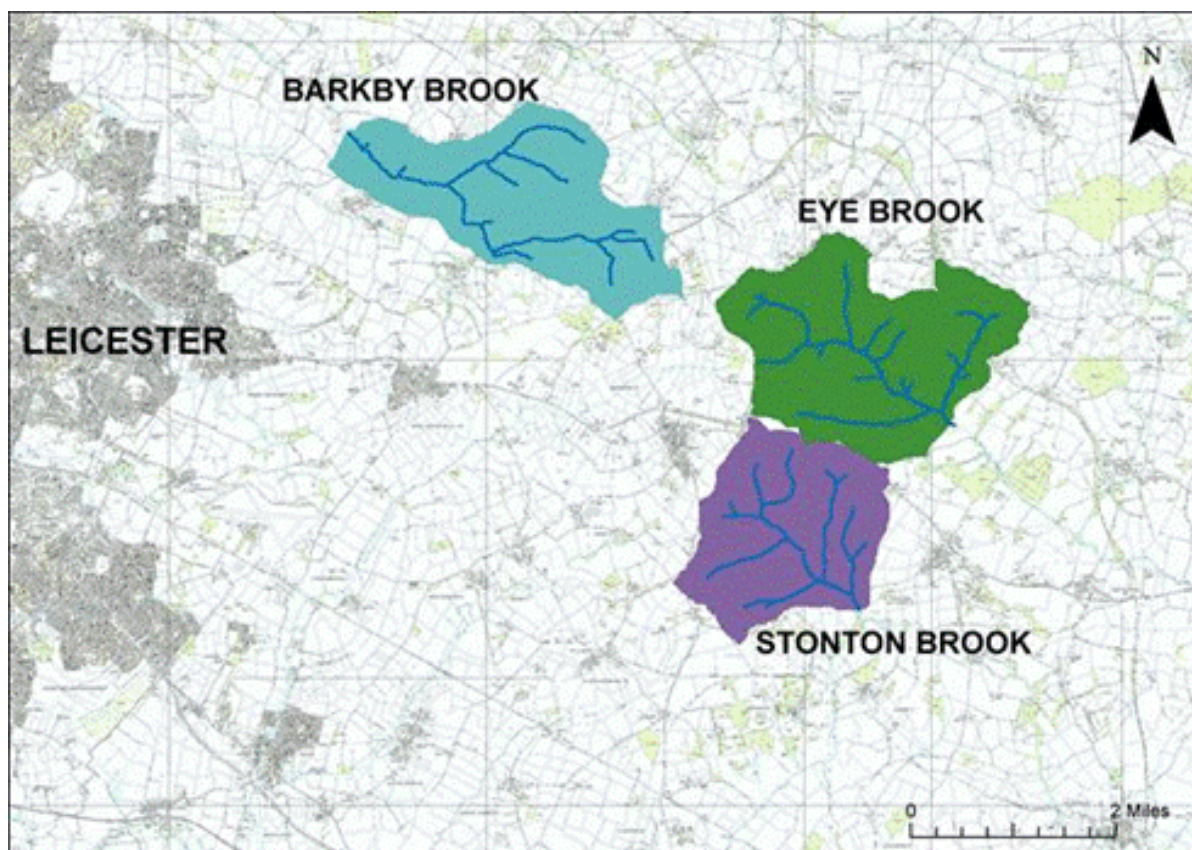


Figure 8: Map of catchment locations (courtesy of Freshwater Habitats Trust)

These catchments are in an agricultural environment characteristic of the English countryside, with a mixture of arable and livestock farming, and where some estate farms earn supplementary income from forestry and game shoots. The fields of the Eye and Stonton catchments are predominantly arable, in contrast to Barkby, which comprises a majority of pasture fields (Figure 9) for livestock grazing (mainly cattle, horses and sheep).

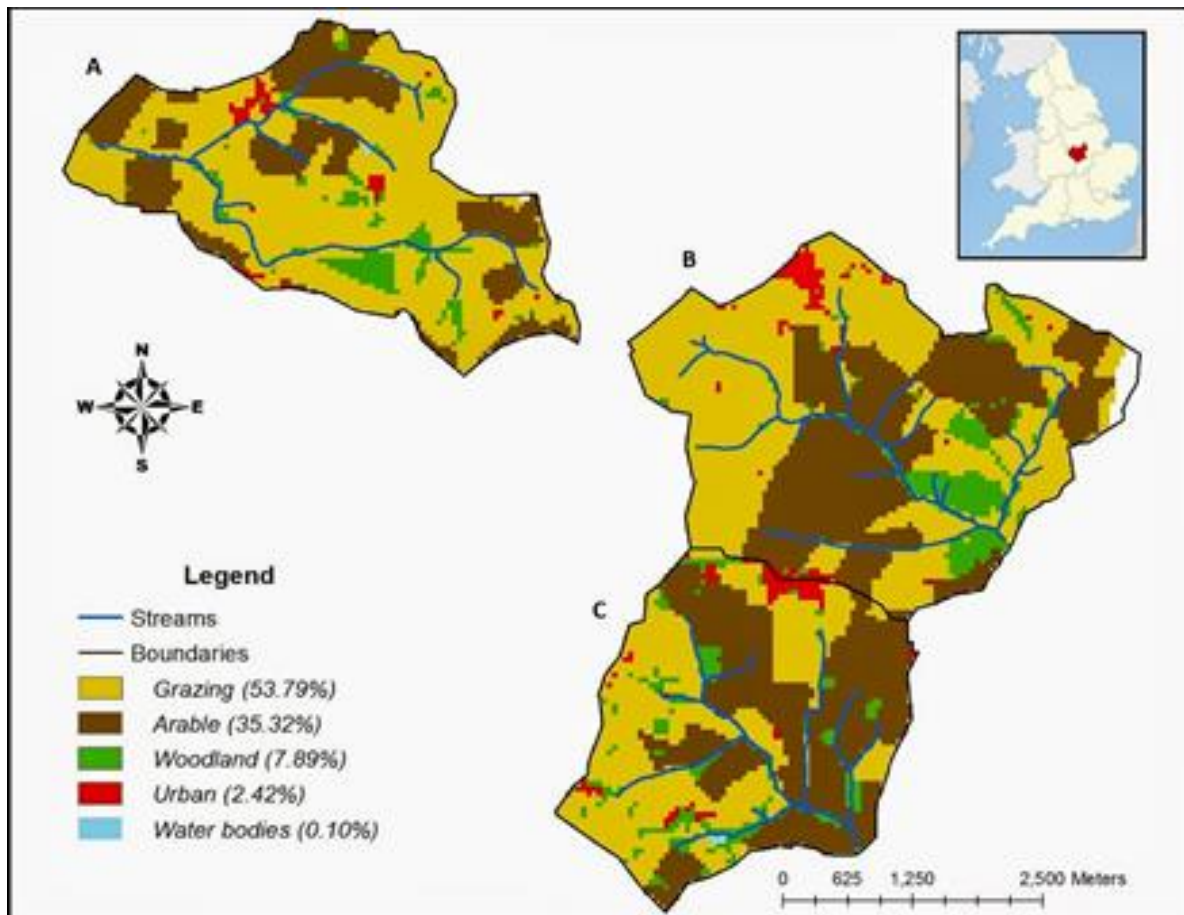


Figure 9: Land-use distribution of the three catchments — A: Barkby, B: Eye and C: Stonton (Biggs *et al.*, 2014).

The area in which these catchments are located is approximately 30 km², including the many tributaries of the three main streams, Eye, Stonton and Barkby. Within each catchment, 60 sampling sites in ponds, ditches and streams (20 each) were allocated by the FHT in collaboration with the Water Friendly Farming Initiative and the Allerton Project of the Game and Wildlife Conservation Trust. The FHT provided an overview of the state of the different water bodies in the three catchments in terms of ecology, environmental variables and biodiversity, which served as baseline data. Water samples, collected by FHT in Feb/March 2013, were sent to the National Laboratory for analysis of environmental variables; nitrogen, phosphorus, ammoniacal nitrogen, chloride, nitrite, conductivity, Turbidity, pH, suspended solids, aluminium, cadmium, chromium, copper, lead, nickel, zinc, calcium, iron, magnesium, sodium, and sulphate. FHT also carried out invertebrate and vegetation surveys as well as general assessment of the water bodies. Thereafter, there was an analysis of any conservation issues that emerged and, where feasible, implementation of mitigation measures related to landscape adjustment (digging/dredging ditches, creating interception ponds and nutrient traps).

The ponds in the catchments are predominantly bordering arable and/or pasture fields, with the exception of a few situated in forests or bordering roads. Throughout the area, ditches run along boundaries of arable fields that are, with a few exceptions, divided by hedgerows. The streams run through these agriculturally varying landscape features of the catchments. Most of the ponds were either fenced off or surrounded by dense vegetation and only a few ponds were accessible by livestock. Where livestock could access the ponds, the soft but compacting sediment had led to some animals drowning, which was apparent from the skeletons that emerged from the water over the years. With a few exceptions, the exact age of ponds is unknown however, some ponds are shown on maps dating back to the 1880s (Digimap, 1880). The majority of ponds carried water all year round, and most seasonal ponds were mainly situated in open fields.

Banks of the catchment streams were often deep and steep and at times showed severe erosion and bank collapse, some of which was a direct result of trampling by livestock. At other sites, bank erosion/collapse, most likely caused by flooding events, accelerated velocity, which then led to further downstream erosion.

Ditches were mostly shallow and some were overgrown with grass or stray arable plants (wheat, barley, oat), with very little water (at times less than 1cm in depth) and no detectable flow.

Despite being situated within a ± 5 km radius, the geology of the Eye, Stanton and Barkby catchments varied, with the bedrock consisting of Whitby Mudstone Formation (WHM), Oadby Member (ODT) and Dyrham Formation (DYS) and Charmouth Mudstone Formation (CHAM), respectively. The geology of Eye and Barkby river bed is DYS, and CHAM in the Stanton river bed (Digimap, 2015). ODT is classified as Jurassic rock, DYS is a ferruginous, silt and sandy mudstone, and CHAM is a siderite mudstone (British Geological Survey, 2015).

2.1.1 Site access permission

The sampling site locations belonged either to one of the three estates in the area or smaller private farms, and were all on private land. All site access permissions were organised by the Loddington based Allerton Project Office of the GWCT. Despite the permissions, some sampling sites were inaccessible due to restricted access dates and times or health and safety issues related to the University (Oxford Brookes) risk assessment.

2.1.2 Site access routes

Maps with site access routes as well as coordinates for GPS tracking were provided by the FHT. Most sites were only accessible by foot, hence the initial plan for in situ analysis failed

because of the weight of the ‘portable’ analyser set: the analyser, a quadrupole mass spectrometer (QMS), and battery weighed 55 kg. This issue became apparent after the initial field visit by the researcher and therefore the research questions and methodology had to be reassessed and adjusted.

2.2 Field work

The first fieldwork season for this project was carried out from 8 April - 22 June 2013, covering 98 of the original 180 sampling sites of the three catchment areas, and encompassing a mixture of water bodies of which 30 were ponds, 44 streams, and 24 ditches. The reduced number of sampling sites was due to an exceptionally dry spring, with ponds, ditches and streams either dried out or with not enough water to get a full sample.

Sites were accessed in stages according to access routes outlined in section 2.1.2 and coordinated to leave enough time to analyse samples the same day. Sampling was not stratified by waterbody type. Instead it was determined by following the most practical route as mapped.

After assessing the results from the first year of field work, which produced inconclusive results, it was decided to shift the second year of field work to late summer (3 August to 17 September 2014) in order to sample during a warmer season, with suspected elevated primary production, compared to the 2013 field season (April - June) where unusually cold and dry weather prevailed. There was also a decision to prioritise ponds in the second phase of field work, including ponds from year one as well as a few new established interception and wildlife ponds (32 ponds in total).

2.3 Baseline data

Baseline data were collected by FHT during a period of three weeks at the end of February and the beginning of March in 2011, 2012 and 2013. Due to a lack of funding, FHT was unable to establish baseline data from 2014 onwards. These samples were sent to the National Laboratory for analysis of water chemistry, environmental variables and heavy metals.

Data obtained from water samples analysed for dissolved CO₂ and CH₄, and the slope values of the air-flux samples were compared to the 2013 baseline data collected by Freshwater Habitats Trust using correlation half-matrix.

2.4 Environmental data

Environmental variables such as temperature (air, water and sediment), pH, and conductivity were recorded at each sampling site immediately prior to sampling, with a view to establishing whether these variables influence the carbon budget in the aquatic environment, and if so, how they influence it.

2.5 Sampling and analysing CO₂ and CH₄ from air and water samples

2.5.1 Background information

A major problem was encountered at the data analysis stage of the project, which resulted in a loss of confidence in the air-flux time series data, due to an unforeseen issue with the resolution of the analytical equipment. Therefore, the general nature of what was done will be described in principle, however the CO₂ and CH₄ air-flux data analysis from ponds, to compare catchment emission rates, could not be carried out.

2.5.2 Air flux samples

During the two field seasons, gas flux measurements in the three catchments were collected from 30 ponds in 2013 and from 32 ponds in 2014. Due to the unpredictable shifting of livestock to different fields and weather influences, some ponds were no longer accessible or had dried out during the 2014 sampling period. The 2014 sampling included five new interception ponds and one new wildlife pond, all of which were excavated and filled in 2013/2014.

Gas flux samples were collected by installing floating flux chambers, (Figure 10) adapted from Vachon, Prairie and Cole (2010), which consisted of a 5 L white plastic bucket with the bucket floor removed and two holes drilled in the lid: one for airing the headspace (placed near the rim) and one to attach a pipe (placed at the centre) to extract the samples using a 60 ml plastic syringes (BD Plastipak) fitted with a 3-way stopcock valve. A tight-fitting polystyrene ring was then mounted, with the base of the ring fixed at the 5 cm mark on the bucket, measured from the rim down, which amounted to a headspace volume of 1950 ml.



Figure 10: Installed floating air-flux chamber at Eye Brooke pond number 03 during the 2013 fieldwork.

The bucket was sealed with the lid, and the centre hole held a hollow rubber bung which served as a flux pipe (nylon) holder and sealant. The outer hole was used to establish ambient air condition within the flux chamber headspace, by removing gas that had accumulated by ebullition and/or diffuse flux while setting the flux chamber afloat. It was aired using a 60 ml syringe (BD Plastipak) fitted with a 3-way stopcock valve stuck through one of the holes in the lid and flushing the chamber with ambient air. This had to be repeated 40 times to ensure ambient air conditions in the 1950 ml head space ($1950 \text{ ml} / 60 \text{ ml} = 32.5$, however, to ensure complete flush with ambient air it was rounded up to 40, as at times the entire 60 ml were not flushed). Thereafter the hole was sealed with a rubber bung. Before each sample, 20 ml of the headspace was routinely taken with the syringe and discarded before a sample was taken, ensuring no contamination of the remaining air in the sampling tube. First a 'zero' sample was taken marking ambient air conditions in the flux chamber. Thereafter five samples were taken at 10 minutes intervals creating a time series. In total six syringes per samplings site were labelled with pond number and bucket code as well as the catchment colour scheme before setting out for the field. All samples were analysed in the laboratory for emissions of CO_2 and CH_4 using membrane inlet QMS.

2.5.3 Water samples

Water samples were obtained using 60 ml plastic syringes (BD Plastipak) at the water surface of ponds, streams, and ditches. Samples were also obtained at 15 cm depth in ponds only. During the 2013 sampling period (15 ponds, 17 streams, and 10 ditches) at Eye; 36 sites in total (6 ponds, 18 streams, and 12 ditches) at Stonton; and 20 sites in total (9 ponds, 9 streams and 2 ditches) at Barkby and from ponds at 15 cm only in the 2014 field season (32 ponds). Syringes were labelled with pond number and bucket code as well as the catchment colour scheme before setting out for the field. The samples were analysed for dissolved concentrations of CO₂, and CH₄ using membrane inlet Quadrupole mass spectrometer (QMS).

2.5.4 Quadrupole mass spectrometry (QMS)

All samples were analysed for CO₂, CH₄, N₂, O₂ and Ar using a membrane inlet QMS (QMS: European Spectrometry Systems, ESS, Northwich, UK, used in 2013, and VG Micromass, UK, used in 2014), which employs a set of four parallel rods for ion separation by mass/charge (m/z) ratio in a residual gas environment established by an internal vacuum system. The vacuum should be at least 5×10^{-4} mBar to work most effectively (e.g. if the vacuum drops, results distort due to increased molecule collisions). The detected mass/charge ratio is transmitted to a detector (Faraday plate and/or electron multiplier) and measured (European Spectrometry Services., 2014). The m/z ratio of 44 (CO₂), 15 (CH₄), 28 (N₂), 32 (O₂) and 40 (Ar), was used and measured concurrently. The external inlet, a stainless-steel tube of 2.5 mm internal diameter, was fitted with a SEM microscope gold grid (24 carat, 3.02 mm diameter), which served as membrane support to prevent the double-layered latex membrane from inverse bulging and/or bursting under high vacuum. Calibration of the QMS was conducted at the beginning, the end and during the course of analysis, when changes in PKR occurred. The QMS internal calibration was run, and analysis could only start if m/z of CO₂, CH₄, N₂, O₂ and Ar were measured at a maximum variation of ± 0.15 (amu) of the atomic weight.

To calibrate the QMS for air flux samples and headspace analysis, sets of 60 ml syringes were filled with either specialist calibration gas (CG) (CryoGas, UK) mixture (96% N₂, and 1% each of CO₂, CH₄, O₂ and Ar) or pure N₂ (CryoGas, UK). CG was fed slowly and as evenly as possible from the syringe, through a nylon pipe attached to the external inlet of the QMS. Before and after each application of CG or N₂, ambient air was flushed through using an ambient air-only syringe to re-establish ambient air conditions between and after calibrations. Air flux samples were then randomly analysed with ambient air flush after each sample using the ambient air-only syringe. Sample numbers and QMS cycle numbers were noted along with date and starting time of the analysis. During the measurement, PKR and any unusual

occurrence (e.g. peaks not related to samples or drops in PKR) were noted, with the corresponding cycle number. Between sample analyses the QMS was re-calibrated once drift occurred and/or PKR changed.

For the water samples, the QMS was calibrated using 60 ml syringes filled with 30 ml deionised (DI) water and 30 ml CG or N₂. The mixture was vigorously shaken for 20 seconds, allowing the CG or N₂ to dissolve from the headspace into the water, and thereafter the air was expelled. The same calibration and analysis protocol was applied as for the air flux samples except the ambient air flush was replaced by flushing with DI water. To avoid solids (sediment and vegetation) clogging the membrane, the syringes were placed in an upright position (spout up) so that acquired but unwanted solids would accumulate at the far end of the syringe spout (Figure 11) and were then slowly turned when ready to analyse, avoiding eddies disturbing the sample anew .



Figure 11: Water sample syringes in upright position to settle sediment and debris at far end from spout to avoid the sample from clogging the QMS sample inlet or puncture the membrane. Between water samples the inlet and membrane were flushed with deionised water (laying syringes with clear water) to in an attempt to remove sample residues.

The samples were randomly analysed, after sorting into separate groups of air flux, headspace and water samples, and then these piles were mixed (Figure 12). Air flux samples were analysed first, followed by headspace and then water samples. This was done to ensure similar analytical conditions throughout each sample medium, as changes in internal pressure would occur once the membrane was exposed to increased moisture content of the headspace and water samples.



Figure 12: Randomly arranged syringes of air-flux samples labelled with pond reference and sample number, for analysis with the quadrupole mass spectrometer.

2.5.5 Sediment analysis

To establish carbon input, organic carbon (OC) and inorganic (IC) content were measured by adapting the analytical loss on ignition (LOI) method of Heiri, Lotter and Lemcke (2001). Pond sediment was obtained using sediment traps, adapted from Nhan *et al.* (2006), collecting the accumulated sediment over a period of at least 3 weeks to then analyse the carbon input per day. Large funnels (21 cm diameter) attached to a 1.5 m bamboo cane were used as an outer structure and driven into the pond sediment, and the funnel rim was levelled with the top sediment layer (Figure 13). After the disturbed sediment had settled, a 0.5 L plastic bowl attached to a weight structure, to keep it in place while submerged, was placed into the funnel. The trap was left for at least 3 weeks and stored at 5°C after collection.

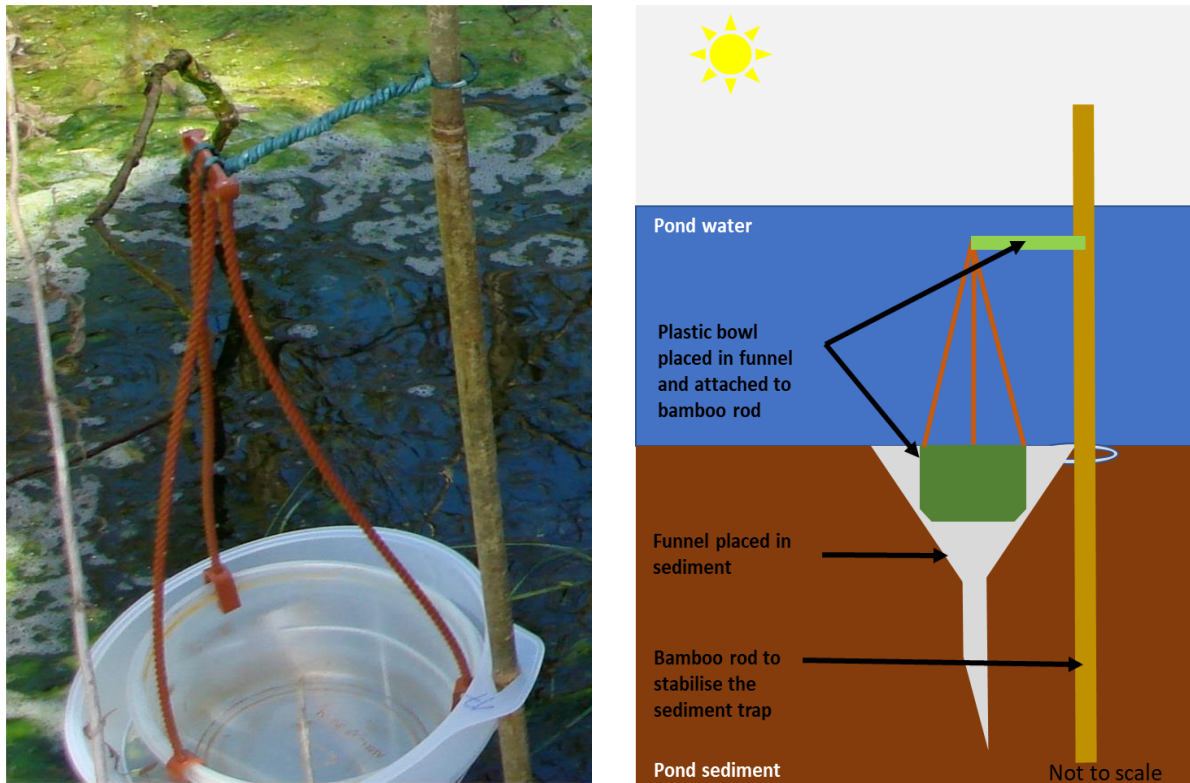


Figure 13: Sediment trap setting before installation in pond sediment and schematic drawing of installed sediment trap.

In the process, the sampling bowl, including lid, was weighed 3 times: while empty, before sampling; with the sample; and again empty, after sample transfer into a crucible.

For the LOI analysis, 1000 ml alumina crucibles were used, which could withstand temperatures of up to 1800°C. Due to the crucibles' porous outer surface, they were heated up to 100°C and then weighed, ensuring that moisture was evaporated before weighing. The spatula, used for sample transfer, was weighed before and after each sample transfer, as was the tissue used for absorption of potential spillage during sample transfer from the bowl into the crucible. To extract the entire sample, the bowl was rinsed with DI and the amount that was used was recorded and deducted during the calculation process for the initial sample weight. In a first drying step, the samples were placed on a sand-bath at 60°C until all moisture evaporated. Thereafter they were heated to 100°C and weighed. Combustion at 550°C and 950°C followed for determination of OC and IC, respectively, where 5 samples at a time were placed into the furnace (Carbolite, UK) and incinerated in two stages (Figure 14). After each stage the furnace would be cooled down to 100°C for weighing. Remaining potash after the last incineration step was transferred into sealable plastic pouches and labelled.



a)



b)



c)



d)

Figure 14: Sediment trap sample preparation for loss on ignition analysis; a) sample transfer from plastic bowl into crucible; b) crucibles placed on sand layered hot plate for drying at 100°C in order to c) evaporate the moisture content; d) sample residue after combustion at 550°C and 950°C.

For the calculation for LOI at 550°C for the proportional organic carbon content of the dried sample the following equation was used

$$LOI_{550} = ((DW_{100} - DW_{550}) / DW_{100}) * 100 \quad (1)$$

where LOI_{550} represents LOI at 550°C (%), DW_{100} represents the dry weight (g) of the sample post 100°C.

To determine the CO_2 content evolved from the carbonate content of the remaining sample after combustion at 550°C, the following equation was used

$$LOI_{950} = ((DW_{550} - DW_{950}) / DW_{100}) * 100 \quad (2)$$

where LOI_{950} represents LOI at 950°C (%), DW_{550} is the dry weight (g) after OC combustion at 550°C, DW_{950} is the dry weight (g) after IC combustion at 950°C and DW_{100} represents the initial dry weight (g) at 100°C before OC combustion. The proportion of $CaCO_3$ and CaO can also be determined by multiplying weight loss at 950°C by a factor of 2.274.

Compared to other methods for soil carbon analysis the LOI is a rather time-consuming method and with its numerous sample preparation steps and carries a much higher risk than, alternative methods like Laser Induced Breakdown Spectroscopy (LIBS) or Inelastic Neutron Scattering (INS). LIBS has sample analysis run times of about one hour; INS takes only a few minutes (Chatterjee *et al.*, 2009). However, due to high acquisition costs, neither the LIBS nor the INS analysers were available at the researcher's institution.

2.6 Data analysis

For the data analysis a regression analysis was then carried out using SPSS software.

3 Lentic and lotic systems in an agricultural environment

3.1 Introduction

Lotic systems are defined by a unidirectional downhill flow with no water column stratification due to recurrent mixing which enables continuous aeration. The lotic benthos is an important habitat for the majority of river organisms due to an abundance of nutrients from mainly allochthonous origin (Wetzel, 2001). In contrast, lentic systems are bodies of water with a stationary appearance and their flow rate depends on river influx and outflux velocity which is influenced by slope gradient and width of the river bed. The epilimnion, the surface part of the lentic water column, is host to the majority of aquatic organisms (Wetzel, 2001). Both of these systems are immensely important for transport and storage of nutrients for aquatic organisms. However, rivers, streams, lakes, and ponds are also exposed and transport polluting compounds from a number of sources (run-off from farming land, efflux from industrial plants and sewage treatment plants, etc.) which can pose a threat to these habitats and the wider ecosystem with at times long-lasting consequences (Bunzel, Kattwinkel and Liess, 2013; Berger *et al.*, 2017).

3.2 Aims

The terrestrial landmass is thought to be a major greenhouse gas sink (GHG), and while freshwaters were thought to be neutral entities, they may in fact be potent producers and emitters of CO₂ and CH₄. Little is known about their contribution to the global carbon budget, especially in agricultural landscapes. This research aimed to determine the rate of organic carbon accumulation in agricultural ponds in relation to nutrients, and to estimate CO₂ and CH₄ production processes in and emission routes from ponds in relation to physico-chemical conditions. In order to obtain data for the aforementioned objectives, samples were obtained to ascertain CH₄ and CO₂ concentrations in, and fluxes from, a number of agricultural impoundments as well as sedimentary carbon inputs. In addition, pH, depth, age, size and temperature were recorded for each sampling location to gain an initial characterisation of variation in water chemistry at each location.

3.3 Materials and methods

3.3.1 Fluxes from ponds

The sampling sites were located in Leicestershire, east of Leicester, and contained three catchments: Eye Brook (Eye), Stonton Brook (Stonton) and Barkby Brook (Barkby). These were in an agricultural environment characteristic of the English countryside, with a mixture of arable and livestock farming, and where farmers earn supplementary income from forestry and game shoots. The fields of the Eye and Stonton catchments (Figure 9) are predominantly arable in contrast to Barkby, which comprises a majority of pasture fields (cattle, horses and sheep).

The area from which these catchments are fed is approximately 30 km², including the many tributaries of the three main streams (Figure 8). The ponds in the catchments were bordering arable and/or pasture fields, with the exception of a few situated in forests, or bordering roads. The first fieldwork was carried out from 8 April to 22 June 2013, covering 98 of the original 180 sampling sites of the three catchment areas, and encompassing a mixture of water bodies: 42 sites in total (15 ponds, 17 streams, and 10 ditches) at Eye; 36 sites in total (6 ponds, 18 streams, and 12 ditches) at Stonton; and 20 sites in total (9 ponds, 9 streams and 2 ditches) at Barkby. Restricted access, livestock and drought limited the number of possible sampling sites. Data from 30 ponds, 44 streams and 24 ditches were collected and analysed.

At each pond, one floating chamber was installed and sampled using a 60 ml syringe (BD Plastipak) fitted with a 3-way valve. Before the 60 ml sample was taken, a 20 ml sample was extracted and discarded to clear the sampling tube of previous sample concentrations. An initial zero sample was taken immediately after ambient air conditions were established by flushing the tube in the headspace a number of times using a 60 ml syringe (BD Plastipak). Thereafter, samples were extracted at intervals of 10 minutes over the course of an hour. All samples were analysed in random order for CO₂ and CH₄ concentrations using QMS in the laboratory.

3.3.2 Water samples: comparison between different water bodies

One water sample was taken at each sampling site, at the water surface (30 ponds, 44 streams and 24 ditches) using 60 ml syringes (BD Plastipak) fitted with 3-way valves. In ponds, a second sample was collected at a depth of 15cm. The samples were analysed in the laboratory on the day of sampling for dissolved concentrations of CO₂, and CH₄ using QMS.

3.3.3 Carbon input

Sediment traps were installed at 29 ponds and collected after 21 days. Once back in the laboratory, they were analysed for OC and IC content, adapting the LOI method of Heiri, Lotter and Lemcke (2001) (see section 2.5.5).

3.4 Results

3.4.1 Air-flux samples of CO₂ and CH₄ emissions from ponds

A major problem, encountered at the data analysis stage of the project, resulted in a loss of confidence in the air-flux time series data. This is thought to be because of an unforeseen issue with the resolution of the analytical equipment. Due to this, the CO₂ and CH₄ air-flux data analysis from ponds, comparing catchment emission rates, could not be carried out. The coefficient of variance calculation (Appendix E) of the air-flux sample data for both, CO₂ and CH₄, of the Eye catchment revealed a very high variance (the coefficient of variance was at an average 40% of the standard deviation of 84 samples with none below 10%) of the sample data which underlined the loss of confidence in the data.

3.4.2 Dissolved CO₂ and CH₄ in water samples

To test whether there was an overall significant difference of concentrations of CO₂ and of CH₄ in the data comparing system types (lentic and lotic, Figure 16) within the three catchments (Figure 15) Kruskal-Wallis test was used, because data distributions were resistant to normalisation and ANOVA computed using transformed data resulted in a model with skewed residuals. A Kruskal-Wallis test revealed significant differences between catchments in both, CO₂ and CH₄ concentrations amongst the three catchments (CO₂: $H_{(corr)}=4.600$, $p=0.003$, $N=129$, $df=2$ and CH₄: $H_{(corr)}=11.600$, $p=0.0003$, $N=129$, $df=2$). To establish which of the catchments differ, the Mann-Whitney U Test was applied as a post-hoc test. The median latencies outcome indicates a significant difference between both CH₄ and CO₂ water samples from Eye and Stonton, and Eye and Barkby catchments. Tests statistics for CH₄ were as follows: ($U=1698$, $U_{larger}=634$, $n_1=53$, $n_2=44$, $p=0.0001$, $Z=3.855$ two-tailed test). For CO₂ differences the test statistics were as follows: ($U=1145$, $U_{larger}=551$, $n_1=53$, $n_2=32$, $p=0.007$, $Z=2.693$ two-tailed test). Water sample CO₂ and CH₄ from Stonton and Barkby catchments were not significantly different using Mann-Whitney U test. Comparing median CO₂ between catchments indicated no significant differences between Eye and Stonton. The median CH₄ at Barkby was significantly different from Eye: ($U=1159$, $U_{(larger)}=537$, $n_1=53$, $n_2=32$, $p=0.004$, $Z=2.82$ two-tailed test); and Stonton versus Barkby: ($U=984$, $U_{(larger)}=424$, $n_1=44$, $n_2=32$, $p=0.003$, $Z=2.94$ two-tailed test).

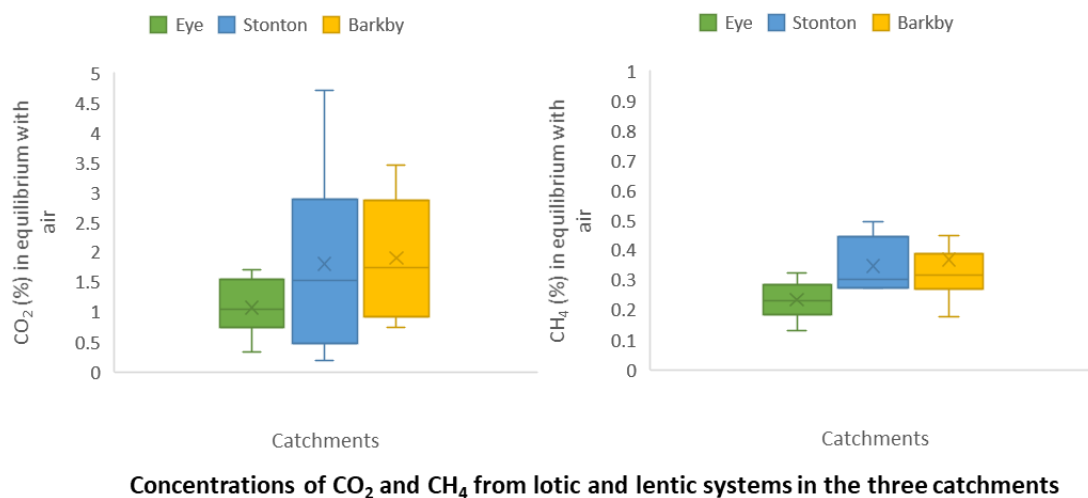


Figure 15: Comparison of dissolved CO₂ and CH₄ concentrations in all system types (ponds, streams and ditches) in all three catchments (n=98). Whiskers represent minimum and maximum values.

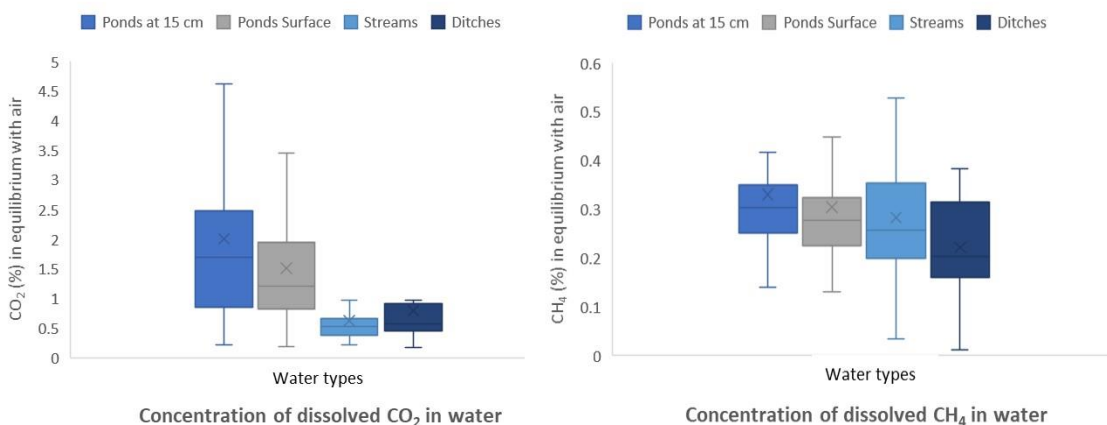


Figure 16: CO₂ and CH₄ concentrations in water samples across all three catchments. Whiskers represent minimum and maximum values.

Comparing different aquatic systems present in the three catchments indicated higher concentrations of carbon dioxide in ponds at 15 cm depth and surface water than in the ditches and streams. Methane showed no significant variation between systems.

3.4.3 Carbon input

Data distributions were not normal, resulting in skewed residuals using transformed data in ANOVA. Instead, the non-parametric Kruskal-Wallis test was applied, which indicated that the mean values for OC and IC among the 3 catchments (Figure 17) were significantly different ($H_{(corr)}=6.53$, $p=0.038$, $N=58$, $df=2$, and $H_{(corr)}=7.51$, $p=0.023$, $N=58$, $df=2$, respectively). Mann-Whitney test results showed a significant difference for OC in Eye vs.

Stonton ($U=92$, $U_{(larger)}=220$, $n_1=26$, $n_2=12$, $p=0.044$, $Z=2.00$) and Eye vs. Barkby ($U=160.5$, $U_{(larger)}=359.5$, $n_1=26$, $n_2=20$, $p=0.027$, $Z=2.20$), whereas Stonton and Barkby were not significantly different. There was a significant difference between Eye and Barkby for IC ($U=149$, $U_{(larger)}=372$, $n_1=26$, $n_2=20$, $p=0.013$, $Z=2.48$) and Stonton and Barkby ($U=175.5$, $U_{(larger)}=64.5$, $n_1=12$, $n_2=20$, $p=0.03$, $Z=2.16$). There was no significant difference between Eye and Stonton.

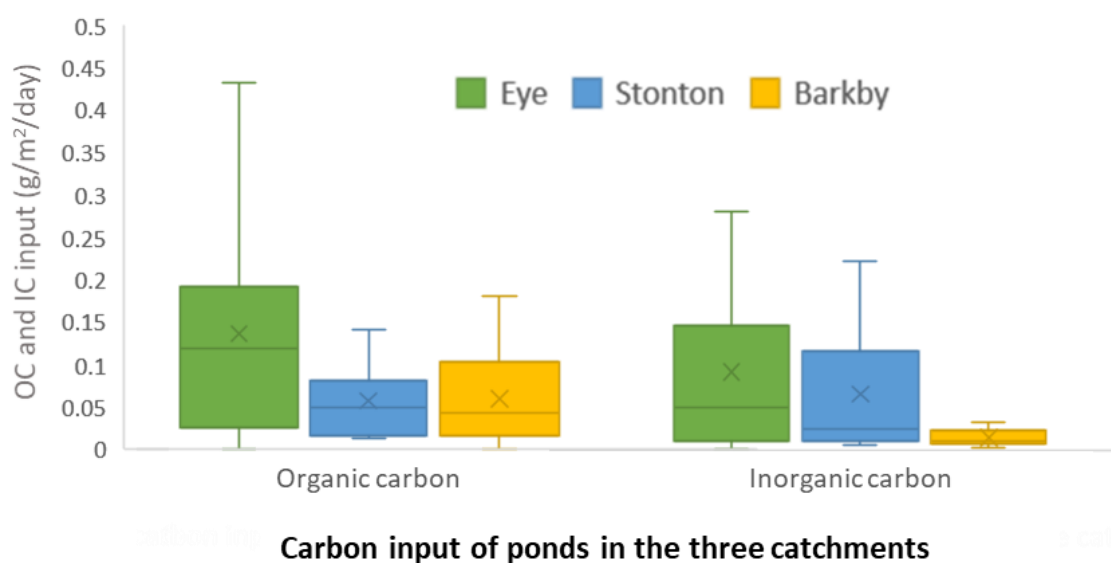


Figure 17: Comparison between OC and IC input in the 3 catchments (Eye: $n=26$, Stonton: $n=12$, Barkby: $n=20$). Whiskers represent minimum and maximum values.

A regression analysis (SPSS) was then carried out on CO_2 and organic carbon input. The regression was significant ($p=0.016$, $F=5.085$, $R=0.571$, $R^2=0.326$), therefore approximately 33% of CO_2 emission is dependent on organic carbon ($t=2.377$, $p=0.027$). The Durbin-Watson statistical test was 1.826, which corresponded to the SPSS model outcome. Although this test is used for time series, it is included in the SPSS analysis output as a useful indicator of autocorrelation between samples (ideal values are close to 2). Model residuals were randomly distributed. OC and IC were not significantly correlated with CH_4 emissions ($p>0.05$, two-tailed test, Pearson's Product Moment Correlation). Data were normally distributed.

3.4.4 Correlation analysis of baseline data and CO_2 and CH_4 data from ponds

The half-matrix analysis was carried out to find out how the data of the measured concentrations of CO_2 and CH_4 dissolved in, and emitted from, ponds would correlate to the baseline data.

CO₂ Water

0.232 CH ₄ Water 0.525 0.301 CO ₂ Slope Air Flux -0.012 -0.199 -0.043 CH ₃ Slope Air Flux -0.009 0.001 -0.257 0.278 BOD 5 Day ATU mg/l 0.385 -0.022 0.084 -0.196 -0.101 Nitrogen : Total as N mg/l 0.004 -0.111 -0.158 0.366 0.902 -0.191 Phosphorus : Total as P mg/l -0.110 -0.078 0.019 -0.163 0.006 -0.027 0.024 Ammoniacal Nitrogen as N mg/l 0.090 -0.148 0.032 0.213 0.331 0.087 0.270 -0.134 Chloride mg/l -0.138 -0.060 -0.250 -0.082 -0.113 0.173 -0.016 0.311 -0.067 Nitrite as N mg/l 0.378 -0.017 0.081 -0.199 -0.158 0.993 -0.254 -0.121 0.104 0.146 Nitrogen : Total Oxidised as N mg/l -0.104 -0.190 -0.241 -0.282 0.120 0.437 0.046 0.475 0.412 0.385 Conductivity at 20°C µS/cm -0.090 0.024 -0.042 0.490 0.373 -0.213 0.432 -0.129 0.157 0.170 -0.220 -0.275 Turbidity NTU 0.349 -0.041 -0.588 0.001 0.097 -0.178 0.093 0.083 0.082 0.133 -0.188 0.323 -0.341 pH 0.265 0.392 0.316 0.028 0.048 0.226 0.020 -0.312 -0.036 -0.025 0.242 -0.164 0.498 -0.492 Solids, Suspended at 105°C mg/l -0.105 -0.114 0.010 0.372 0.034 -0.129 0.053 -0.031 -0.103 0.053 -0.125 -0.344 0.694 -0.401 0.200 Aluminium µg/l -0.097 -0.164 0.037 0.353 0.010 -0.098 0.052 -0.078 -0.070 0.030 -0.087 -0.395 0.658 -0.446 0.120 0.971 Chromium µg/l 0.235 0.335 0.234 -0.212 -0.183 -0.092 -0.153 -0.173 -0.300 -0.235 -0.072 -0.371 0.059 -0.199 0.281 -0.002 -0.008 Copper µg/l -0.180 -0.116 -0.111 0.265 -0.030 -0.056 -0.026 -0.052 -0.004 0.081 -0.041 -0.273 0.637 -0.223 0.119 0.902 0.924 0.034 Lead µg/l -0.017 -0.014 0.370 0.367 0.048 -0.230 0.097 0.077 -0.196 -0.079 -0.245 -0.422 0.443 -0.622 0.074 0.675 0.672 0.128 0.410 Nickel µg/l 0.175 -0.010 0.414 0.532 0.037 -0.158 0.064 -0.130 0.061 -0.106 -0.154 -0.323 0.464 -0.478 0.273 0.590 0.517 -0.085 0.324 0.682 Zinc µg/l -0.173 -0.133 -0.191 -0.299 0.011 0.435 -0.050 0.337 0.306 0.408 0.403 0.939 -0.241 0.304 -0.013 -0.362 -0.426 -0.376 -0.283 -0.500 -0.273 Calcium mg/l 0.048 -0.174 -0.058 0.377 0.407 -0.167 0.513 0.187 -0.074 0.270 -0.220 -0.120 0.730 -0.337 0.144 0.689 0.643 -0.088 0.494 0.576 0.465 -0.186 Iron µg/l -0.205 0.068 -0.182 -0.302 -0.018 -0.067 -0.052 0.539 -0.243 0.149 -0.117 0.462 -0.335 0.172 -0.099 -0.238 -0.295 -0.072 -0.254 -0.106 -0.345 0.328 -0.138 Magnesium mg/l 0.177 -0.073 0.098 0.126 0.387 0.070 0.323 -0.080 0.954 -0.094 0.076 0.372 0.108 0.066 0.005 -0.189 -0.162 -0.220 -0.091 -0.232 -0.026 0.329 -0.101 -0.157 Sodium mg/l -0.306 -0.231 -0.235 -0.253 -0.174 0.227 -0.241 0.453 -0.059 0.205 0.198 0.657 -0.377 0.170 -0.361 -0.094 -0.099 -0.353 -0.050 -0.118 -0.279 0.588 -0.176 0.543 -0.050 Sulphate as SO ₄ µg/l 0.378 -0.017 0.081 -0.199 -0.157 0.992 -0.254 -0.121 0.104 0.145 1.000 0.385 -0.220 -0.188 0.242 -0.125 -0.087 -0.071 -0.041 -0.245 -0.154 0.402 -0.220 -0.117 0.076 0.197 Nitrate as N	n=27 P 0.050 0.010 0.001 27-2= 25 r 0.381 0.487 0.597
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3.5 Discussion

The air flux results yielded plots with positive, negative and nearly neutral straight lines, but the data points were widely scattered and returned weak r^2 values. This eliminated the possibility that straight lines would reliably indicate an increase of concentration over time. To investigate this, the first step was to query the sampling methodology, and to test it using the next set of data from year 2 fieldwork. The outcome of this investigation was inconclusive, and strongly suggested that the problem lay not with the sampling methodology, but with the analytical method underpinning all the measurements. The next step of the investigation involved reanalysing the data from both year 1 and year 2, and employing LINEST (Figure 19) as a function (Excel), calculating linear least squares to establish uncertainty estimates.

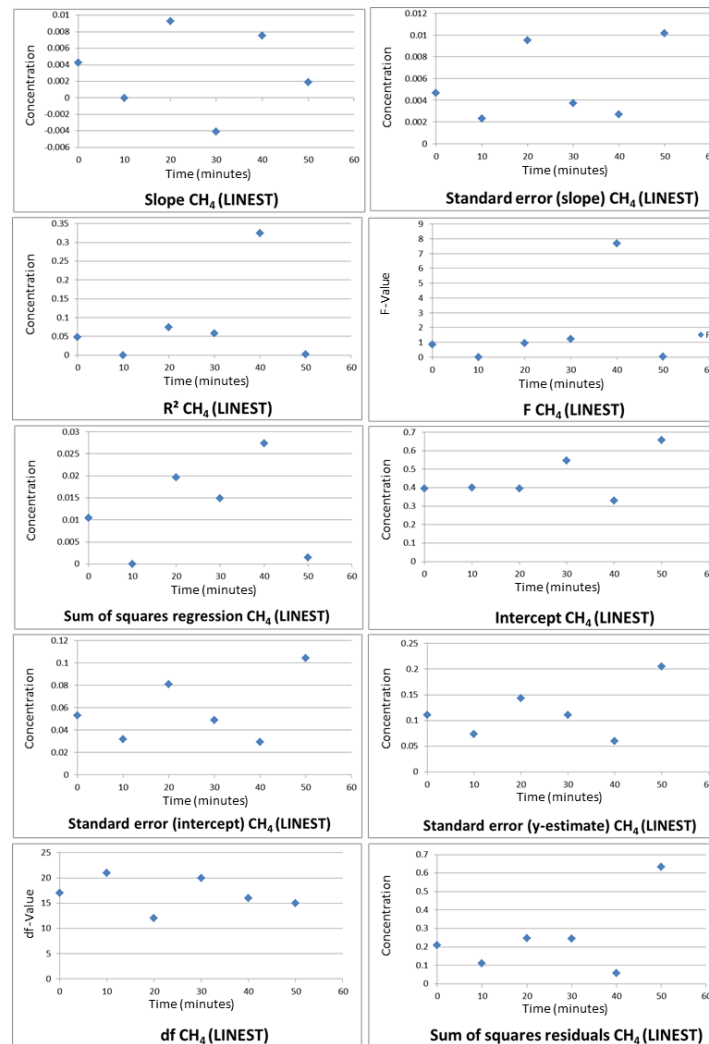


Figure 19: Plotted LINEST results of CH₄ emission data of pond 1 in the Eye Brook catchment.

The example of the plotted LINEST results from the CH₄ emission data of pond 1 in the Eye Brook catchment show unreliable r^2 values which represent the pattern found in the vast majority of the analysed air-flux data. The coefficient of variance calculation (Appendix E) on the air-flux sample data for both, CO₂ and CH₄, of the Eye catchment revealed a very high variance (the coefficient of variance was at an average 40% of the standard deviation of 84 samples with none below 10%) of the sample data which underlined the loss of confidence in the data.

In the final analysis, it became apparent that the detection level of the analytical method was not adequate: a better resolution was needed to detect lower concentrations of CO₂ and CH₄ than the analyser was capable of measuring.

Initially the lowest values of the time-series raw data representing nitrogen, argon, oxygen, CO₂, and CH₄ were deducted. As each analysed sample consisted of several data points, the first four measurements were excluded from the data analysis. This was done to avoid noise

derived from cleaning the membrane with ambient air applied between samples. The time-series data were analysed in three sets, using maximum, median and minimum values to establish whether the r^2 results would improve using a different combination of the data.

In a truly mathematical sense, a straight line is defined by its gradient and its intercept. Hence, lines with positive gradient run from lower left, up to higher right. Conversely, lines with a negative gradient run from higher left to lower right. The intercept (C) is the point where the line crosses the y-axis. These controls are called parameters, and the equation for a straight-line is $y=mx+C$, where m is the gradient, and C is the intercept (Figure 20).

Straight-lines were used to model the rise in concentration of methane or carbon dioxide with time, accumulated in the flux chamber installed on each pond used in this study. Indeed, the diffusion of these gases from a source such as a water body, into the air in the flux chamber can be derived from theory, as following a straight line. In this case the intercept is the ambient air concentration at the beginning of measuring air flux, and the gradient is the increase or decrease of the accumulated concentration over time. For a run starting with zero concentration of gas in the flux chamber, the intercept will be zero. If some of the gas exists already in the atmosphere of the collector, there will be an intercept.

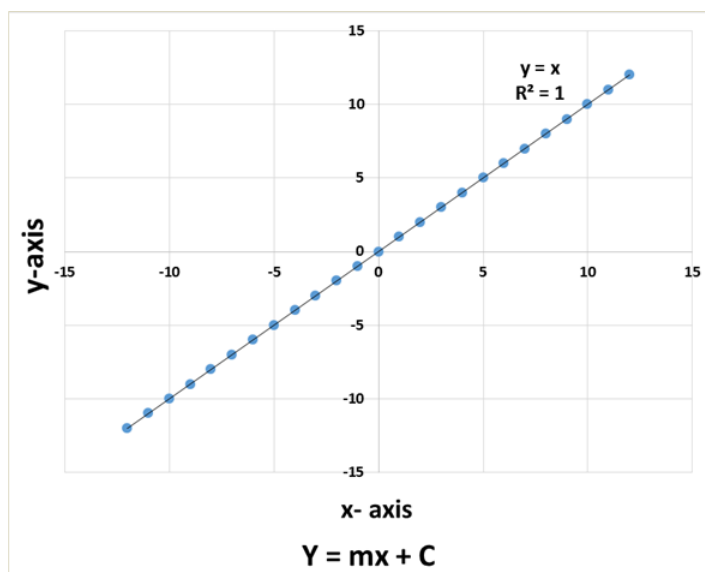


Figure 20: Plotted straight line of the equation $y=mx+C$

The goodness of fit to $y=mx+c$ decreases the greater the scatter (Figure 21 and Figure 22), and finding a reliable gradient (m) to calculate the intercept (c) decreases the further distance from the straight line the scatter is spread. The trend line on a 2D scatter plot can help to give an indication of whether the plotted ordered variables are correlated and, if so, in a positive, negative or no-trend display. The trendline can be used to predict further data points and at times it is sufficient to approximate the data without calculating the exact data points.

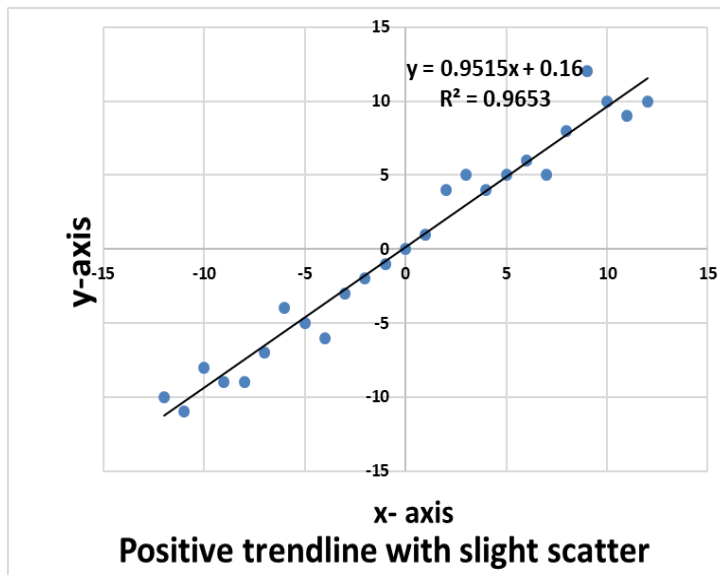


Figure 21: Plotted straight line with some minimal scatter with a strong r^2

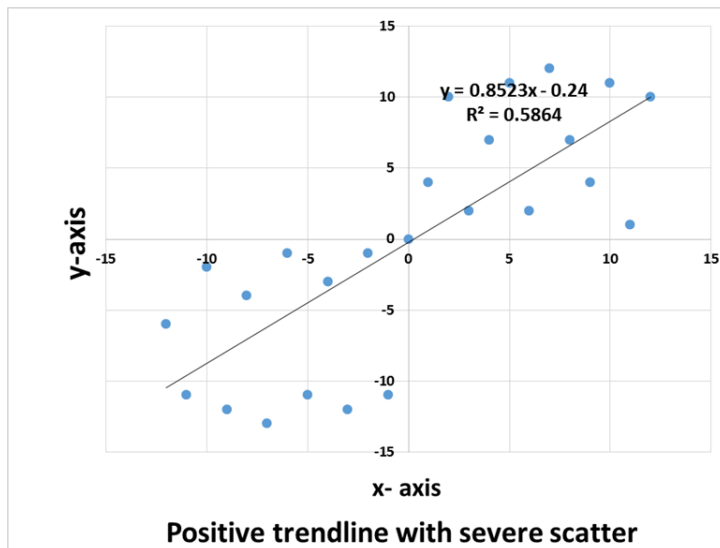


Figure 22: Plotted straight line with severe scatter with a weak r^2

This phenomenon explains the problem that occurred in the data for the pond air-flux samples of this study. The scatter graphs plotted for the air-flux samples show a very diverse scatter with, at times, negative sloping trendlines. The scatter in the data resulted in very low r^2 values in all but one graph of the air-flux samples. The majority of the air flux results resemble the graphs in Figure 22 and very few had straight lines similar to the one in Figure 21. The graphs of ponds number 8 and 18 (Figure 23) in the Eye Brook catchment are exemplars of the many air-flux samples with a very similar scatter pattern.

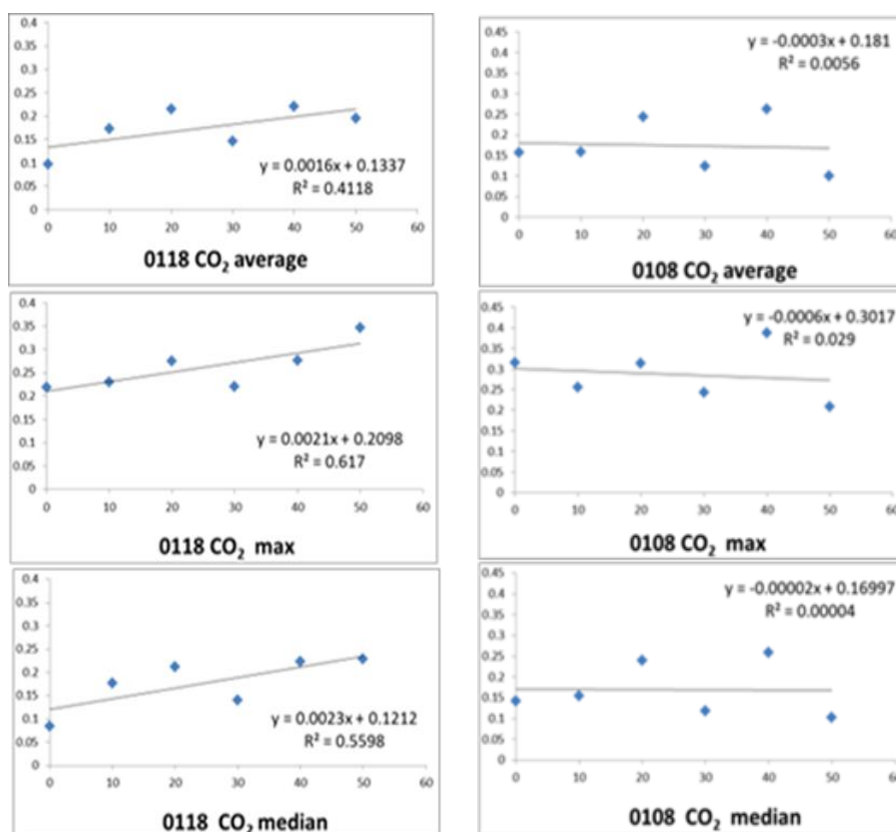


Figure 23: Examples of analysed data points of air-flux samples from pond 8 and 18 of the Eye Brook Catchment for CO₂, representing the many graphs created with most of them showing severe scattering (y-axis = Concentration of CO₂ in ppm; x-axis = time).

3.5.2 Water samples

The significant differences between CH₄ concentrations in water samples from the Eye catchment compared with those from Barkby and Stonton may be traced back to the influence of one pond (No. 01018) in the Eye catchment, which was an outlier (high CH₄). However, no conclusive explanation can be given for these high emissions since this pond was the middle pond of three cascading ponds (Nos. 01017, 01018, and 01019). These ponds were surrounded by arable fields, an orchard plantation and a grass field (not grazed). Visual influx was into pond 01017, from what looked like a slow running subterranean well or seepage from the top field (grass). Ponds 01018 and 01019 were consecutively feed from cascading water of pond overflow. All three ponds were surrounded by shrubs and small trees, with the densest growth around pond 01019. Pond 01018 seemed not particularly to differ in its appearance from ponds 01017 and 01019, and further investigation is needed regarding the aforementioned problem with the analytical method.

3.5.3 Carbon input

The loss on ignition (LOI) analysis was carried out to compare LOI data with the air-flux and water data, in terms of organic matter input over a period of time vs. CO₂ and CH₄ emission

rates from, and dissolved CO₂ and CH₄ in water. However, on a standalone basis the data retrieved just state the status quo of organic input twice over a three-week period during the growth season. Over all three catchments, OC input exceeds IC input (Figure 17). The comparison between OC and IC input in the three catchments (Figure 17) indicates higher inputs of OC compared to IC. The Kruskal-Wallis test and Mann-Whitney U-tests confirmed that the initial observations of higher carbon inputs in the Eye catchment were significant, with higher OC than IC. Stonton had slightly lower OC than IC levels, whereas in the Barkby catchment OC input was about three times higher than IC. The higher OC input vs. IC input could have its origin in that most of the ponds were surrounded by trees and shrubs which could account for increased organic matter accumulation in the pond sediment and could play an important role for carbon burial (Downing *et al.*, 2008). The overall lower IC input might be derived from limited sediment influx from streams, as only seven ponds in the three catchments could be identified as being fed by stream influx amongst other inflow. All other ponds in the study area are fed by a mix of drainage/seepage from neighbouring fields and/or underground wells. Further, sediment run-off from the shore line might be limited in ponds surrounded by shrubs and trees, as their root system would, to a certain extent, prevent erosion, and will also act as a natural fence inhibiting livestock disturbing the shore line.

A recent paper by Prairie *et al.* (2018) states that the carbon burial to emission relationship is assumed more complex than previously thought and would need further investigation. This is especially true in places with transitioning agricultural landscape management, such as the Eye Brook catchment, where catchment conscious farming practices are implemented.

3.5.4 Correlation half-matrix

Some correlation between CO₂ water (dissolved in) and nitrogen would be expected as both play an essential role in the growth of pond flora, algae, and phytoplankton (Lilkanen and Martikainen, 2003; Brooker, Bohrer and Mouser, 2014) and CH₄ water (dissolved in) and nitrogen at the decay stage of pond plant organisms (Bridgham *et al.*, 2013). The correlation of CH₄ water (dissolved in) and suspended solids, and CH₄ slope air-flux with turbidity would strongly point to an anoxic environment possibly caused by low light penetration slowing or even inhibiting photosynthesis (Bretz and Whalen, 2014). The CO₂ and CH₄ slope air-flux and zinc correlation is interesting as CH₄ production could be a result of a photocatalytic reaction of CO₂ and zinc oxide-copper(I) (Bae *et al.*, 2017). A correlation between CO₂ water (dissolved in) and CO₂ slope air-flux would be expected.

3.6 Recommendations to improve the methodology

3.6.1 Loss on ignition

Collect samples in spring, when leaf litter is at its lowest, and in autumn, at the height of leaf litter.

Use sample trays with a minimum of 1.5 L capacity, and to prevent collecting shore soil run-off, place them at least 2 metres away from the shore but near to where air flux and water samples will be sampled.

Just before installing sediment traps and the temperature and conductivity data logger, ‘zero’ the pond by taking air flux and water samples, and samples for N and P values as well as environmental variables (e.g. air and water temperature, pH, conductivity, wind speed, in- and outflow, distance from a ‘given’ mark (tree, fence post, boulder etc.) to the shore-water boundary, general water condition, vegetation, and observed pond fauna).

Leave traps for three weeks and take air flux and water samples before removing the traps (avoiding any disturbance). Thereafter take air flux and water samples for analysis every week until autumn when traps are installed again for three weeks. Repeat air flux and water sampling until spring or until the anniversary of the date when the traps were first installed.

During that time take regular measurements of the temperature (air and water), pH, conductivity, P and N. For ponds with a direct run-off from adjacent arable fields, request a spray schedule from the farmer showing dates when fertilisers, fungicides, herbicides and pesticides were sprayed. Obtain the name of the products used. For adjacent fields with livestock grazing, find out the numbers and kinds of livestock in the fields (sheep, cows, calves, horses etc.), and the rotating schedule showing how long the livestock was in the field.

For all adjacent fields, check the soil type to establish the likeliness of run-off.

Note the distance of arable fields’ herbal leys to the water course in fields adjacent to the pond. Check whether pasture fields are fenced off towards the water course in fields adjacent to the pond.

List numbers, species and size of trees and shrubs and other prevalent vegetation around the pond with direct leaf litter input. Note whether livestock can access the pond for drinking. Establish the size and depth of the pond and register the shallowness of the shore. Test the base of the pond (e.g. clay, gravel, man-made (concrete, liner etc.) or mud). Establish the shade pattern: how much sunlight penetrates the canopy, if present. Register in-pond growth such as algae, duck weed and water plants, as well as mammals (otters,

water voles etc.), water birds (ducks, geese, swans, moor hens, herons etc.), fish, newts and invertebrates.

Establish whether there are surface and subsurface wells feeding into the pond, and if possible, measure their temperature, pH and conductivity.

3.6.2 Sampling with flux chambers

Flux chambers should be a one-piece bucket (not a sawn-off base with attached lid), as a fastened lid would still not count as being airtight. The bucket should only have one hole cut out, with a rubber stopper tightly fitted where an inlet pipe can be attached. The inlet pipe should be, ideally, about 1.5 m longer than the distance from the chamber to the shore, for ease of obtaining the sample.

It is best to install the chamber using an extendable rod to let the rim submerge 3-5 cm (Cole *et al.*, 2010) aided by a fitted buoyancy ring. It is not necessary to flush the chamber with ambient air, as less disturbance occurs using the extendable pole than standing in the pond to place the chamber, and a 'zero' sample is taken for reference after installing the chamber. When taking a sample, a three-way valve should be attached to the tube and another to a syringe (glass for gas sampling) to then connect them. First, a sample is taken that is equivalent to the volume of the pipe and discarded by adjusting the three-way valve to expel it into the ambient air (not back into the chamber). Then a 25 ml sample is taken, with the valve closed immediately so that no air can escape from the tube or the syringe. After the syringe is detached (with the valve) and a hypodermic needle attached, the sample is injected into the labelled storage vial (Wheaton vials with rubber septa and aluminium crimp).

3.6.3 Analysis of dissolved CO₂ and CH₄ in water

Obtain water samples using a syringe at a depth of 15 cm down the water column with an attached 15 cm-long tube equipped with a small grid filter (≤ 1 mm). Sampled water should then be transferred into light-blocking vials, avoiding production continuum (add bactericide if necessary; e.g. if samples are not analysed the same day). The samples should be analysed within a few hours of collection.

4 Milham Ford Nature Park experiment

4.1 Background/introduction

The floating chamber method for assessing CH₄ and CO₂ emission from aquatic environments can incur problems, especially when the water body lacks depth and size to sample from a dinghy. This is the case for the ponds located in the three catchments (near Loddington, Leicestershire) where the main research of this work took place. Occasionally, to set the flux chambers afloat, it was not possible to avoid stepping into the pond and disturbing the sediment. This is not a major issue with new clayey ponds as the sediment is firm due to little or negligible organic matter accumulation. However, in old ponds with a substantial layer of organic matter the sediment is rather deep and very muddy. In such ponds, stepping into the pond to take samples is suspected to disturb and release sedimentary and water column CH₄ and CO₂, which is likely to have an influence on the measurements obtained from the floating chambers. To avoid these ebullitive emissions being recorded in the initial measurement, all flux chambers were flushed with ambient air before sampling to establish ambient air equilibrium within the headspace. However, ebullition may continue throughout the sampling period, and may not be distinguished from the initial release caused by stepping in to take the sample, because ebullitive release of gases is caused intermittently by aquatic fauna or during settling of sediment. In every case, the utmost care was taken when it was necessary to step into the pond. However, it remains unclear whether or to what extent this may have directly affected the data and results.

To test this a pond was chosen that had a bridge installed over it. The Milham Ford Nature Park is under the care of Oxfordshire County Council, and after gaining permission, experiments were conducted on 22 June 2014 (Permission to sample was granted a couple of weeks earlier as well. However, due to faulty equipment these samples had to be discarded and time constraints would not allow for another round of sampling to be carried out after 22/06/2014). Two sets of 90 samples were collected, each over a period of 3 hours, generating a total of 180 air flux samples for methodological comparison. The first set was sampled from the bridge, without stepping into the pond (hereafter referred to as ‘bridge’ samples) and the second set was sampled as described above (in the Background/introduction) while standing in the pond as far as possible from the floating chamber, to minimise disturbance of the underlying sediment (hereafter referred to as ‘step-in’).

4.2 Aims

This experiment sought to determine whether pond sediment disturbance has an impact on emissions sampling. The hypothesis is that ‘bridge’ samples will have lower concentrations of CH₄ and CO₂ compared with ‘step-in’ samples.

4.3 Materials and methods

4.3.1 Study site

Sampling took place on 22 June 2014 from a pond at the Milham Ford Nature Park in Oxford. This was one of two ponds which, in 2008, were newly created wildlife ponds in a playing field of a former school compound. The park is open to the public and available for normal park activity, including children’s playground activities and dog walking. The ponds are also used in other experimental work and serve as educational ponds for the surrounding schools and universities. The ponds are surrounded by managed lawn, some shrubs and a few trees. Water from what is believed to be an underground drain enters the upper pond (where there is no bridge) from the north-east side of the field. Flora and fauna are well established, suggesting that this is representative of a UK wildlife pond. In 2011, buildings surrounding the pond caused a major sewage incident, which required the draining and jet-washing of the ponds (Wang, Wang and Zhang, 2013). The trophic status is not known, although it is most likely mesotrophic rather than eutrophic, as there is no algal bloom despite the sewage spill. The water was clear at the time of data collection, with a depth of about 30 cm varying +/- 1-2 cm. Sediment is fine silt (FSi as per ISO 14688-1) with a protracted settling time once disturbed. The pond surface area has seasonal variation and is estimated to have been between 120 m² and 140 m² at the time of sampling. The ponds were shallow, most likely due to a persistent dry spell before sampling began. Sediment organic matter content was assumed to be low as these are relatively new ponds (created in 2011) in an open landscape, with trees at least 8 m away from the margin, preventing direct litter fall. This suggests that most of the organic matter comes from the aquatic flora and fauna as well as plants along the shoreline, which are understood to be partially managed.

4.3.2 Environmental variables

Air and water temperatures were measured using a digital thermometer (Digitron 206T, UK), and conductivity meter readings were recorded from the water samples (Jenway, Model 470, UK). Water temperature was measured at surface-water level and at a depth of 15 cm. Conductivity was measured in situ and directly in the water using a handheld conductivity meter and probe (Jenway, Model 470, UK), which was pre-calibrated. Once the reading had

stabilised, the conductivity (μS) was noted. The pH was measured using a handheld pH meter and probe (Jenway, Model 350, UK) calibrated using pH 7 and pH 4 buffer solutions as described in Müller *et al.* (1998). Measurements were taken in situ, at hourly intervals during the entire sampling period, adapting the method of Bowes, Smith, et al. (2011): a 30 ml plastic bottle was rinsed with sample water each time and pH values were noted when equilibrium was reached. Wind speed was measured using a mechanical wind meter (Deuta Anemo, Germany) placed on the shore at 20 cm above water surface level.

4.3.3 Bridge vs. step-in experiment

In this experiment, CH_4 and CO_2 were sampled using headspace chambers which were set afloat, first from the bridge and later by stepping into the pond with the least possible disturbance of the sediment, using the same sampling location in both instances.

Five floating chambers were installed (Figure 24), fitted with a sampling tube (nylon, 2 m long and 2 mm inner diameter).



Figure 24: Set-up of floating air-flux chambers for the experiment in the Milham Ford pond.

Samples of 60 ml volume were extracted using plastic syringes (BD Plastipak) fitted with a 3-way valve (Figure 25). After ambient air conditions were established by flushing the headspace 40 times with a syringe (BD Plastipak), an initial 60 ml ‘zero’ sample was taken. However, before extracting a sample, a 20 ml sample was extracted and discarded to clear the tube of previous sample concentrations. Thereafter samples were extracted at intervals of 10 minutes over the course of 3 hours. The first set of data was from the bridge, collected between 10:05 and 12:55, yielding 180 samples (collected in 60 ml plastic syringes labelled with chamber number, sample number, and sampling method, i.e., bridge or step-in) from the 5 installed chambers. This was then repeated from 13:35 to 16:25, this time installing the chambers at the same place but stepping into the pond as carefully as possible to avoid sediment disturbance. Thereafter the samples were taken to the laboratory for analysis using QMS.



Figure 25: Buckets with labelled and colour coded syringes and alarm clocks were placed above the corresponding air-flux chambre on the foot bridge for obtaining samples for the bridge vs step in experiment at the Milham Ford pond.

4.3.4 Quadrupole mass spectrometry analysis of air-flux and water samples

The syringes were sorted into air flux, water and headspace samples. These subsets were analysed in random order within sample types. Air flux samples were analysed first followed by headspace samples and then water samples. This order enabled ideal conditions for QMS analysis, starting with a dry membrane and leaving it to dry overnight after the water samples were analysed as per the protocol in the main methods section (2.5).

4.4 Results

4.4.1 Environmental variables

Measurements recorded in hourly intervals, while sampling from the bridge (10:05-12:55) and step-in (13:35-16:25), respectively, were as follows: air temperature between 20.9 and 24.0°C, and 22.7 and 25.3°C; water surface temperature between 16.8 to 20.4°C, and 21.2 to 21.9°C, and at 15 cm depth between 16.6 and 18.1°C, and 18.3 and 19.1°C; pH measurements between 6.99 to 7.75, and 7.43 to 7.79; and conductivity between 363 and 437µS, and 415 and 449µS.

4.4.2 Emissions into the atmosphere (bridge versus step-in experiment)

The pond was a net source of CO₂ to the atmosphere (Figure 26). Sampled CO₂ (n=115 in both experiments) ranged from 369 to 867 ppm and CH₄ (n=113) ranged from 106 to 1392 ppb. Figure 26 shows CO₂ and CH₄ efflux (both bridge and step-in samples). The R²s of the bridge sample datasets were significantly higher than those of data collected by the step-in method (two-tailed equal variance t-test comparing bridge with step-in, $t_{calc} = 2.765$, $t_{crit} = 2.447$, df= 4, P= 0.033; Tukey HSD test P=0.033). Comparing the trendlines for CO₂ samples collected using bridge and step-in methods confirms that the bridge method is preferable; the Pearson's R of the bridge samples was 0.899 (Figure 26d). The Pearson's R for the step-in samples was 0.705 (Figure 26f) (the critical value of R for n=90 is 0.211). Comparing trendlines for CH₄, the bridge samples had a Pearson's R of 0.282 (Figure 26c) and the step-in samples of 0.044 (Figure 26e).

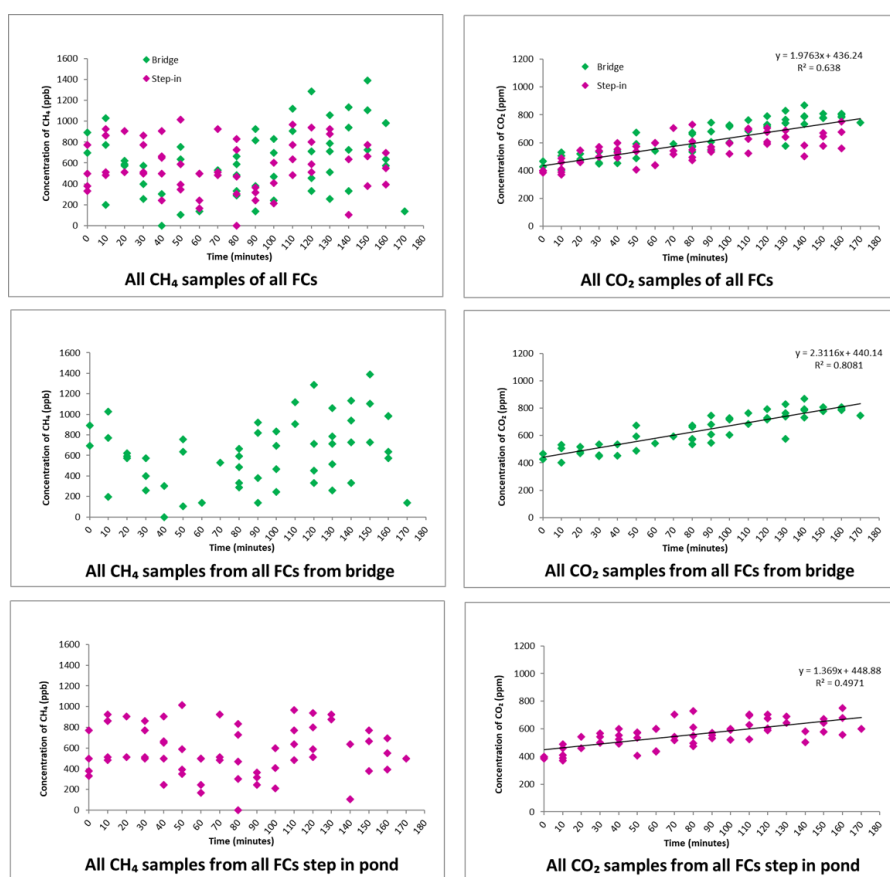


Figure 26: Mean CH₄ and CO₂ concentrations (ppm) comparing 'bridge' and 'step-in' methods: all samples of all flux chambers analysed for a) CH₄ and b) CO₂; 'bridge' samples for c) CH₄ and d) CO₂; 'step in' samples for e) CH₄ and f) CO₂. All R² values for CO₂ are significant at the 95% confidence interval (88 degrees of freedom). CH₄ trendlines are not shown, because the R values were not significant except for the bridge samples which had respectively little or no correlation ($R = 0.211$, $r^2 = 0.044$).

Methane results from the bridge indicated that the pond was a net source of CH₄ to the atmosphere over the sampling period, but r^2 values were low ($r^2 = 0.400, 0.067, 0.002, 0.011, 0.464$, chambers 1-5 respectively). However, CH₄ data collected using the step-in method were scattered, also with low r^2 values, although not significantly lower than those of the bridge samples ($r^2 = 0.223, 0.000, 0.003, 0.000, 0.062$, chambers 1-5, respectively) ($t_{\text{calc}} = 1.665$, $t_{\text{crit}} = 3.182$, $df = 4$, $P = 0.195$).

4.5 Discussion

The analysis of CH₄ and CO₂ emissions, measured from samples collected using different sampling protocols, has illustrated that sediment disturbance prior to sample collection reduces the reliability of the measurement data. Despite the lower CO₂ values of the 'step-in' experiment the r^2 (0.4971) is weaker than the one for the CO₂ values of the 'bridge' samples ($r^2 = 0.8081$). CO₂ data were more homogenous than CH₄ regardless of sampling

method. Due to this, the r^2 values were deemed the more meaningful measure and, at the time, it was decided that more care should be taken when setting-up sampling equipment in ponds. A possible explanation for the aforementioned outcome could derive from increased air temperature which ultimately increased the water temperature during the sampling period (see 4.4.1). The rise in water temperature can increase the emission rate of GHG to the atmosphere (Negandhi, Laurion and Lovejoy, 2016; Kuhn *et al.*, 2018). These results suggest that sampling from a bridge or a boat (e.g. Huttunen *et al.*, 2003) is preferable to the step-in method, because the latter risks sediment disturbance. Future sampling will be carried out without stepping into the sediment as a result of this experiment.

5 Discussion

5.1 General discussion

Anthropogenic pressure and population growth, which increase demand for food and lead to enhanced and sophisticated food production and farming, have received rather bad press over the last few decades. This is especially true with regard to the use of resources and the environmental impact on waterways and impoundments (Holden *et al.*, 2017). It is therefore prudent to take a closer look at the side effects and impact on the environment of such practices, and to investigate whether water bodies in a farming environment sequester or emit greenhouse gases. The ‘lifeline’ of run-off nutrients from arable and pasture fields, and the biogeochemical processes initiated by it, would indicate, in theory that lentic systems act as carbon sequestrers. This research aimed to determine the rate of organic carbon accumulation in agricultural ponds in relation to nutrients, CO₂ and CH₄ production processes in and emission routes from ponds in relation to physico-chemical conditions.

To investigate these objectives, a number of experiments were carried out involving analysis of CO₂ and CH₄ concentrations dissolved in and emitted from watercourses and impoundments, as well as a loss on ignition analysis and in conjunction with environmental variables.

In the initial research proposal, gas and water samples were to be collected and analysed using a portable QMS. At the time there was a limited understanding by the researcher of any risks to data integrity that might be involved in this process.

Initial investigation suggested that the inconclusive data were the result of a poor sampling regime, and that more care needed to be taken in collecting the samples. As a result, the data collected during the first field session in 2013 were deemed unfit for analysis, and the decision was made to focus on improving the sampling methodology. An interim experiment (see chapter 4) was carried out before the second field season in a ‘controlled’ environment. More specifically, one set of samples was to be collected in a way that would avoid disturbing water and sediment. This was because such disturbance could offset CH₄ ebullition. This could also generate unwanted CO₂ emission as a result of the oxidation of CH₄ along the water column, which reacts with dissolved O₂ (Guérin and Abril, 2007; Holgersson and Raymond, 2016). To this end, the researcher located a pond with a bridge, where first samples could be taken with minimal disturbance of water and sediment. Thereafter, sampling was undertaken in the conventional way, via shore access, where some

disturbance of water and sediment was inevitable. The outcome of this experiment entailed an improved sampling method for the next field season. Despite the adjusted sampling regime, this did not have the desired outcome in the year 2 data. Ultimately, it was clear that the focus needed to change entirely — it was imperative to investigate the QMS itself.

By the time this was fully realised, the project was in its third year. A second set of unanalysable data led to some very instructive — but highly disappointing — lessons learned, along with the forfeiture of the opportunity to earn a PhD as originally intended.

To ensure that the lessons of this project were not learned in vain, the methodology was revisited and the raw data were reanalysed in an effort to find the fault in the analysis. The findings from this extensive re-investigation showed unequivocally that the data were unfit for statistical analysis.

It would be irresponsible and unethical to assume that, since the data are inconclusive, there is actually no or no significant emission of CO₂ and CH₄ — there is no statistical proof of this, since data analysis could not be carried out. Re-analysis was done due to substantial differences in the means of the standard deviation of the CO₂ and CH₄ Air-flux data. It was therefore thought necessary to apply the coefficient of variation test (Appendix E) which revealed a very high variance (the coefficient of variance was at an average 40% of the standard deviation of 84 samples with none below 10%) of the sample data which underlined the loss of confidence in the data.

It was feasible, however, to hypothesise that the reason for this extensive variation in the air-flux data could lie with the QMS and not as initially thought due to a poor sampling regime. There was, however, no comparison of analytical methods undertaken at the beginning of the project, which would have determined the reproducibility of the experiments set out in the methodology. Reproducibility testing was not done at the beginning of the project, since the QMS had recently been serviced by ESS. In hindsight this was a missed opportunity to detect a possible issue early in the project.

With hindsight, it is clear that this should have been done at the start of the project. Nevertheless, advice was sought to establish whether it was feasible and logical to undertake an intertechnique comparison, using the QMS and GC, to test whether the analyser used for this project was faulty and/or the methodology was flawed. While it was agreed that this was a good approach, it would require a substantial financial commitment, which was simply not available.

5.2 Concluding remarks

Thus, despite tremendous disappointment, the decision was made to refocus the project on the lessons learned, in the hope that it would benefit the scientific community more broadly, even if being awarded a PhD was now deemed an impossibility. The inconclusive data was initially thought to derive from a poor sampling regime, but ultimately linked to the analytical method and instruments. Although data analysis could not be completed, descriptive analyse of the processes undertaken are provided. Recommendations are given (section 3.6) for ensuring reliability before commencing sampling, and for improving the methodology. The most vital lesson, undoubtedly, was the importance of testing the validity of all analytical methods and instruments before commencing sampling and analysis, to confirm reproducibility and reliability compared to previous studies. Moreover, testing for precision must give satisfactory results (e.g. supporting the argument) before commencing the next step. Once reliability and precision are confirmed, only then should the analytical instrument be calibrated.

6 References

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7 Appendices

Appendix A:

Summaries of data analyses

a) Comparing dissolved CO₂ and CH₄ in water in the three catchments

CO₂

CO₂ Eye vs Stonton			
Mann-Whitney U Test			
Comparing Two Independent Samples			
Sample size #1	53	Sample size #2	44
Mann-Whitney U Test			
W1 Sum of Ranks (series 1)	2787	U (larger)	1356.000
W2 Sum of Ranks (series 2)	1966	U	976.000
Mean W1	2597	Mean W2	2156.000
Standard Deviation W	138.002	Multiplicity Factor	0.000
Z	1.377	p-level	0.169
Kolmogorov-Smirnov Test			
Maximal Difference	0.281	p-level	0.036
Wald-Wolfowitz Runs Test			
Runs count R	97.000	Z	4.882
p-level	0.000		
Rosenbaum Criterion			
Rosenbaum Q	2	Critical value (5%)	#N/A

CO₂ Eye vs Barkby			
Mann-Whitney U Test			
Comparing Two Independent Samples			
Sample size #1	53	Sample size #2	32
Mann-Whitney U Test			
W1 Sum of Ranks (series 1)	1968	U (larger)	537
W2 Sum of Ranks (series 2)	1687	U	1159
Mean W1	2279	Mean W2	1376
Standard Deviation W	110.248	Multiplicity Factor	0
Z	2.821	p-level	0.005
Kolmogorov-Smirnov Test			
Maximal Difference	-0.404	p-level	0.002
Wald-Wolfowitz Runs Test			
Runs count R	85.000	Z	5.070
p-level	0.000		
Rosenbaum Criterion			
Rosenbaum Q	3	Critical value (5%)	#N/A

CO₂ Stonton vs Barkby			
Mann-Whitney U Test			
Comparing Two Independent Samples			
Sample size #1	44	Sample size #2	32
Mann-Whitney U Test			
W1 Sum of Ranks (series 1)	1414	U (larger)	424
W2 Sum of Ranks (series 2)	1512	U	984
Mean W1	1694	Mean W2	1232
Standard Deviation W	95.051	Multiplicity Factor	0.000
Z	2.946	p-level	0.003
Kolmogorov-Smirnov Test			
Maximal Difference	-0.449	p-level	0.001
Wald-Wolfowitz Runs Test			
Runs count R	76.000	Z	4.437
p-level	0.000		
Rosenbaum Criterion			
Rosenbaum Q	1	Critical value (5%)	#N/A

CO₂				
Kruskal-Wallis ANOVA				
Comparing Multiple Independent Samples				
	Sample size	Sum of Ranks		
CO ₂ Eye	53	3324		
CO ₂ Stonton	44	2390		
CO ₂ Barkby	32	2671		
Kruskal-Wallis ANOVA				
H	11.600523	N	129	
Degrees Of Freedom	2	p-level	0.0030268	
H (corrected)	11.600523			
Median Test				
Overall Median	0.7062327	Chi-square	14.322256	
p-level	0.0007762			
	Variable #1	Variable #2	Variable #3	Total
<= Median (observed)	30	28	7	65
<= Median (excepted)	26.5	22	16	
observed-excepted	3.5	6	-9	
> Median (observed)	23	16	25	64
> Median (excepted)	26.5	22	16	
observed-excepted	-3.5	-6	9	
Total:observed	53	44	32	129

CH₄

CH ₄ Eye vs Stonton				
Mann-Whitney U Test				
Comparing Two Independent Samples				
Sample size #1	53	Sample size #2	44	
Mann-Whitney U Test				
W1 Sum of Ranks (series 1)	2065	U (larger)	634	
W2 Sum of Ranks (series 2)	2688	U (stat)	1698	
Mean W1	2597	Mean W2	2156	
Standard Deviation W	138.002	Multiplicity Factor	0.000	
Z (same as tstat)	3.855	p-level	0.000	
Kolmogorov-Smirnov Test				
Maximal Difference	-0.399	p-level	0.001	
Wald-Wolfowitz Runs Test				
Runs count R	97.000	Z	4.882	
p-level	0.000			
Rosenbaum Criterion				
Rosenbaum Q	16	Critical value (5%)	#N/A	

CH ₄ Eye vs Barkby				
Mann-Whitney U Test				
Comparing Two Independent Samples				
Sample size #1	53	Sample size #2	32	
Mann-Whitney U Test				
W1 Sum of Ranks (series 1)	1982	U (larger)	551	
W2 Sum of Ranks (series 2)	1673	U	1145	
Mean W1	2279	Mean W2	1376	
Standard Deviation W	110.248	Multiplicity Factor	0.000	
Z	2.694	p-level	0.007	
Kolmogorov-Smirnov Test				
Maximal Difference	-0.354	p-level	0.010	
Wald-Wolfowitz Runs Test				
Runs count R	85.000	Z	5.070	
p-level	0.000			
Rosenbaum Criterion				
Rosenbaum Q	10	Critical value (5%)	#N/A	

CH ₄ Stonton vs Barkby				
Mann-Whitney U Test				
Comparing Two Independent Samples				
Sample size #1	44	Sample size #2	32	
Mann-Whitney U Test				
W1 Sum of Ranks (series 1)	1741	U (larger)	751	
W2 Sum of Ranks (series 2)	1185	U	657	
Mean W1	1694	Mean W2	1232	
Standard Deviation W	95.051	Multiplicity Factor	0.000	
Z	0.494	p-level	0.621	
Kolmogorov-Smirnov Test				
Maximal Difference	0.145	p-level	0.800	
Wald-Wolfowitz Runs Test				
Runs count R	76.000	Z	4.437	
p-level	0.000			
Rosenbaum Criterion				
Rosenbaum Q	5	Critical value (5%)	#N/A	

CH ₄				
Kruskal-Wallis ANOVA				
Comparing Multiple Independent Samples				
	Sample size	Sum of Ranks		
CH ₄ Eye	53	2616		
CH ₄ Stonton	44	3439		
CH ₄ Barkby	32	2330		
Kruskal-Wallis ANOVA				
H	16.128122	N	129	
Degrees Of Freedom	2	p-level	0.0003146	
H (corrected)	16.128122			
Median Test				
Overall Median	0.2781794	Chi-square	16.435678	
p-level	0.0002698			
	Variable #1	Variable #2	Variable #3	Total
<= Median (observed)	38	15	12	65
<= Median (excepted)	26.5	22	16	
observed-excepted	11.5	-7	-4	
> Median (observed)	15	29	20	64
> Median (excepted)	26.5	22	16	
observed-excepted	-11.5	7	4	
Total:observed	53	44	32	129

b) Loss in ignition analyses

Organic carbon (OC)

OC					
Kruskal-Wallis ANOVA					
	Sample size	Sum of Ran			
Eye OC (g/day)	26.0000	930.5000			
Stonton OC (g/day)	12.0000	295.0000			
Barkby OC (g/day)	20.0000	485.5000			
Kruskal-Wallis ANOVA					
H	6.5374	N	58.0000		
Degrees Of Freedom	2.0000	p-level	0.0381		
H (corrected)	6.5376				
Median Test					
Overall Median	0.0667	Chi-square	7.3795		
p-level	0.0250				
	Variable #1	Variable #2	Variable #3	Total	
<= Median (observed)	8.0000	7.0000	14.0000	29.0000	
<= Median (excepted)	13.0000	6.0000	10.0000		
observed-excepted	-5.0000	1.0000	4.0000		
> Median (observed)	18.0000	5.0000	6.0000	29.0000	
> Median (excepted)	13	6	10		
observed-excepted	5	-1	-4		
Total:observed	26	12	20	58	

Inorganic carbon (IC)

IC					
Comparing Multiple Independent Samples					
	Sample size	Sum of Ran			
Eye IC (g/day)	26	886			
Stonton IC (g/day)	12	402.5			
Barkby IC (g/day)	20	422.5			
Kruskal-Wallis ANOVA					
H	7.516612	N	58		
Degrees Of Freedom	2	p-level	0.023323		
H (corrected)	7.520776				
Median Test					
Overall Median	0.01545	Chi-square	4.917949		
p-level	0.085523				
	Variable #1	Variable #2	Variable #3	Total	
<= Median (observed)	10	5	14	29	
<= Median (excepted)	13	6	10		
observed-excepted	-3	-1	4		
> Median (observed)	16	7	6	29	
> Median (excepted)	13	6	10		
observed-excepted	3	1	-4		
Total:observed	26	12	20	58	

Appendix B:

Milham Ford Nature Park experiment, bridge vs. step-in pond method, comparing air flux samples of CO₂ and CH₄.

1 Page

Appendix C:

Comparison of air-flux samples of CH₄ and CO₂ concentration vs. time from data of 1 flux chambers per pond from year 1 data from the three catchments (numbering: 03 Barkby, 02 Stonton, and 01 Eye followed by pond number).

3 Pages

Appendix D:

Comparison of air-flux samples of CH₄ and CO₂ concentration vs.time from data of 3 flux chambers per pond from year 2 data from the three catchments (numbering: 03 Barkby, 02 Stonton, and 01 Eye followed by pond number).

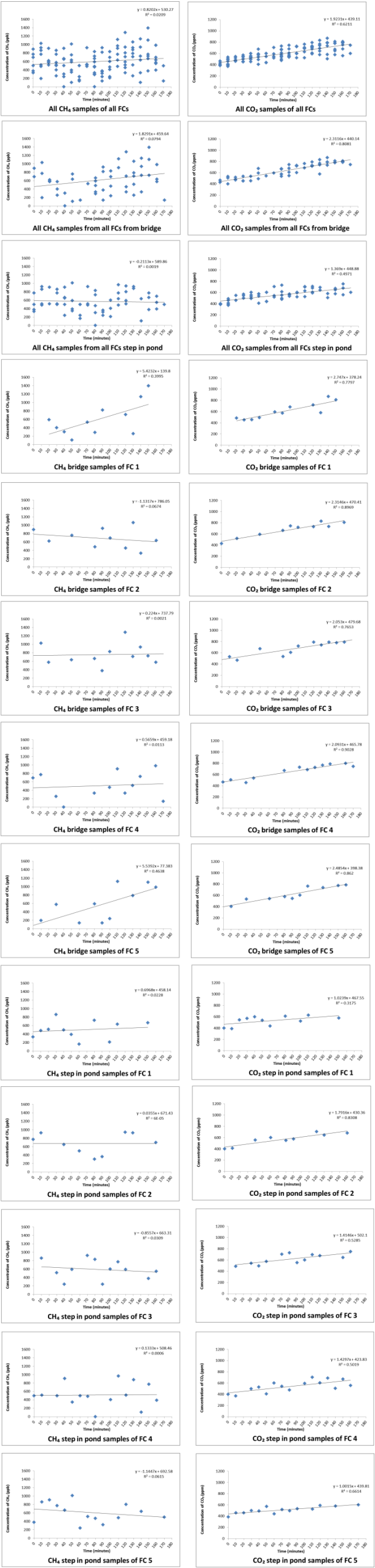
6 Pages

Appendix E:

Coefficient of variance results from Eye Brook of the air-flux sample data.

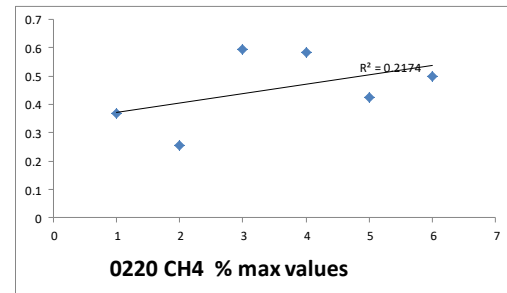
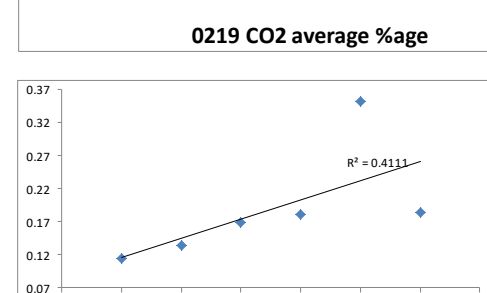
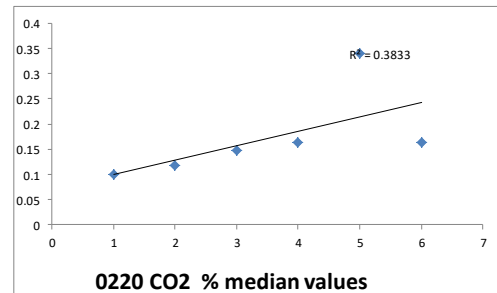
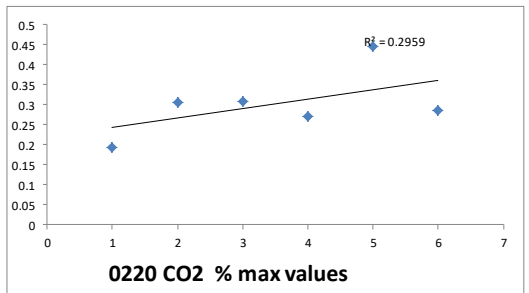
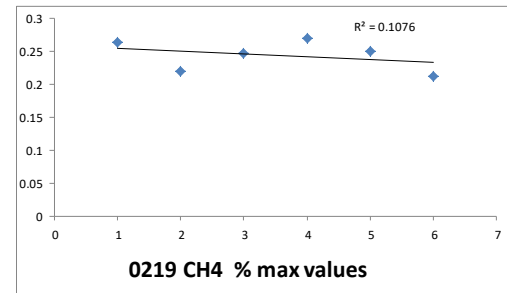
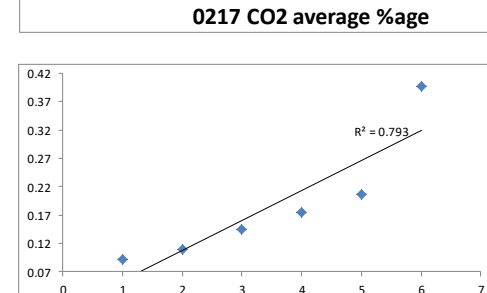
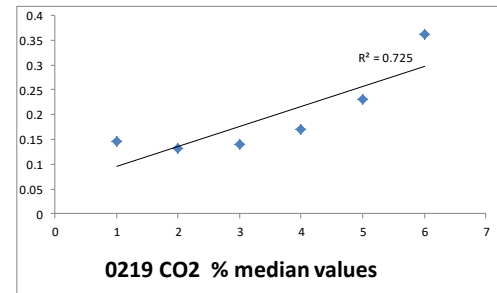
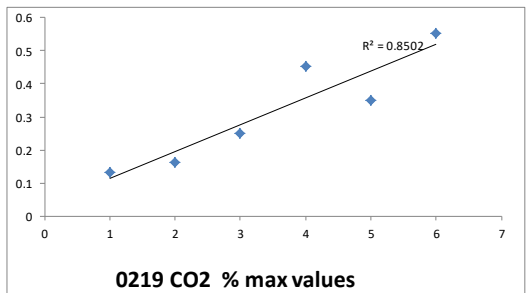
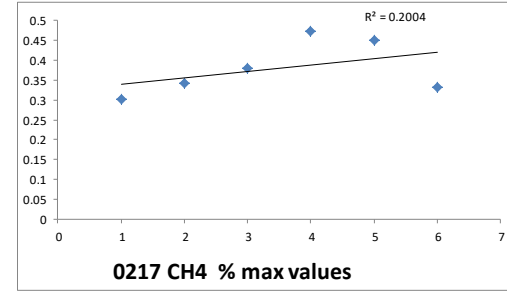
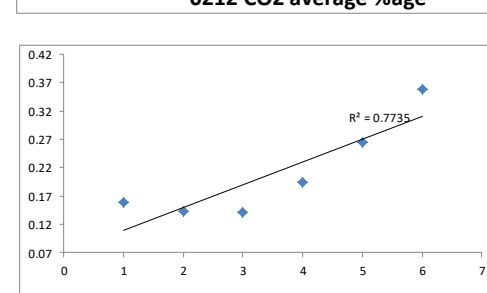
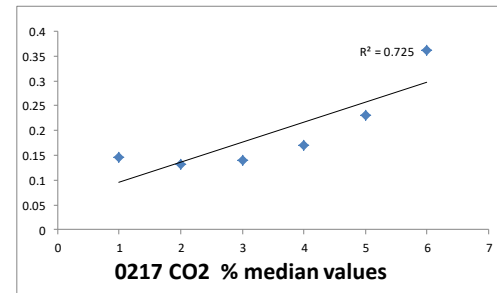
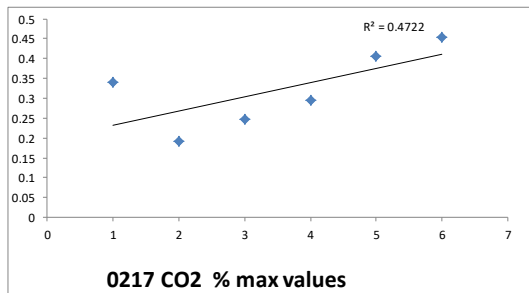
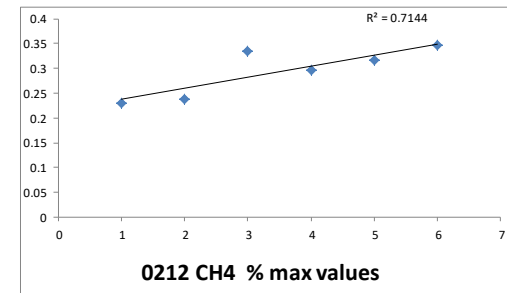
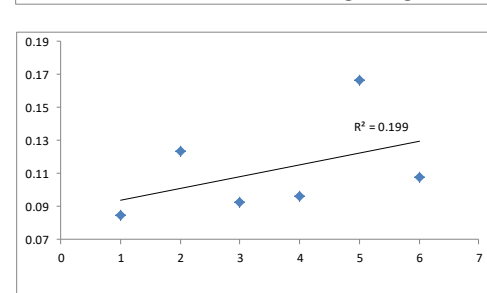
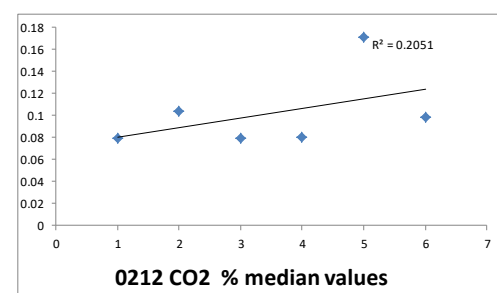
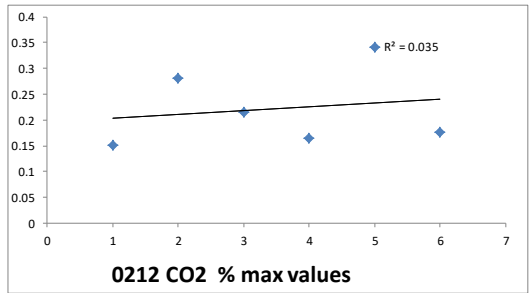
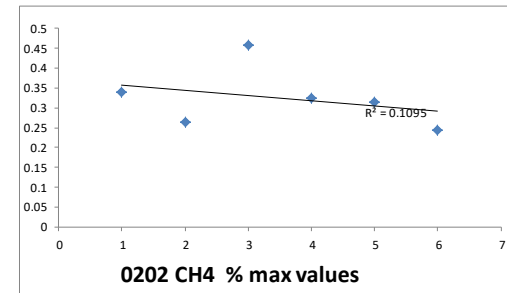
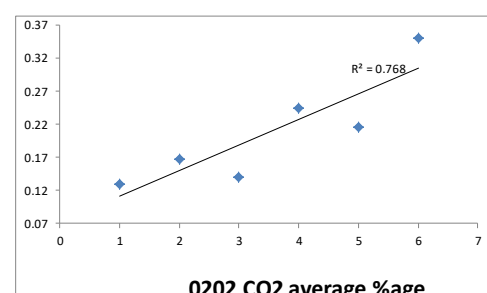
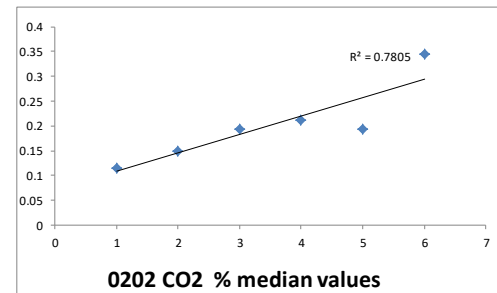
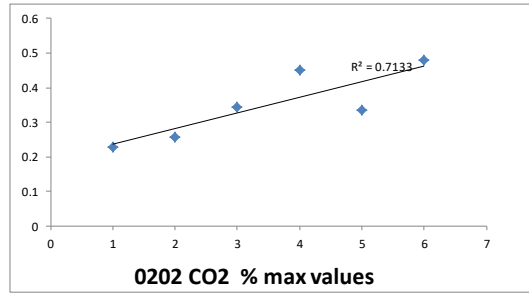
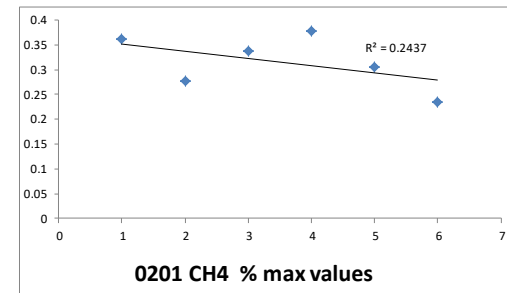
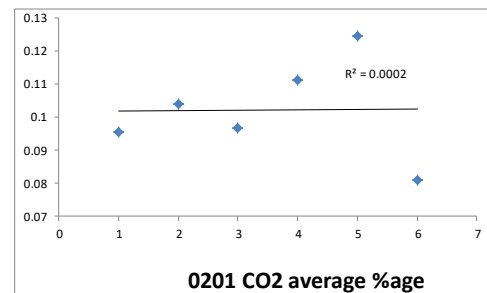
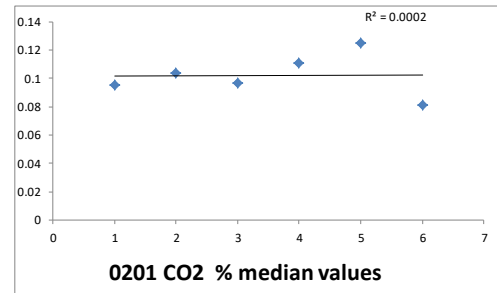
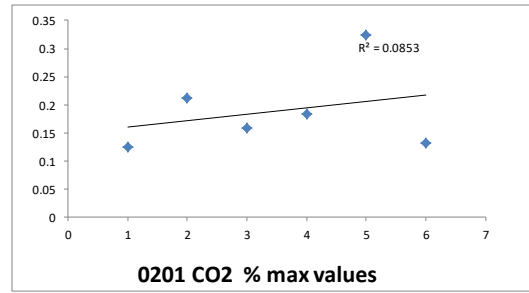
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Appendix B: Milham Ford Nature Reserve experiment, bridge vs. step-in pond method, comparing air flux samples of CO₂ and CH₄ for concentration vs. time from data of 5 flux chambers.

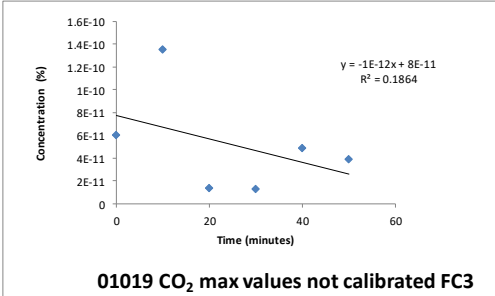
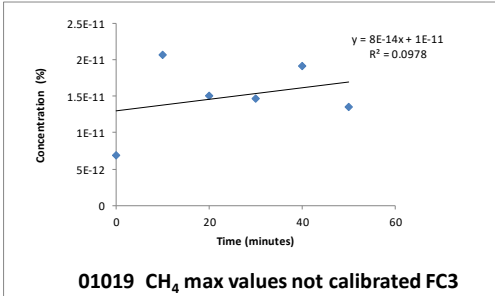
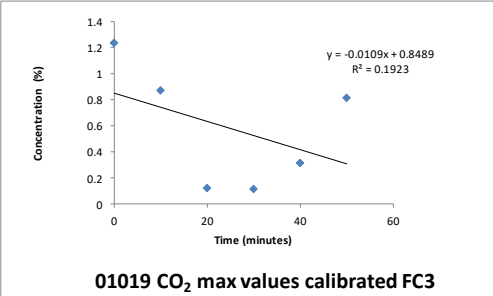
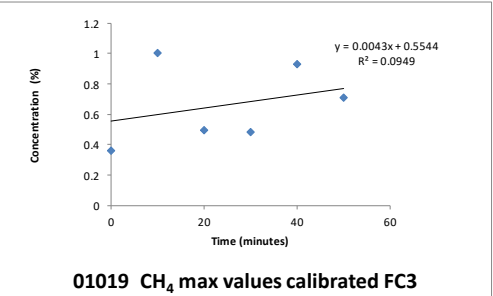
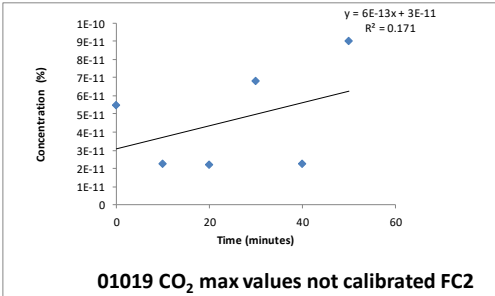
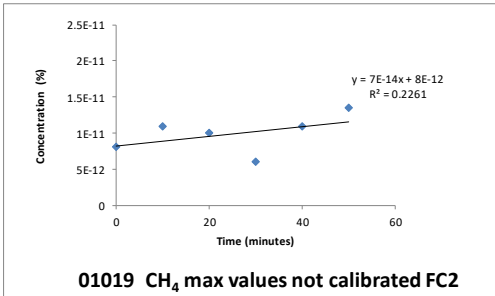
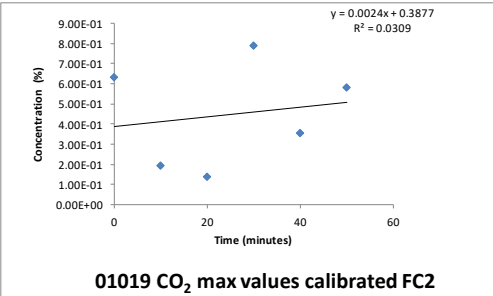
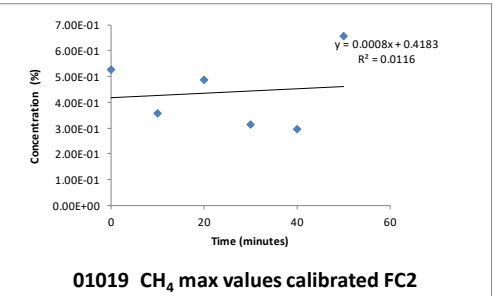
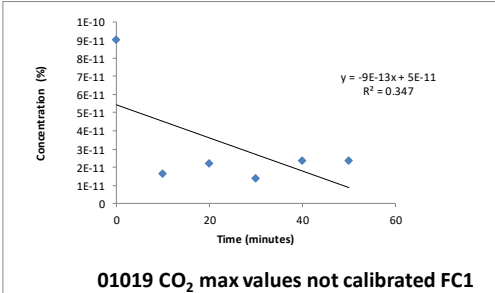
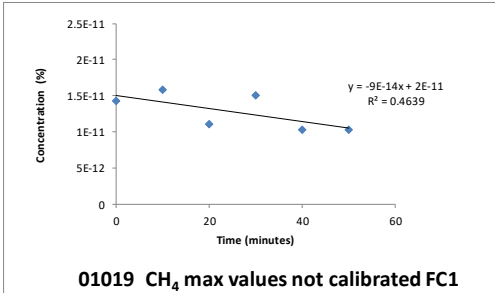
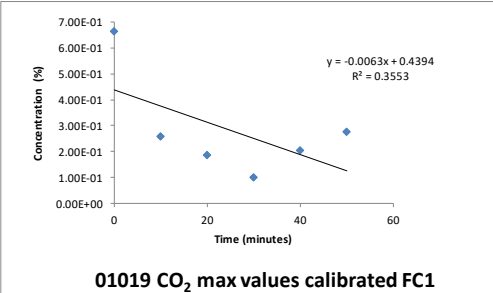
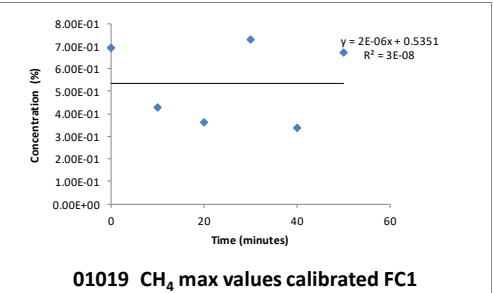
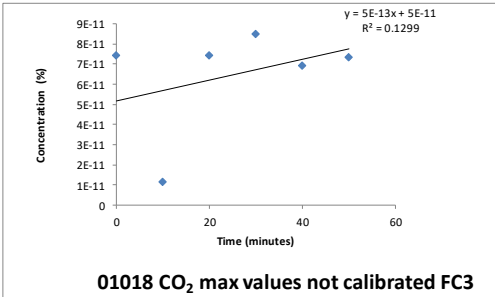
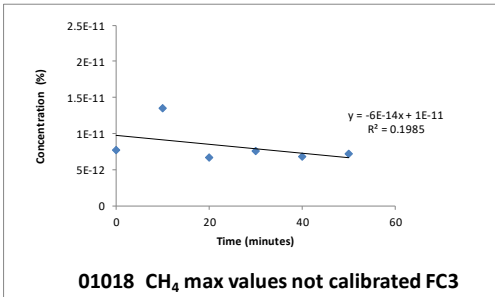
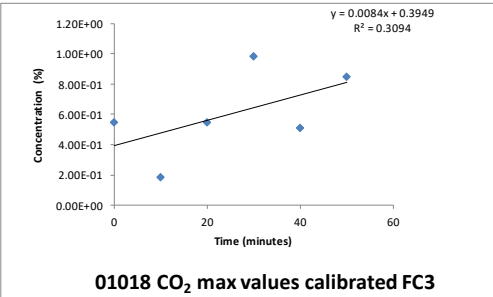
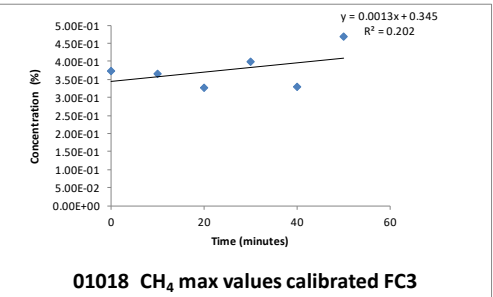
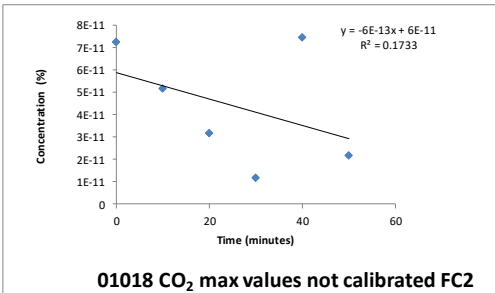
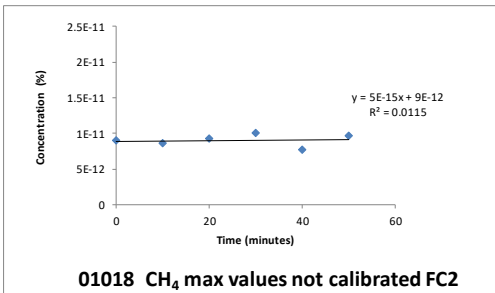
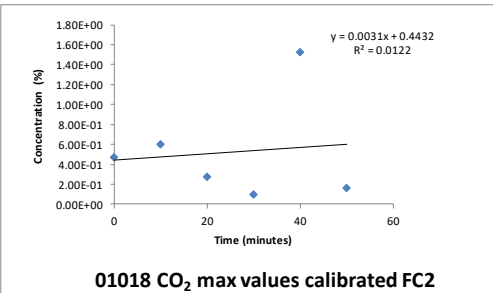
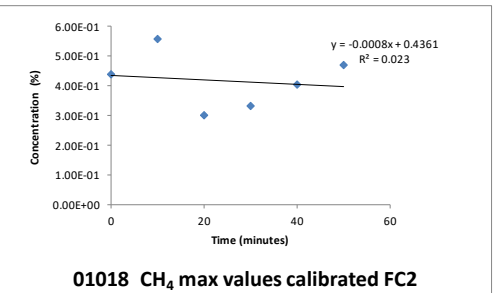
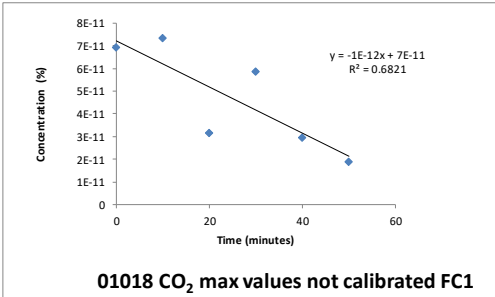
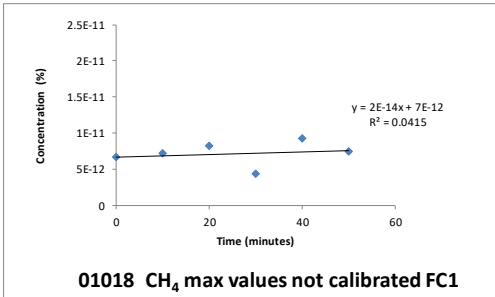
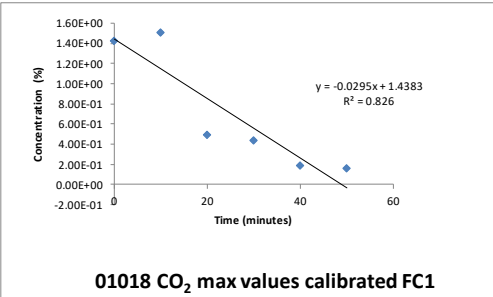
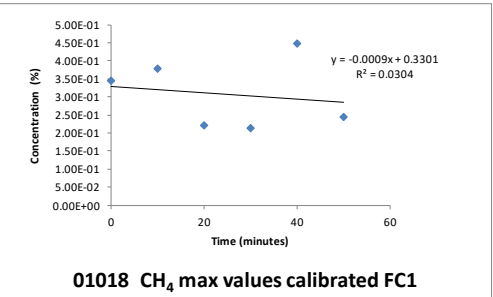
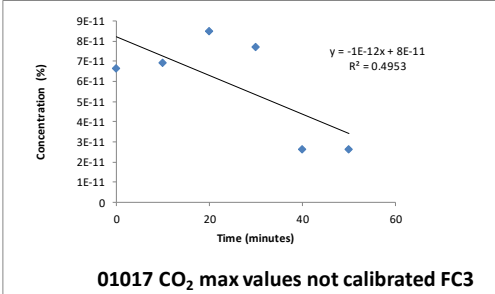
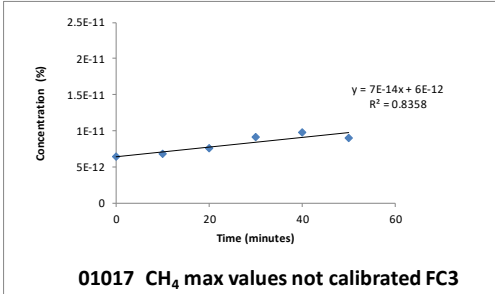
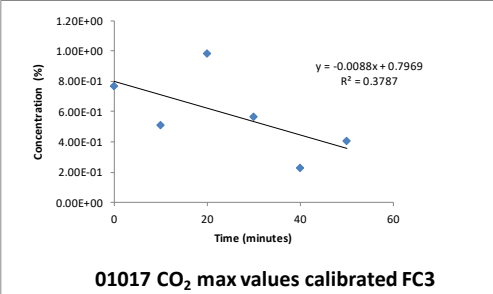
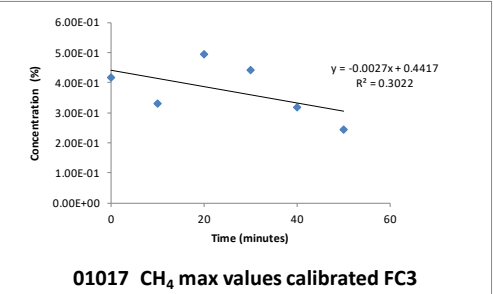
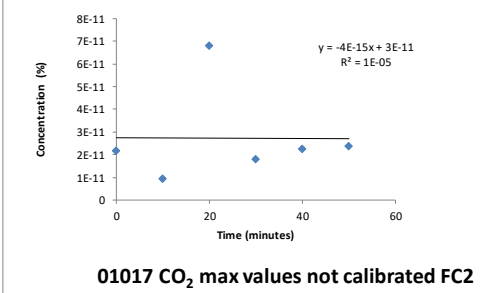
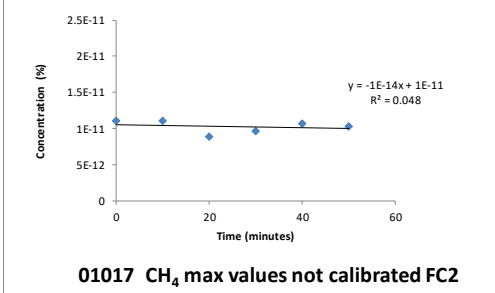
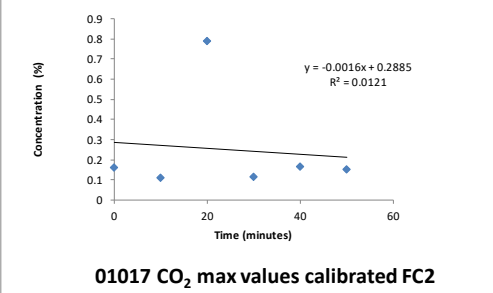
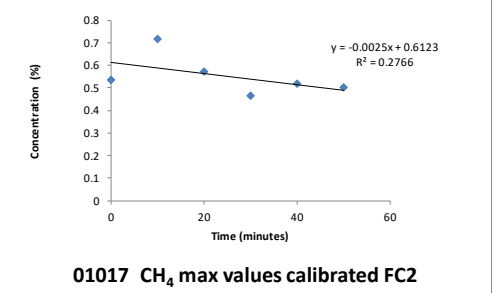
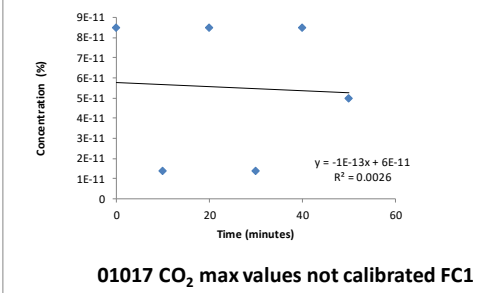
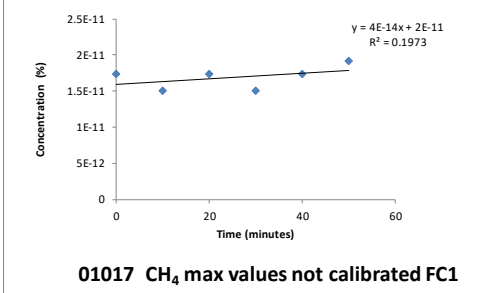
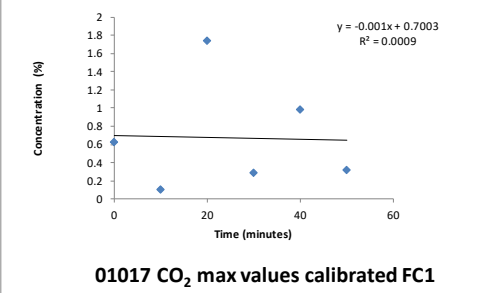
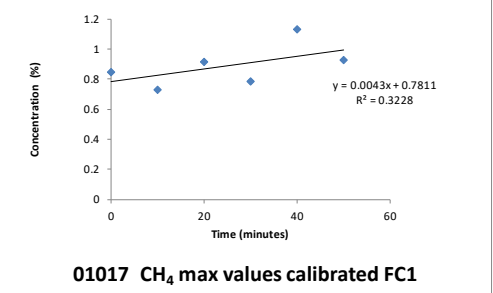


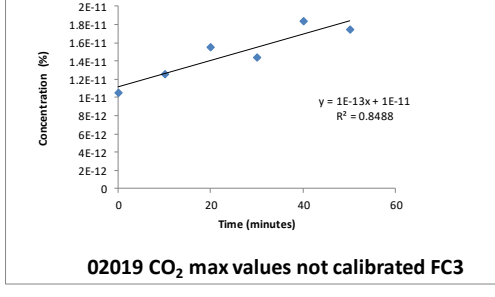
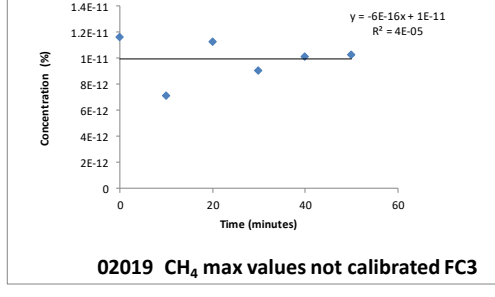
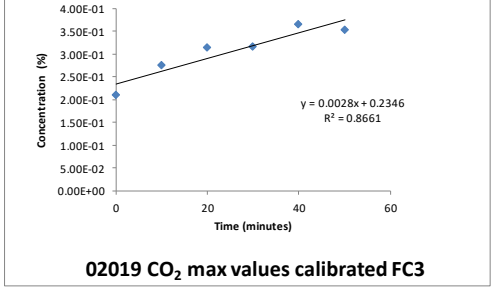
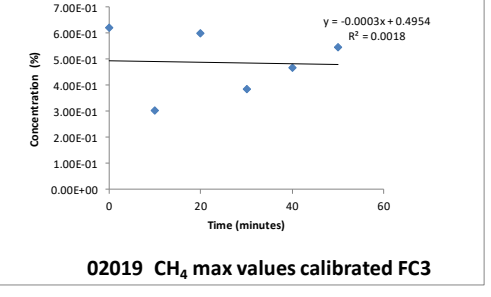
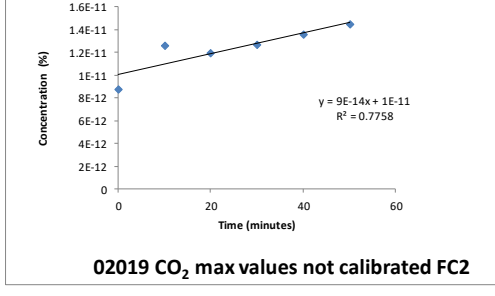
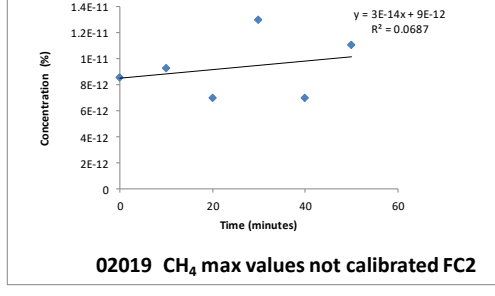
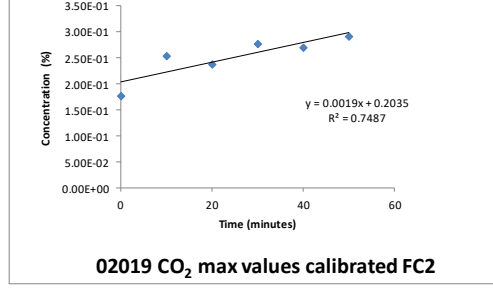
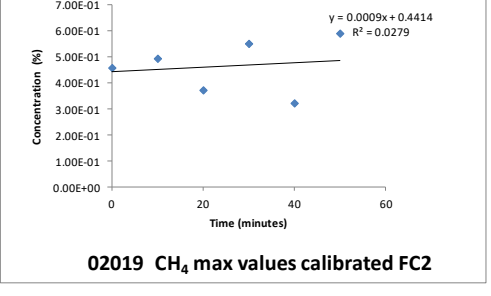
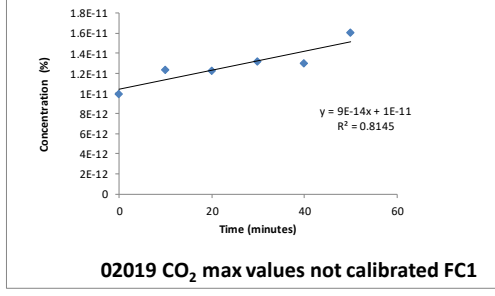
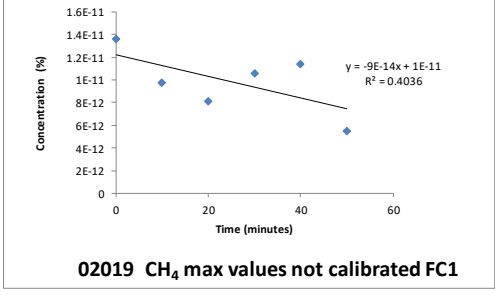
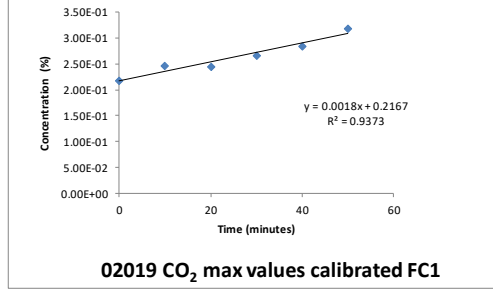
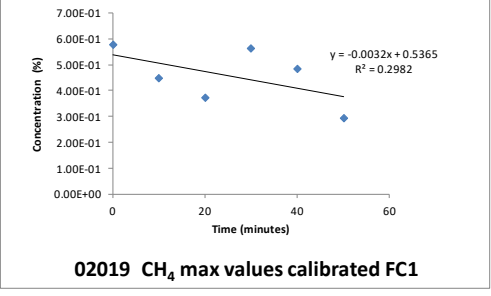
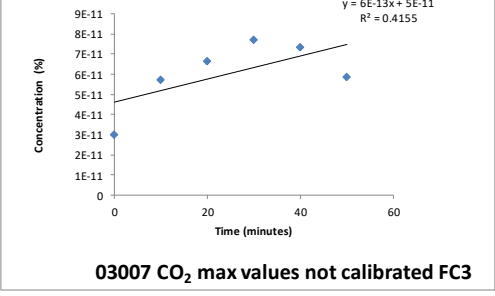
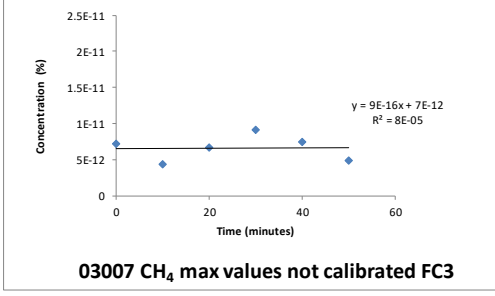
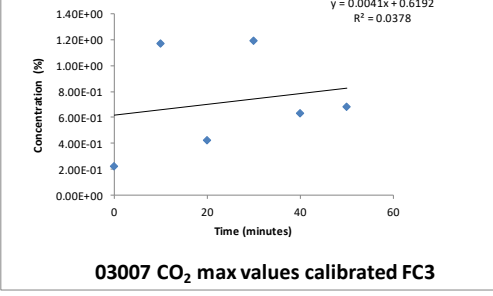
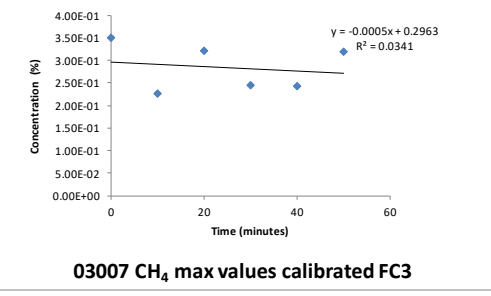
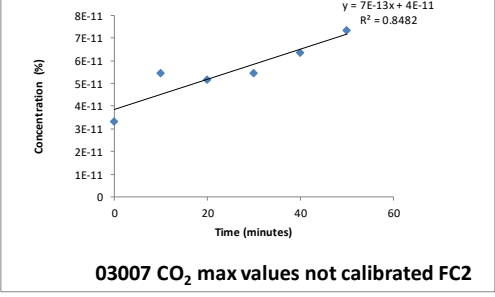
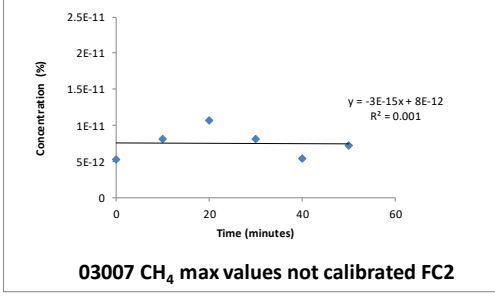
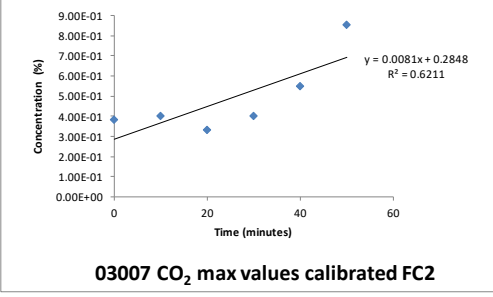
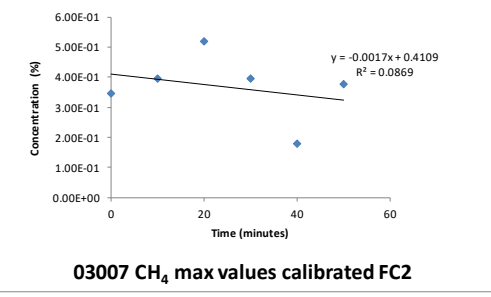
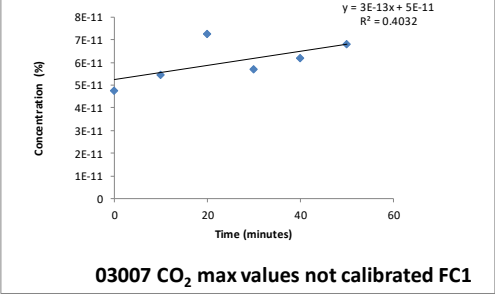
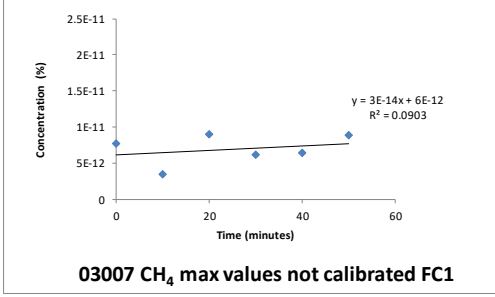
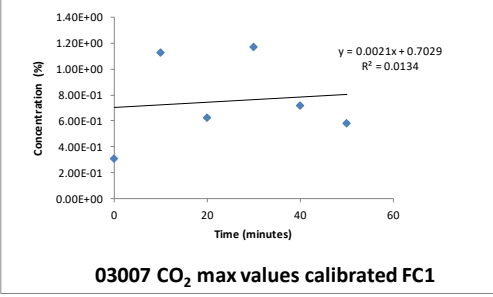
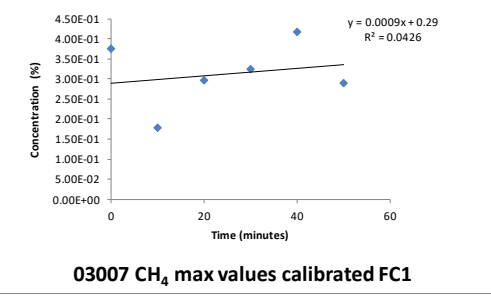
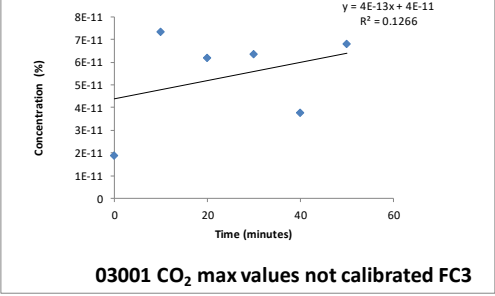
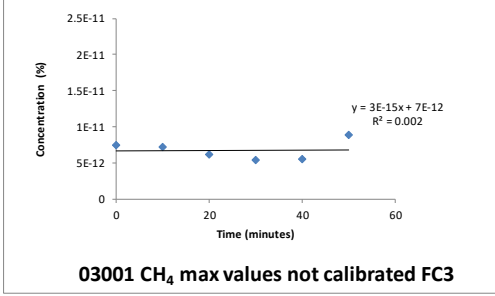
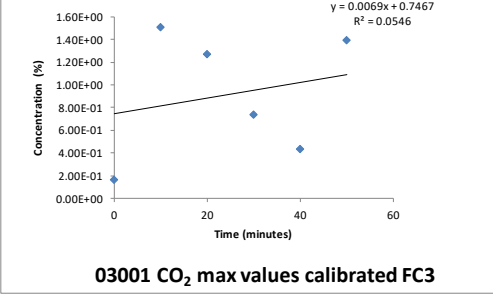
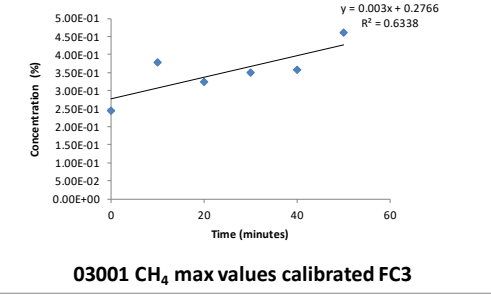
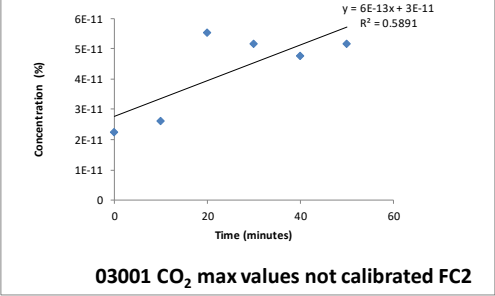
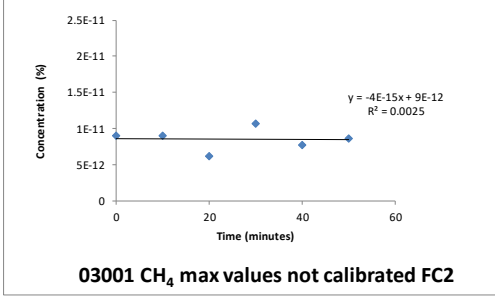
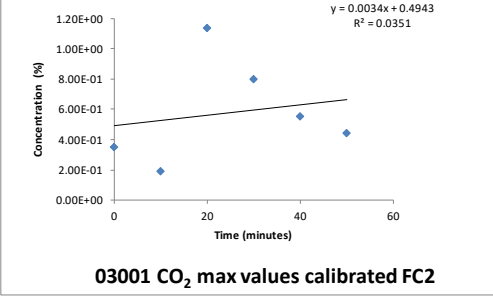
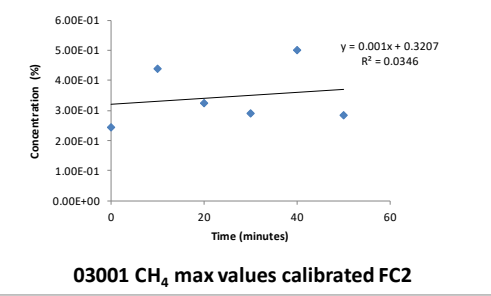
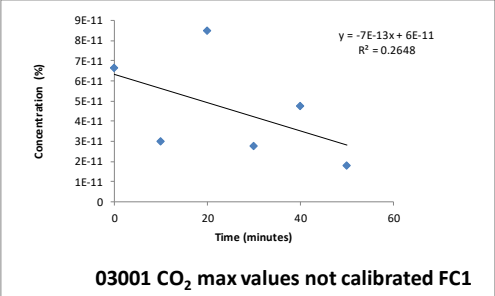
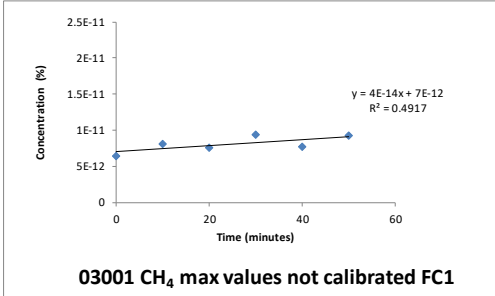
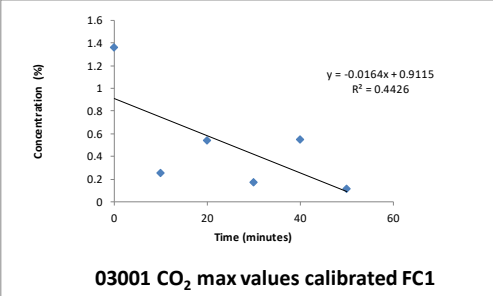
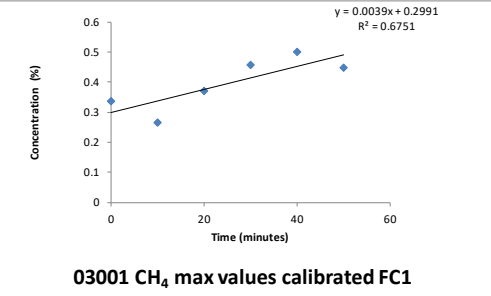
Appendix C: Comparison of air-flux samples of CH₄ and CO₂ concentration vs. time from data of 1 flux chambers per pond from year 1 data from the three catchments (numbering: 03 Barkby, 02 Stonton, and 01 Eye followed by pond number).



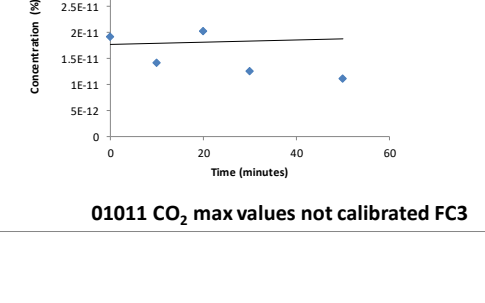
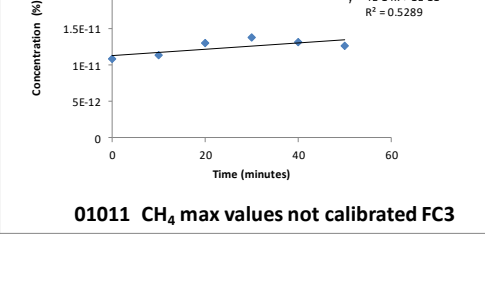
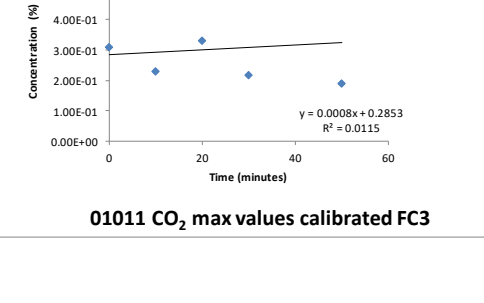
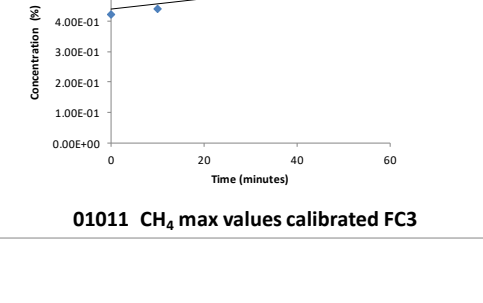
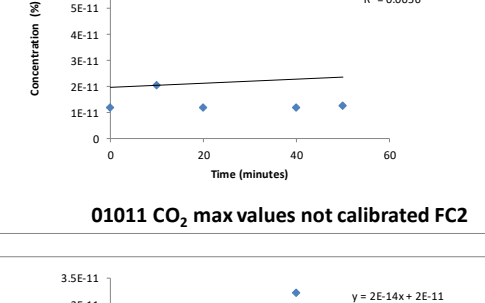
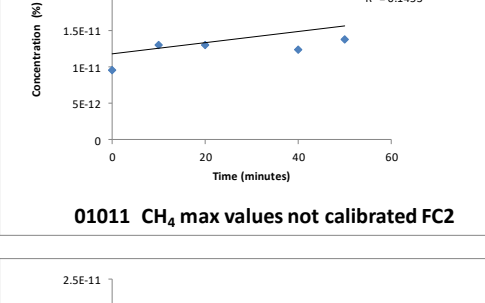
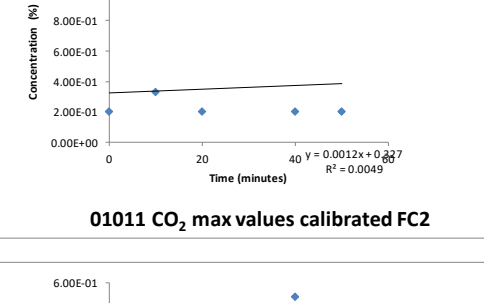
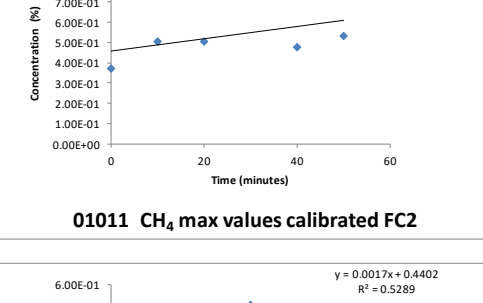
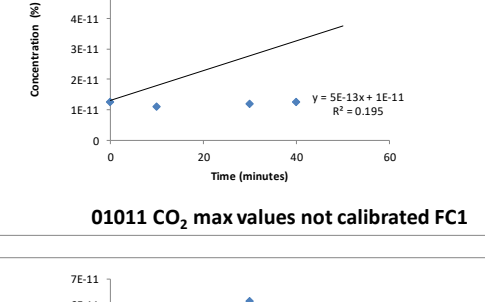
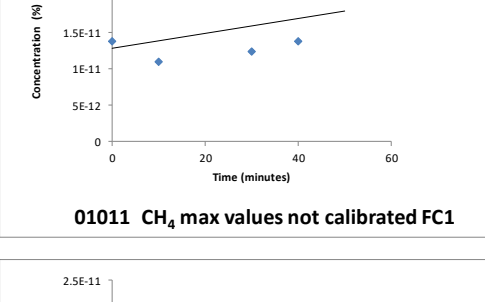
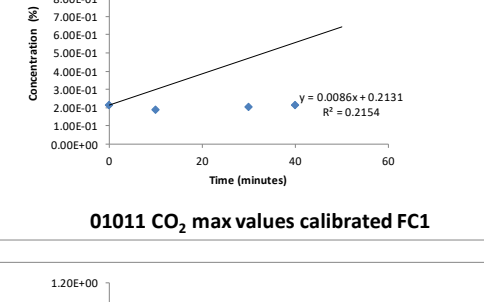
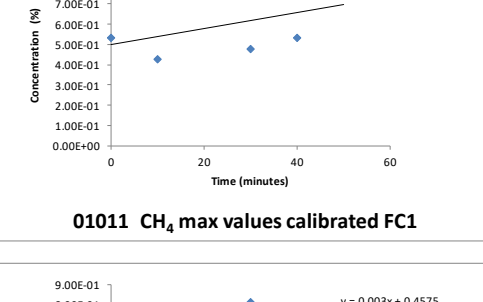
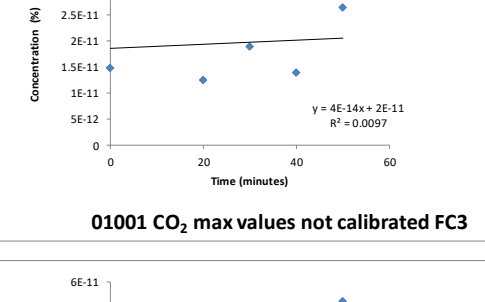
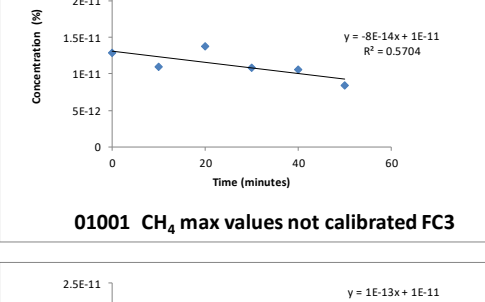
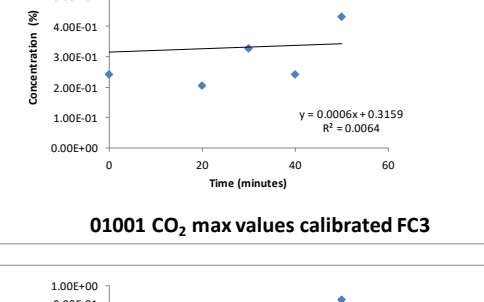
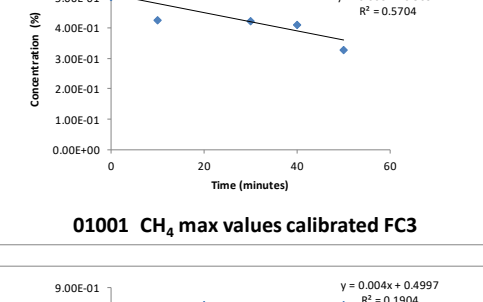
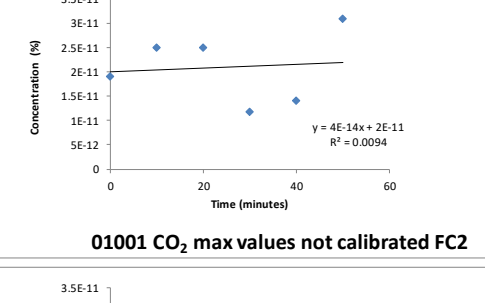
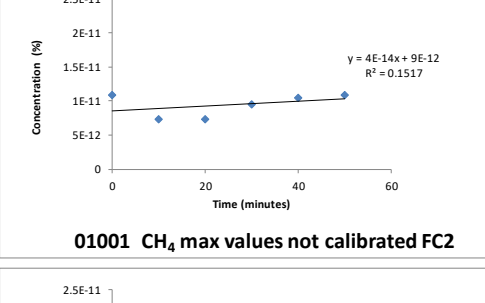
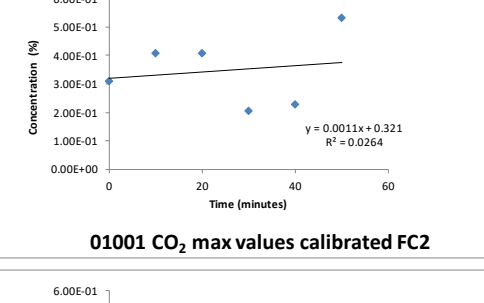
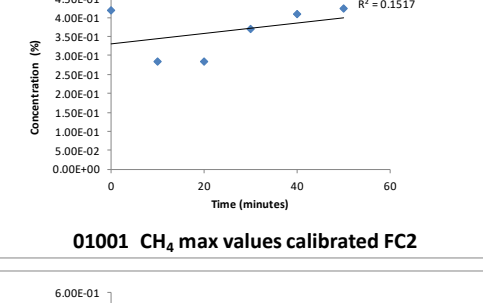
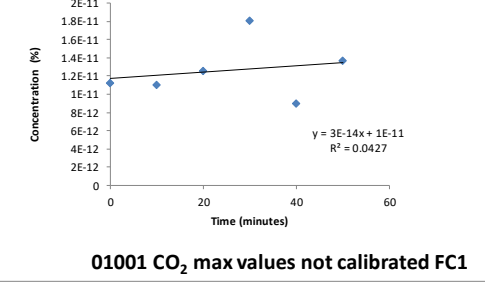
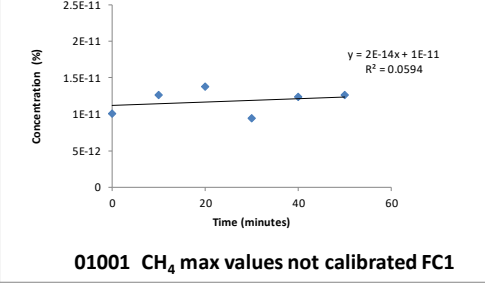
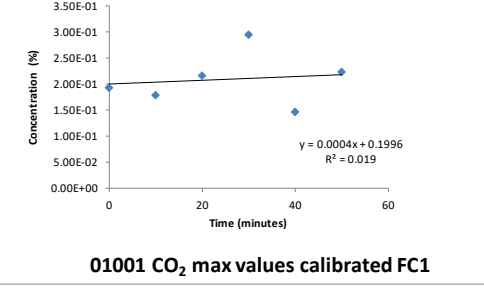
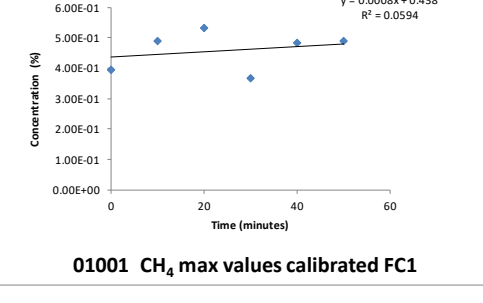
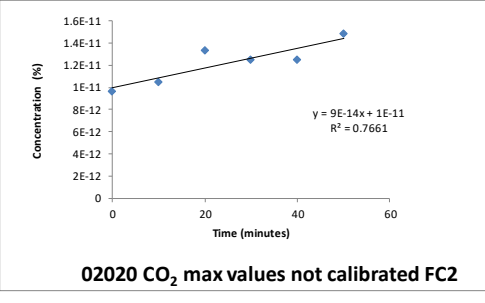
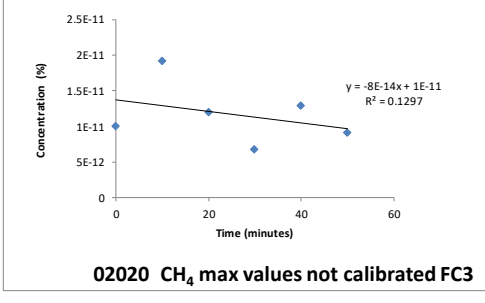
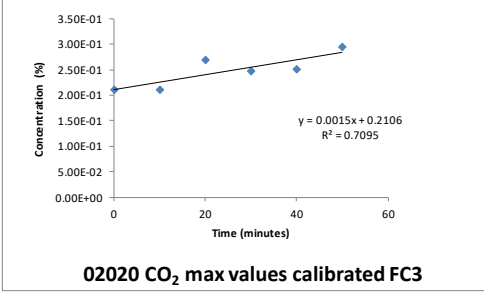
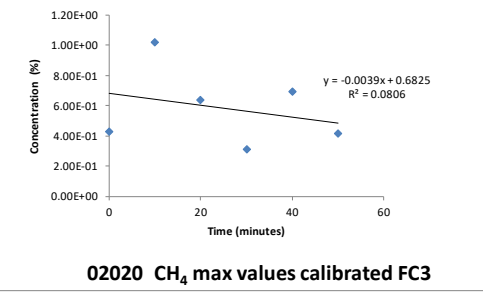
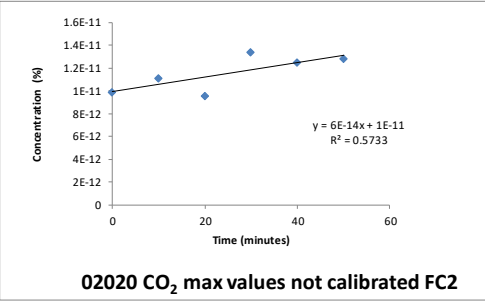
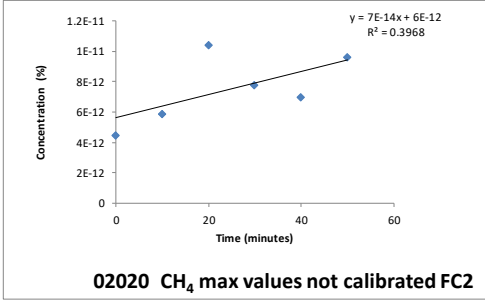
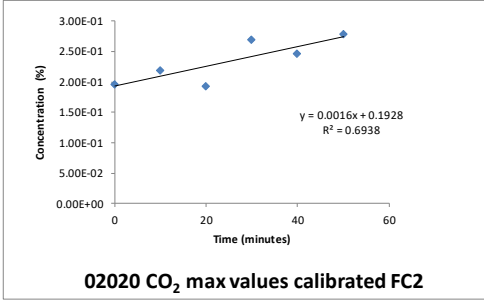
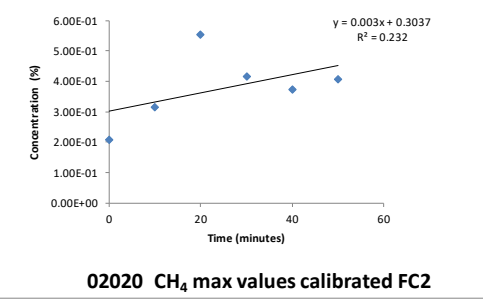
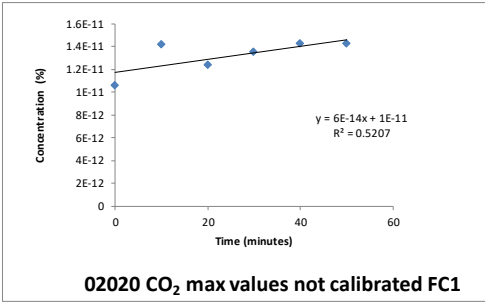
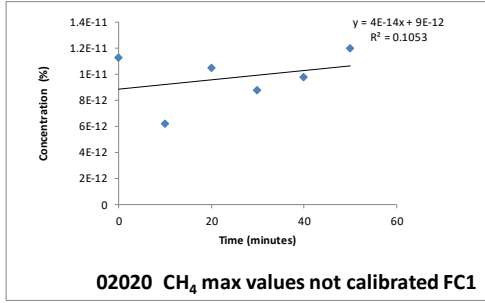
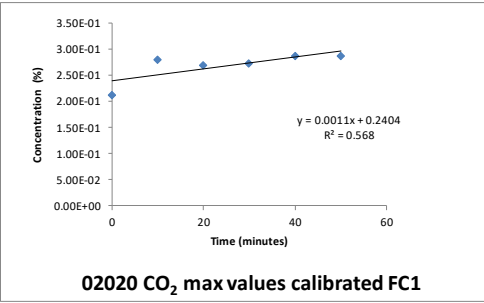
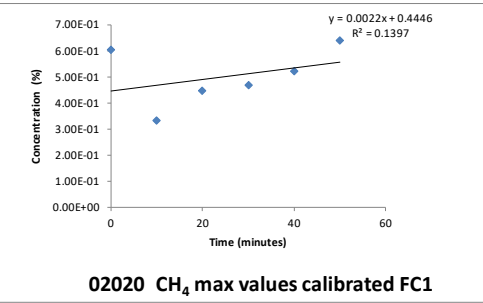


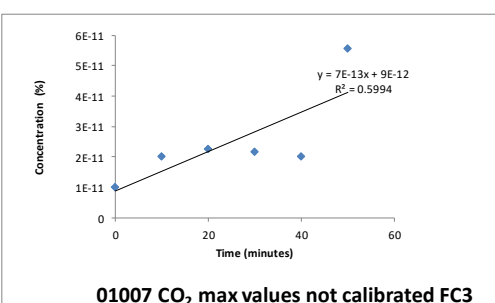
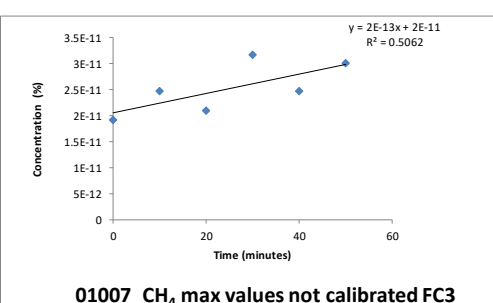
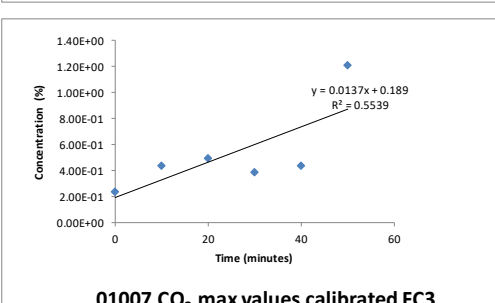
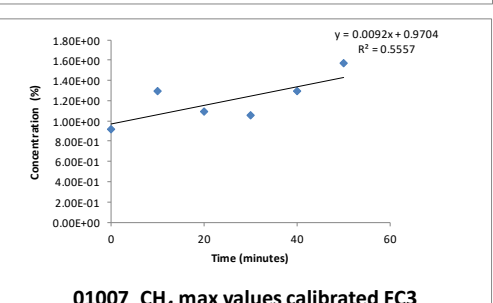
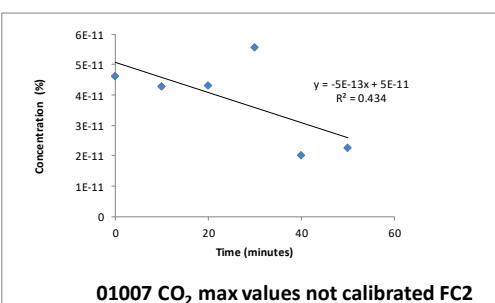
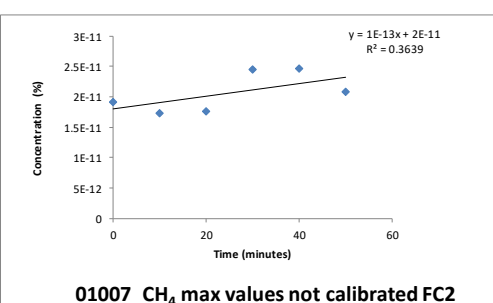
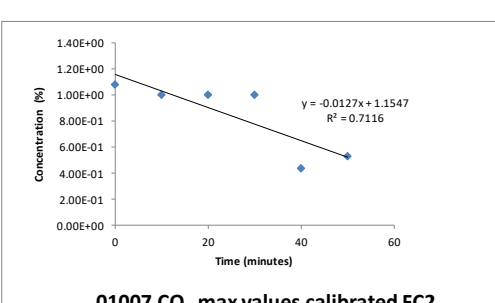
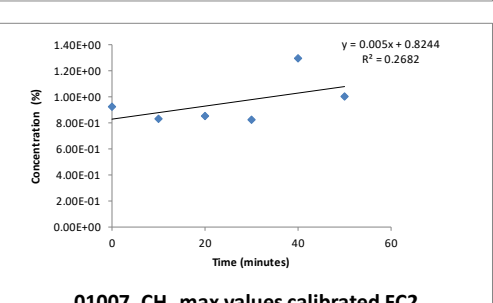
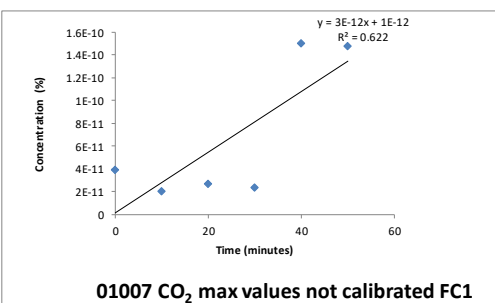
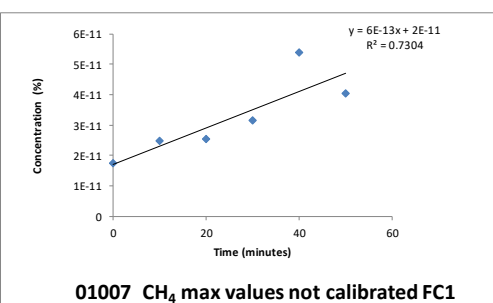
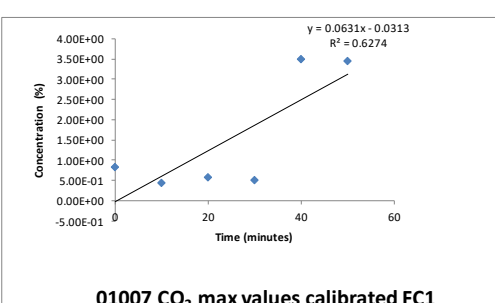
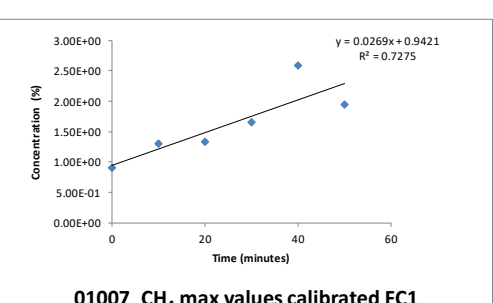
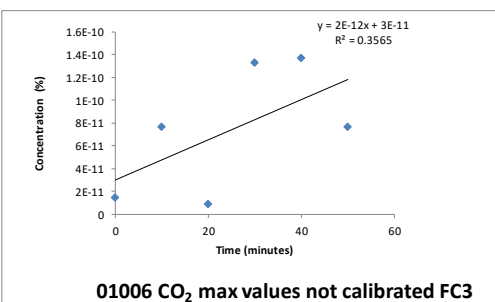
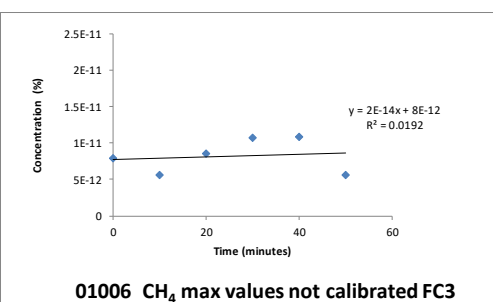
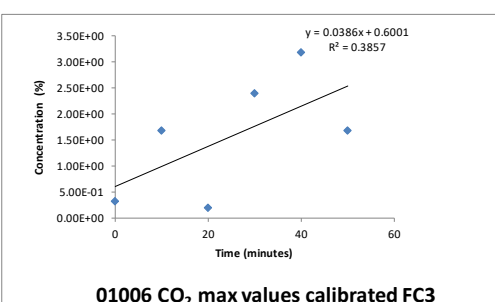
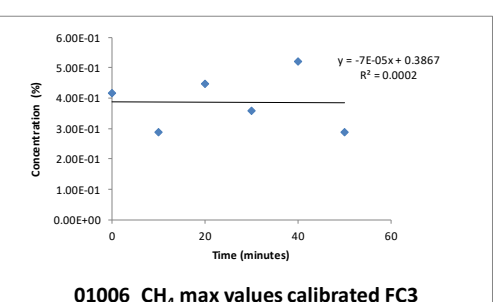
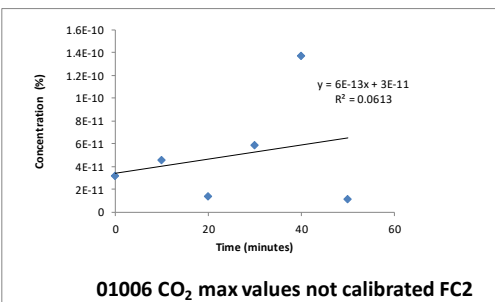
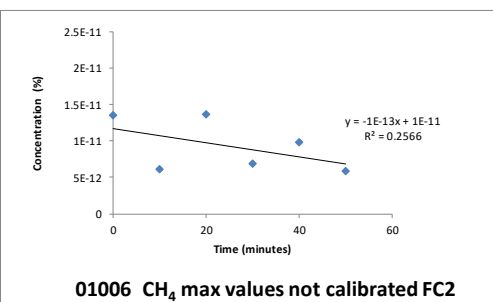
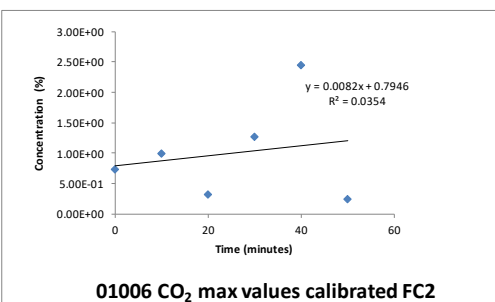
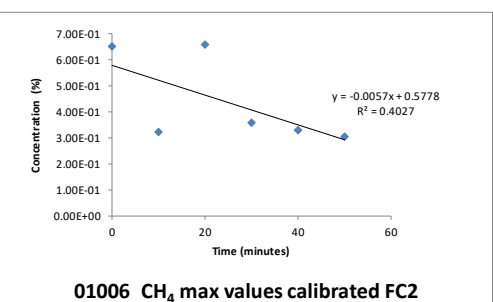
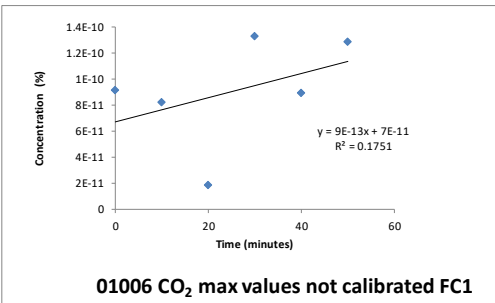
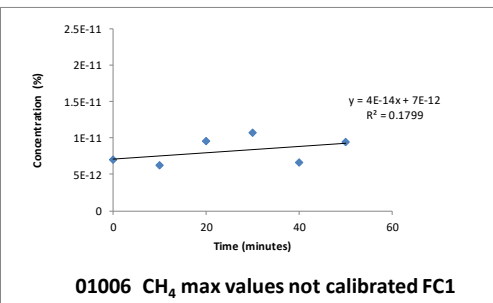
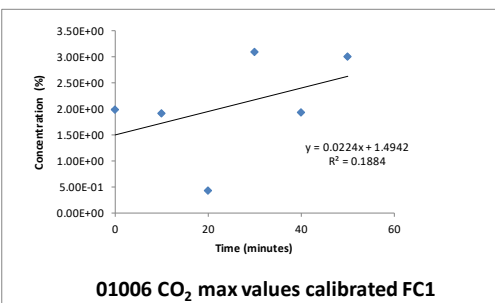
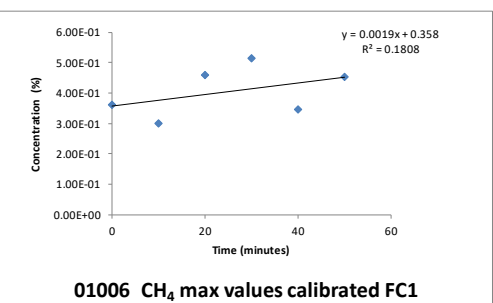
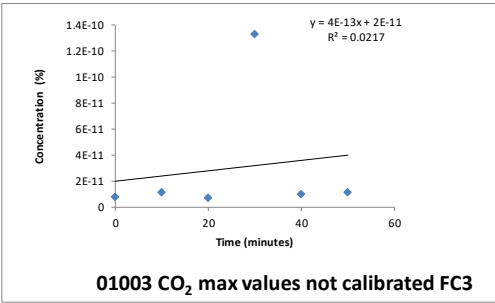
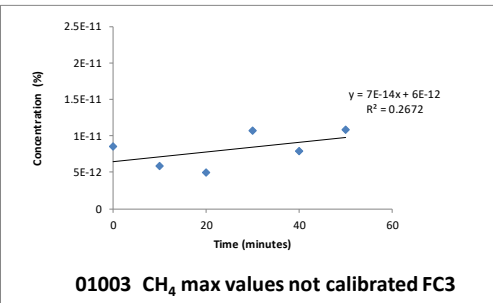
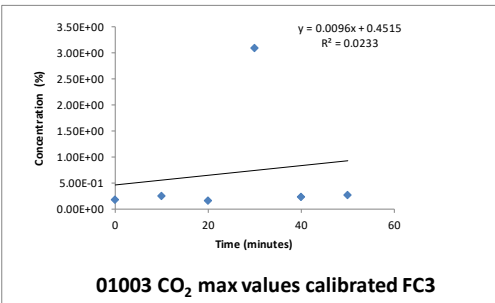
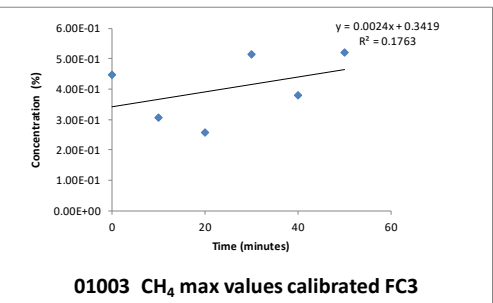
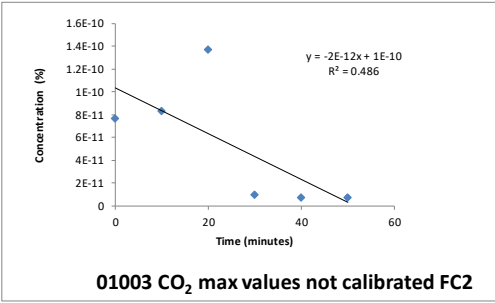
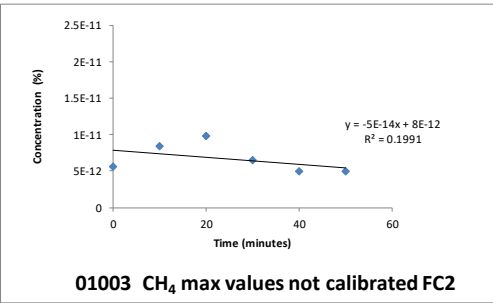
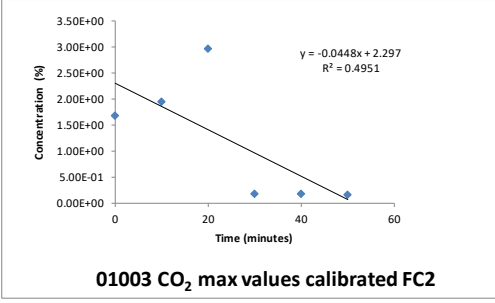
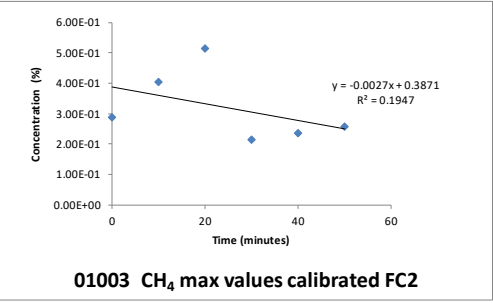
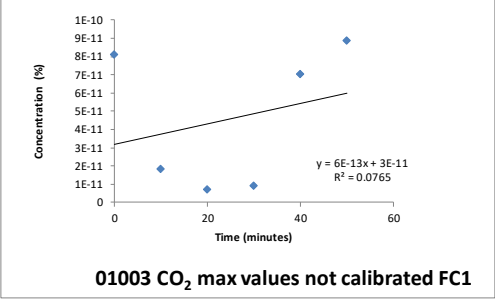
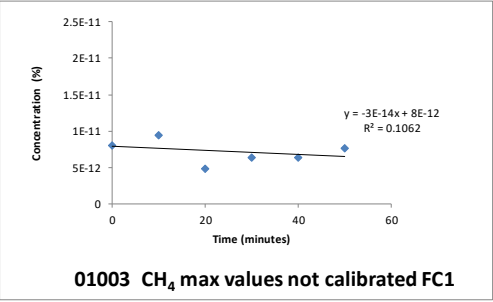
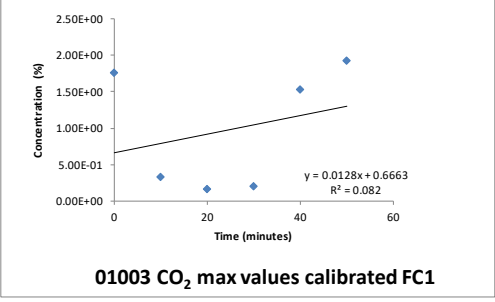
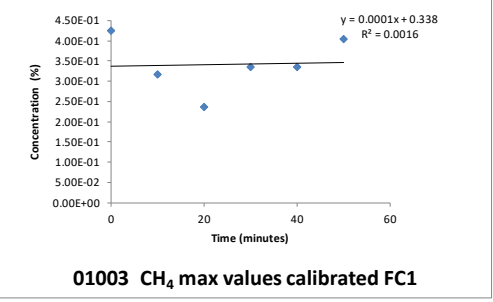


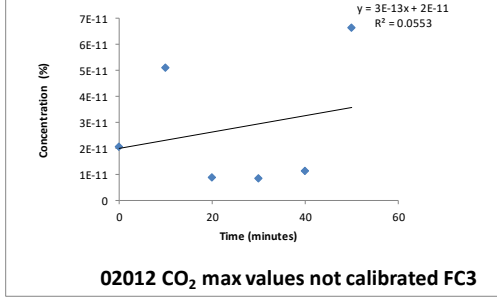
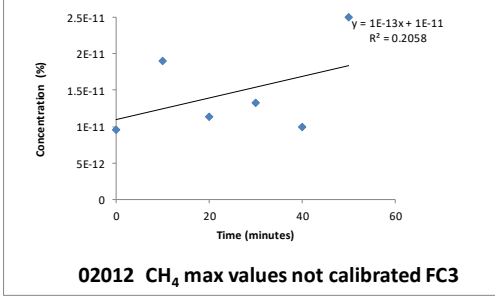
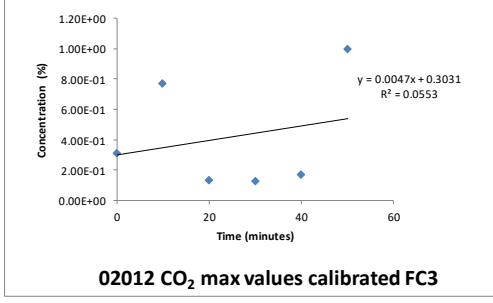
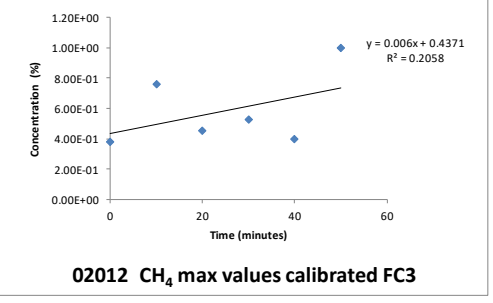
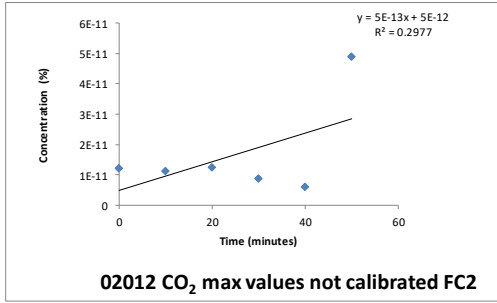
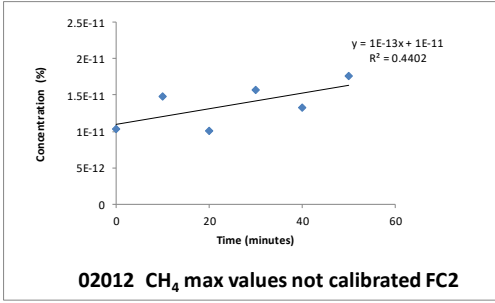
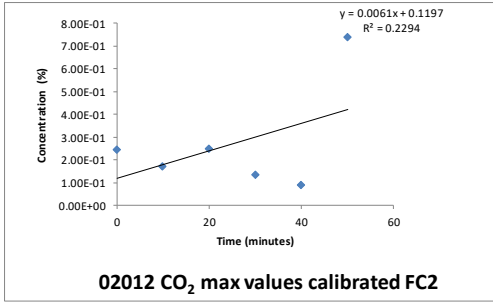
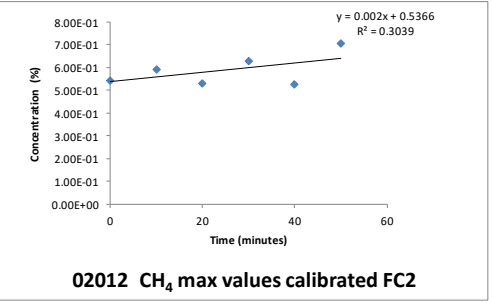
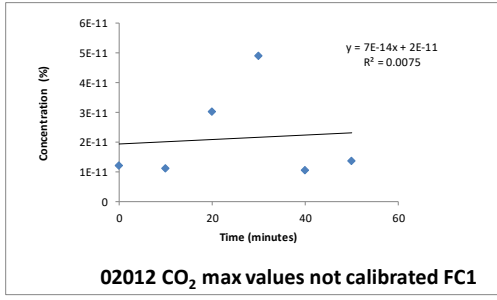
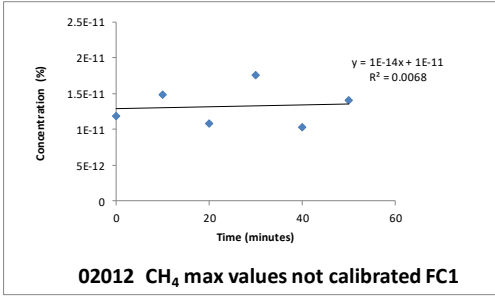
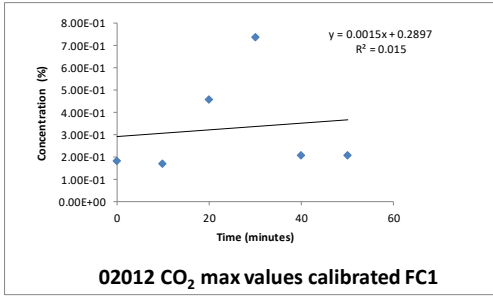
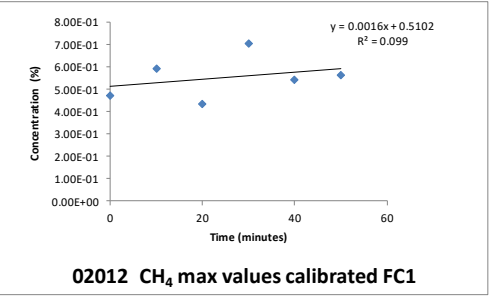
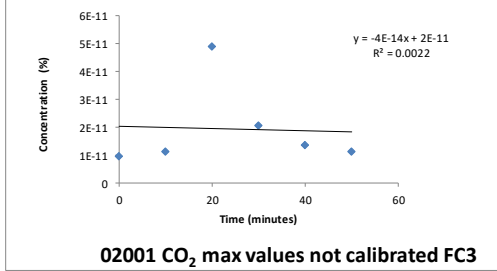
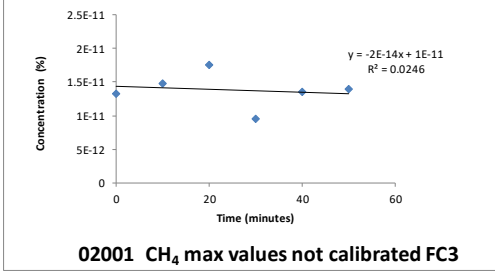
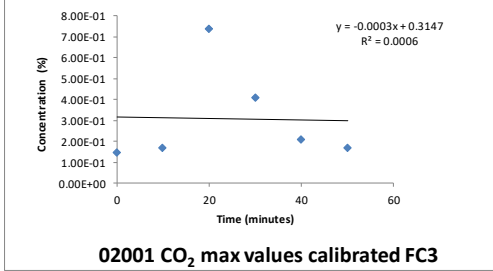
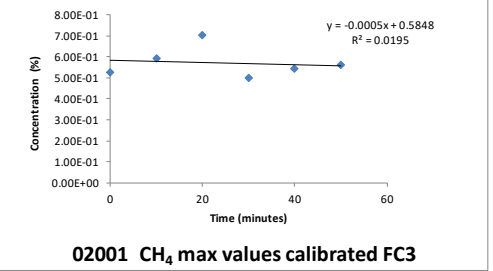
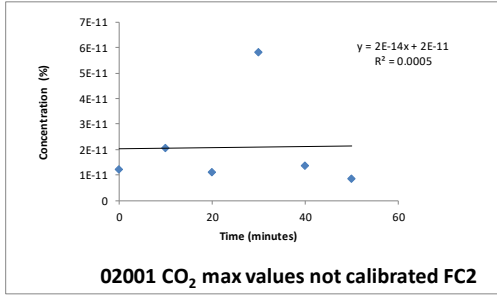
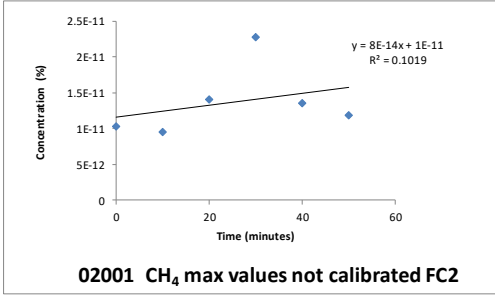
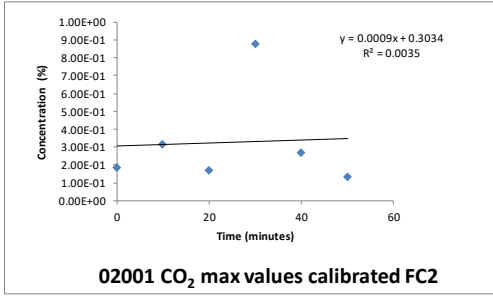
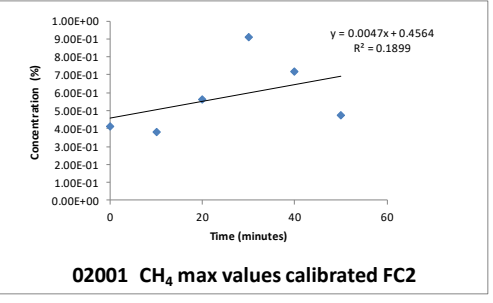
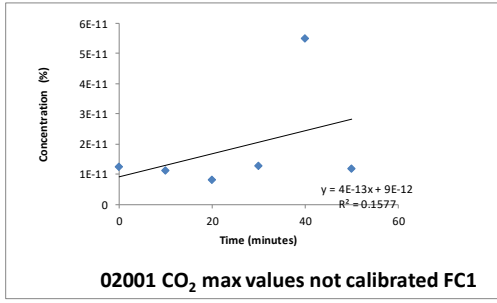
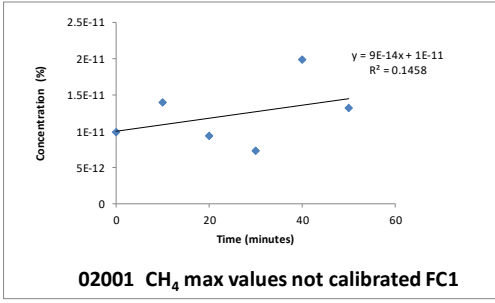
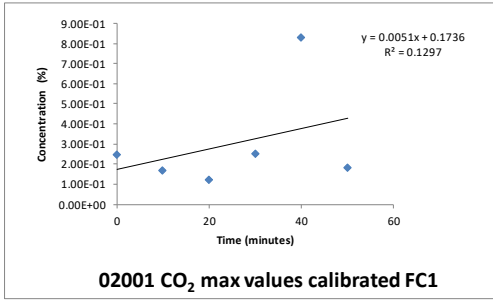
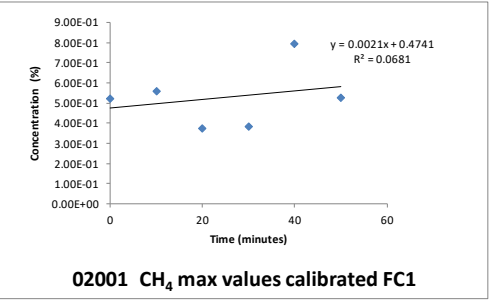
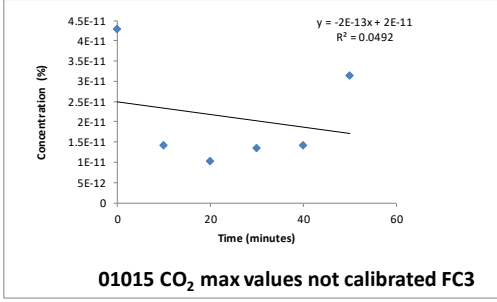
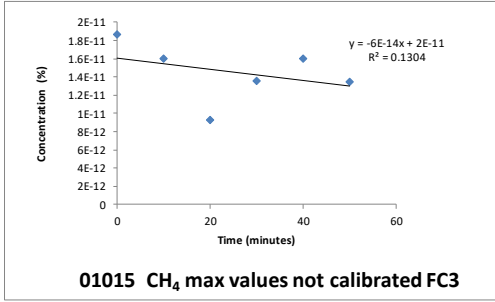
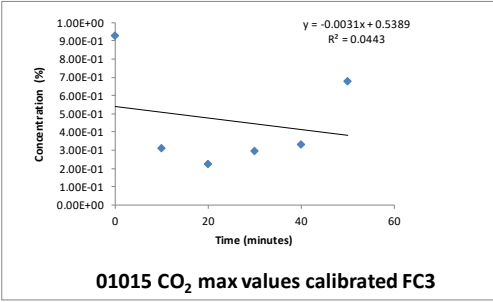
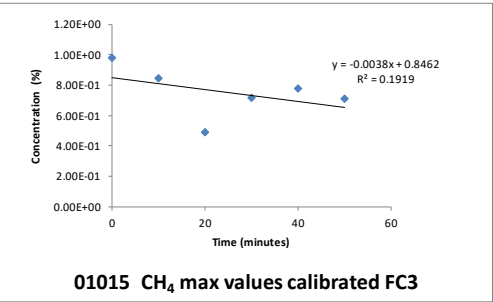
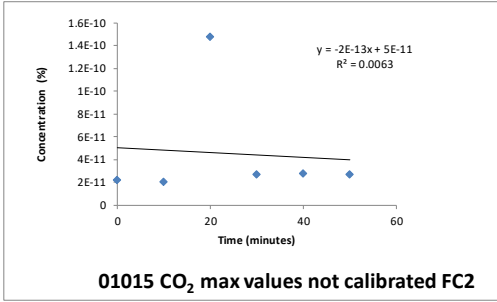
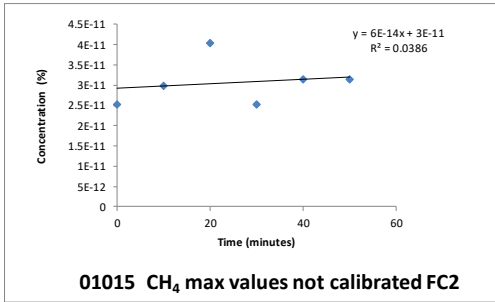
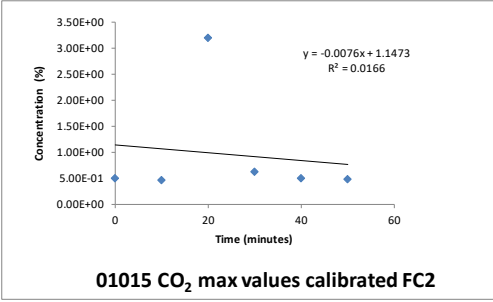
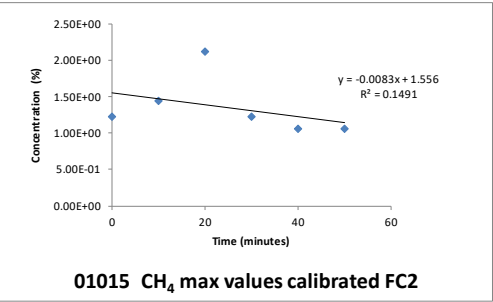
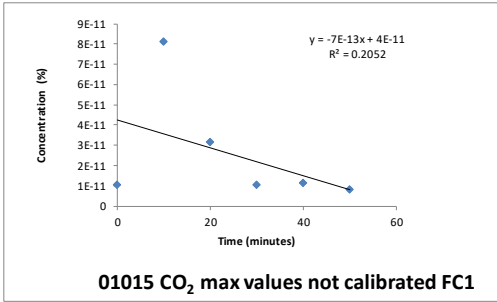
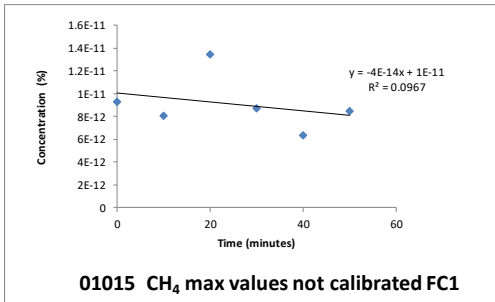
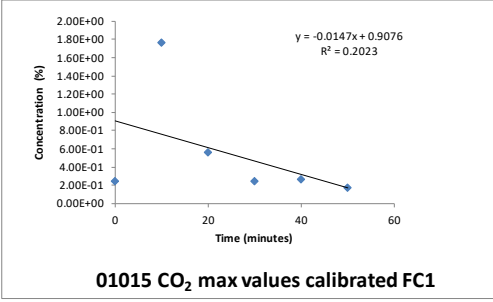
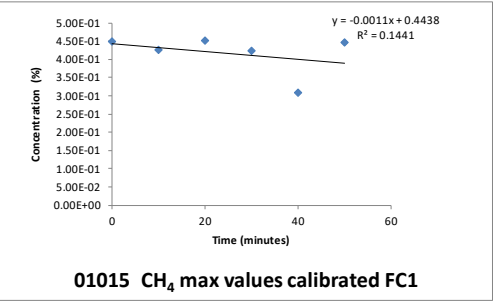


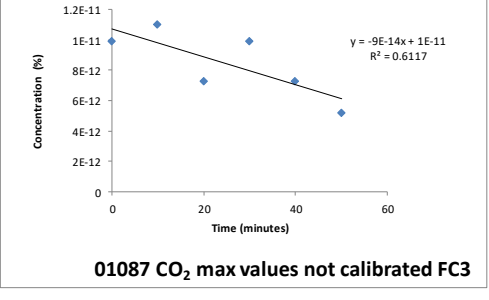
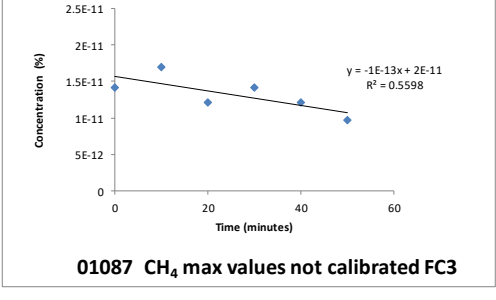
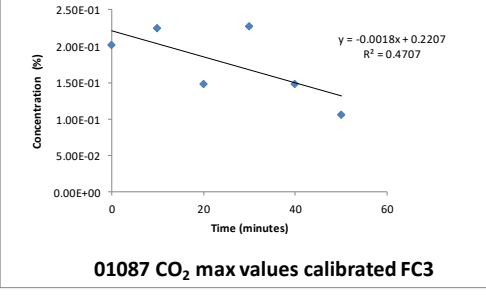
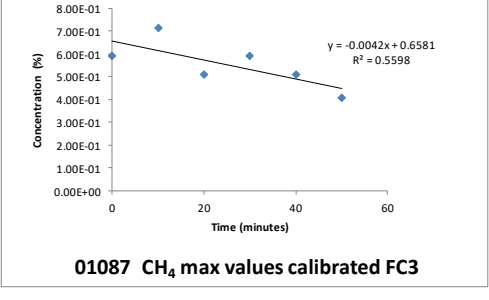
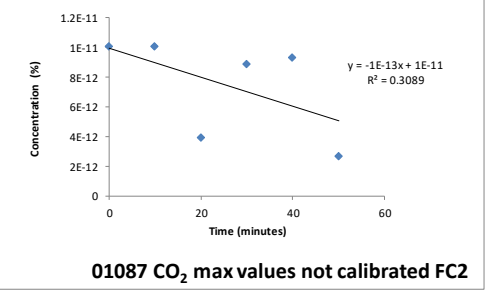
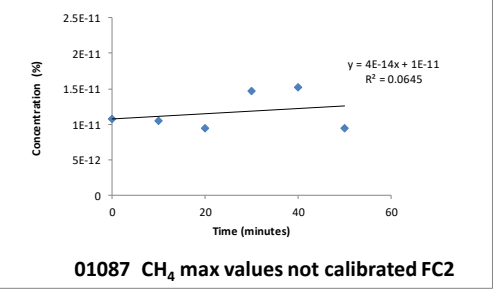
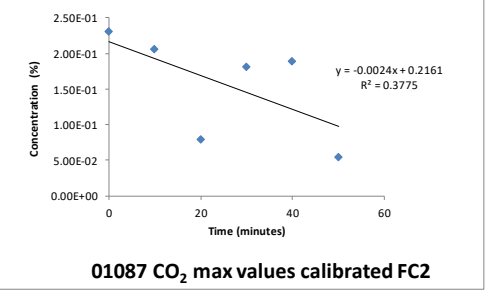
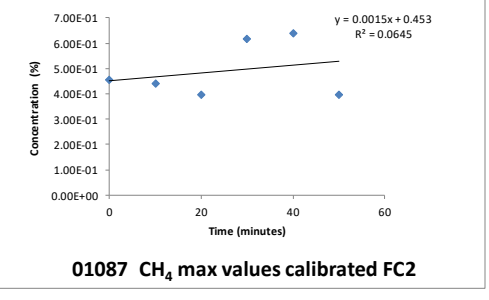
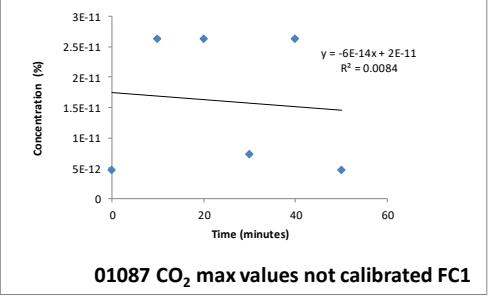
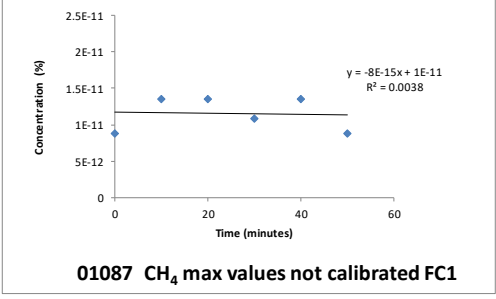
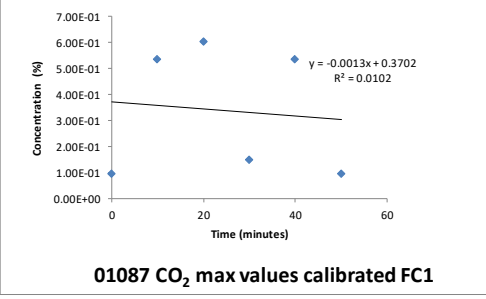
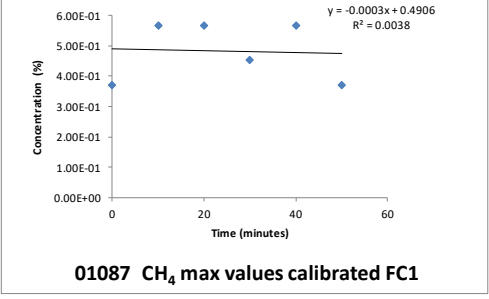
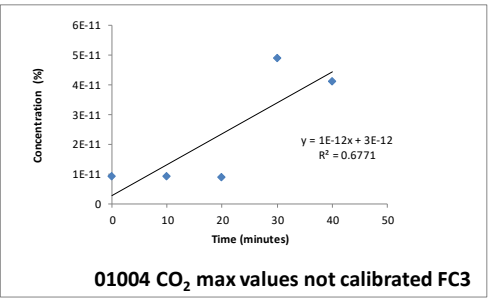
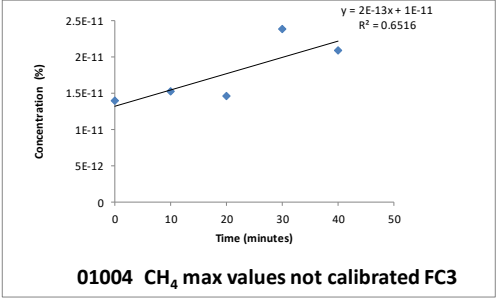
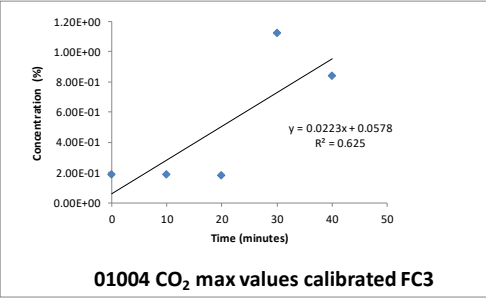
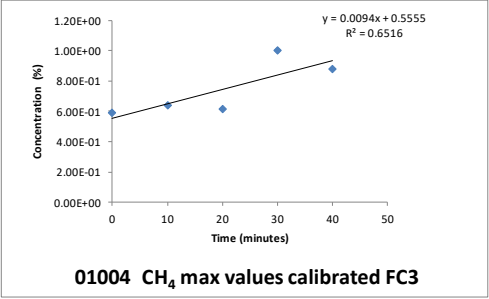
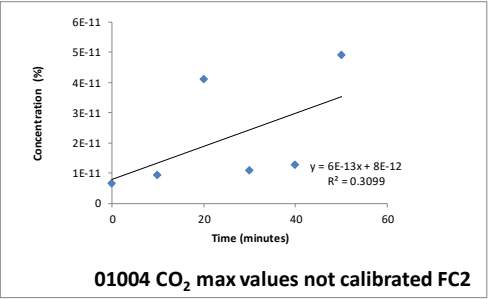
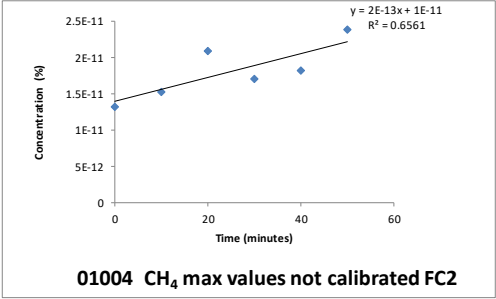
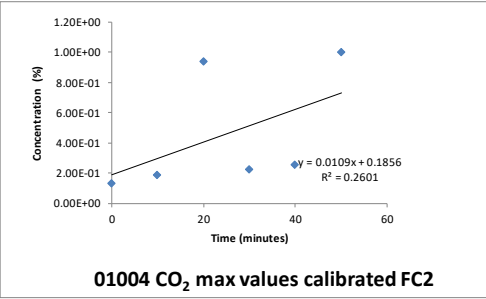
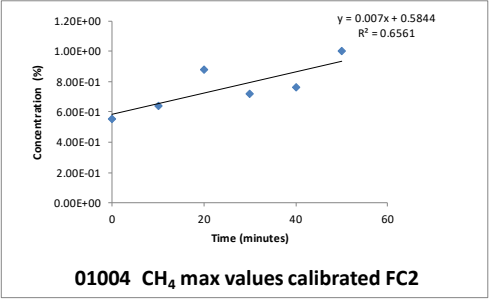
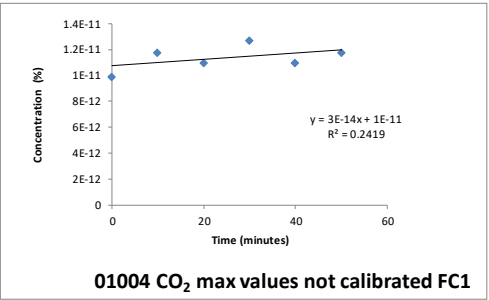
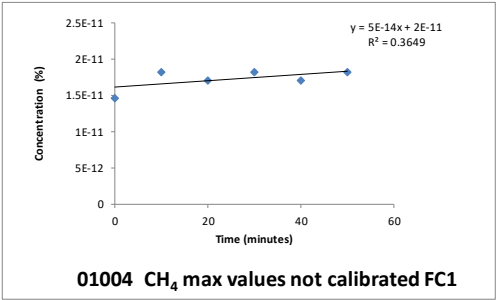
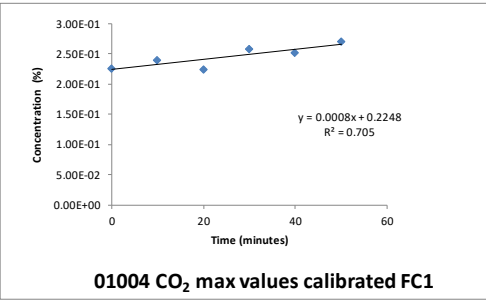
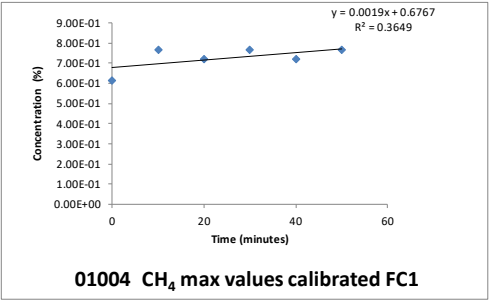


Appendix D: Comparison of air-flux samples of CO₂ and CH₄ concentration vs.time from data of 3 flux chambers per pond from year 2 data from the three catchments (numbering: 03 Barkby, 02 Stonton, and 01 Eye followed by pond number).









Appendix E:

Coefficient of variance
results from Eye Brook of
the air-flux sample data.

Eye Brook Pond number	Sample No		CO ₂	% of StDev	CH ₄	% of StDev		Eye Brook Pond number	Sample No		CO ₂	% of StDev	CH ₄	% of StDev
101	0	Mean	0.183		0.410			111	0	Mean	0.144		0.452	
101		Standard deviation	0.074		0.116			111		Standard deviation	0.074		0.090	
101		Coefficient of variance	0.407	40.7	0.283	28.3		111		Coefficient of variance	0.509	50.9	0.198	19.8
101	1	Mean	0.149		0.377			111	1	Mean	0.188		0.327	
101		Standard deviation	0.047		0.074			111		Standard deviation	0.077		0.089	
101		Coefficient of variance	0.318	31.8	0.197	19.7		111		Coefficient of variance	0.409	40.9	0.271	27.1
101	2	Mean	0.182		0.367			111	2	Mean	0.241		0.429	
101		Standard deviation	0.092		0.170			111		Standard deviation	0.088		0.139	
101		Coefficient of variance	0.504	50.4	0.462	46.2		111		Coefficient of variance	0.365	36.5	0.324	32.4
101	3	Mean	0.171		0.480			111	3	Mean	0.201		0.441	
101		Standard deviation	0.079		0.116			111		Standard deviation	0.079		0.189	
101		Coefficient of variance	0.462	46.2	0.241	24.1		111		Coefficient of variance	0.395	39.5	0.428	42.8
101	4	Mean	0.201		0.314			111	4	Mean	0.122		0.505	
101		Standard deviation	0.116		0.081			111		Standard deviation	0.055		0.210	
101		Coefficient of variance	0.576	57.6	0.257	25.7		111		Coefficient of variance	0.450	45.0	0.415	41.5
101	5	Mean	0.103		0.598			111	5	Mean	0.125		0.606	
101		Standard deviation	0.047		0.245			111		Standard deviation	0.046		0.170	
101		Coefficient of variance	0.453	45.3	0.410	41.0		111		Coefficient of variance	0.367	36.7	0.280	28.0
103	0	Mean	0.144		0.293			114	0	Mean	0.171		0.348	
103		Standard deviation	0.044		0.147			114		Standard deviation	0.067		0.354	
103		Coefficient of variance	0.307	30.7	0.503	50.3		114		Coefficient of variance	0.390	39.0	1.017	101.7
103	1	Mean	0.210		0.325			114	1	Mean	0.294		0.444	
103		Standard deviation	0.076		0.115			114		Standard deviation	0.120		0.438	
103		Coefficient of variance	0.362	36.2	0.354	35.4		114		Coefficient of variance	0.410	41.0	0.985	98.5
103	2	Mean	0.245		0.362			114	2	Mean	0.174		0.393	
103		Standard deviation	0.106		0.122			114		Standard deviation	0.058		0.400	
103		Coefficient of variance	0.434	43.4	0.337	33.7		114		Coefficient of variance	0.333	33.3	1.020	102.0
103	3	Mean	0.358		0.463			114	3	Mean	0.402		0.309	
103		Standard deviation	0.110		0.137			114		Standard deviation	0.106		0.313	
103		Coefficient of variance	0.307	30.7	0.295	29.5		114		Coefficient of variance	0.263	26.3	1.012	101.2
103	4	Mean	0.222		0.586			114	4	Mean	0.172		0.774	
103		Standard deviation	0.076		0.183			114		Standard deviation	0.064		0.781	
103		Coefficient of variance	0.342	34.2	0.312	31.2		114		Coefficient of variance	0.373	37.3	1.009	100.9
103	5	Mean	0.339		0.420			114	5	Mean	0.221		0.339	
103		Standard deviation	0.110		0.124			114		Standard deviation	0.060		0.347	
103		Coefficient of variance	0.323	32.3	0.296	29.6		114		Coefficient of variance	0.273	27.3	1.023	102.3
104	0	Mean	0.128		0.565			115	0	Mean	0.164		0.433	
104		Standard deviation	0.044		0.118			115		Standard deviation	0.050		0.184	
104		Coefficient of variance	0.344	34.4	0.210	21.0		115		Coefficient of variance	0.308	30.8	0.425	42.5
104	1	Mean	0.106		0.529			115	1	Mean	0.121		0.665	
104		Standard deviation	0.050		0.190			115		Standard deviation	0.057		0.239	
104		Coefficient of variance	0.467	46.7	0.359	35.9		115		Coefficient of variance	0.473	47.3	0.359	35.9
104	2	Mean	0.110		0.555			115	2	Mean	0.117		0.230	
104		Standard deviation	0.038		0.194			115		Standard deviation	0.050		0.088	
104		Coefficient of variance	0.349	34.9	0.349	34.9		115		Coefficient of variance	0.425	42.5	0.382	38.2
104	3	Mean	0.134		0.535			115	3	Mean	0.170		0.417	
104		Standard deviation	0.050		0.168			115		Standard deviation	0.064		0.134	
104		Coefficient of variance	0.375	37.5	0.314	31.4		115		Coefficient of variance	0.374	37.4	0.322	32.2
104	4	Mean	0.093		0.686			115	4	Mean	0.117		0.447	
104		Standard deviation	0.057		0.186			115		Standard deviation	0.060		0.136	
104		Coefficient of variance	0.617	61.7	0.271	27.1		115		Coefficient of variance	0.516	51.6	0.305	30.5
104	5	Mean	0.084		0.720			115	5	Mean	0.103		0.414	
104		Standard deviation	0.031		0.240			115		Standard deviation	0.049		0.174	
104		Coefficient of variance	0.375	37.5	0.334	33.4		115		Coefficient of variance	0.476	47.6	0.421	42.1
106	0	Mean	0.096		0.570			116	0	Mean	0.227		0.576	
106		Standard deviation	0.052		0.254			116		Standard deviation	0.109		0.192	
106		Coefficient of variance	0.541	54.1	0.446	44.6		116		Coefficient of variance	0.481	48.1	0.333	33.3
106	1	Mean	0.298		0.351			116	1	Mean	0.293		0.650	
106		Standard deviation	0.118		0.113			116		Standard deviation	0.073		0.160	
106		Coefficient of variance	0.397	39.7	0.321	32.1		116		Coefficient of variance	0.248	24.8	0.246	24.6
106	2	Mean	0.148		0.469			116	2	Mean	0.295		0.507	
106		Standard deviation	0.052		0.157			116		Standard deviation	0.122		0.143	
106		Coefficient of variance	0.354	35.4	0.335	33.5		116		Coefficient of variance	0.413	41.3	0.282	28.2
106	3	Mean	0.200		0.458			116	3	Mean	0.401		0.569	
106		Standard deviation	0.066		0.110			116		Standard deviation	0.163		0.143	
106		Coefficient of variance	0.329	32.9	0.241	24.1		116		Coefficient of variance	0.405	40.5	0.251	25.1
106	4	Mean	0.235		0.461			116	4	Mean	0.145		0.562	
106		Standard deviation	0.106		0.122			116		Standard deviation	0.076		0.161	
106		Coefficient of variance	0.452	45.2	0.264	26.4		116		Coefficient of variance	0.524	52.4	0.287	28.7
106	5	Mean	0.449		0.385			116	5	Mean	0.422		0.654	
106		Standard deviation	0.117		0.084			116		Standard deviation	0.083		0.171	
106		Coefficient of variance	0.261	26.1	0.218	21.8		116		Coefficient of variance	0.197	19.7	0.262	26.2
107	0	Mean	0.195		0.461			117	0	Mean	0.179		0.409	
107		Standard deviation	0.094		0.103			117		Standard deviation	0.107		0.174	
107		Coefficient of variance	0.484	48.4	0.224	22.4		117		Coefficient of variance	0.597	59.7	0.426	42.6
107	1	Mean	0.297		0.685			117	1	Mean	0.263		0.239	
107		Standard deviation	0.136		0.111			117		Standard deviation	0.081		0.073	
107		Coefficient of variance	0.456	45.6	0.162	16.2		117		Coefficient of variance	0.308	30.8	0.307	30.7
107	2	Mean	0.170		0.644			117	2	Mean	0.161		0.642	
107		Standard deviation	0.050		0.188			117		Standard deviation	0.063		0.190	
107		Coefficient of variance	0.294	29.4	0.291	29.1		117		Coefficient of variance	0.389	38.9	0.296	29.6
107	3	Mean	0.172		0.718			117	3	Mean	0.143		0.447	
107		Standard deviation	0.075		0.196			117		Standard deviation	0.044		0.200	
107		Coefficient of variance	0.438	43.8	0.273	27.3		117		Coefficient of variance	0.306	30.6	0.446	44.6
107	4	Mean	0.403		0.667			117	4	Mean	0.152		0.319	
107		Standard deviation	0.122		0.185			117		Standard deviation	0.055		0.178	
107		Coefficient of variance	0.302	30.2	0.277	27.7		117		Coefficient of variance	0.359	35.9	0.559	55.9
107	5	Mean	0.415		0.695			117	5	Mean	0.188		0.396	
107		Standard deviation	0.120		0.247			117		Standard deviation	0.044		0.173	
107		Coefficient of variance	0.289	28.9	0.356	35.6		117		Coefficient of variance	0.236	23.6	0.438	43.8
108	0	Mean	0.170		0.355			118	0	Mean	0.154		0.344	
108		Standard deviation	0.128		0.144			118		Standard deviation	0.066		0.101	
108		Coefficient of variance	0.758	75.8	0.407	40.7		118		Coefficient of variance	0.427	42.7	0.294	29.4
108	1	Mean	0.144		0.465			118	1	Mean	0.122		0.241	
108		Standard deviation	0.089		0.189			118		Standard deviation	0.030		0.178	
108		Coefficient of variance	0.613	61.3	0.407	40.7		118		Coefficient of variance	0.250	25.0	0.740	74.0
108	2	Mean	0.119		0.572			118	2	Mean	0.168		0.308	
108		Standard deviation	0.084		0.210			118		Standard deviation	0.032		0.181	
108		Coefficient of variance	0.705	70.5	0.368	36.8		118		Coefficient of variance	0.189	18.9	0.588	58.8
108	3	Mean	0.173		0.361			118	3	Mean	0.237		0.231	
108		Standard deviation	0.083		0.134			118		Standard deviation	0.084		0.124	
108		Coefficient of variance	0.481	48.1	0.370	37.0		118		Coefficient of variance	0.355	35.5	0.539	53.9
108	4	Mean	0.127		0.383			118	4	Mean	0.159		0.476	
108		Standard deviation	0.056		0.117			118		Standard deviation	0.042		0.139	
108		Coefficient of variance	0.436	43.6	0.305	30.5		118		Coefficient of variance	0.263	26.3	0.291	29.1
108	5	Mean	0.172		0.252			118	5	Mean	0.275		0.249	
108		Standard deviation	0.070		0.062			118		Standard deviation	0.115		0.081	
108		Coefficient of variance	0.405	40.5	0.245	24.5		118		Coefficient of variance	0.421	42.1	0.325	32.5
109	0	Mean	0.144		0.338			119	0	Mean	0.127		0.395	
109		Standard deviation	0.089		0.127			119						