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**From Source to Sea: Spatial and Temporal Fluxes of
the Greenhouse Gases N₂O, CO₂ and CH₄ in the River
Tay Catchment**

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Declaration

I declare that this thesis and the papers within it have been composed by myself and that no part of this thesis has been submitted for any other degree or qualification. The work described is my own unless otherwise stated.

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March 2013

Abstract

River networks act as a link between components of the terrestrial landscape, such as soils and groundwater, with the atmosphere and oceans, and are now believed to contribute significantly to global budgets of carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O). The idea of rivers being an inert conduit for carbon and nitrogen to reach the coast has been challenged recently, with considerable processing of carbon and nitrogen occurring in both the water column and bed sediments in the various aquatic components that make up a river network, including lakes, streams, rivers and estuaries. Although understanding of the cycling of carbon and nitrogen has improved markedly in the last 20 years, there is still much uncertainty regarding the production and emission of greenhouse gases (GHGs) linked to this processing across river catchments and few studies have quantified GHG fluxes from source to sea.

Therefore this study aimed to a) understand the spatial and temporal saturations and fluxes of GHGs from both the freshwater River Tay catchment (Scotland) and the River Tay estuary, and b) understand what controls the production of GHGs within both a freshwater lake and across multiple sites in the freshwater river using laboratory incubations of sediment.

Hotspots of in-stream production and emission were evident both in the freshwater catchment and the estuary, with significant temporal and spatial variability in saturation and emission (density) for CH₄, CO₂ and N₂O.

CH₄ emission densities, across the freshwater river sites, ranged from 1720 to 15500 μg C m⁻² d⁻¹ with a freshwater catchment wide mean of 4640 μg C m⁻² d⁻¹, and in general decreased from upland to lowland sites along the main river stem, with notable peaks of emission in a lowland tributary and at the outflow of a lowland loch. This corresponds well with the main drivers of spatial variability which include allochthonous inputs from gas rich soil waters and *in-situ* production in fine grained organic rich sediments. CH₄ production was observed to be higher in the lowland tributaries (R. Isla 4500 μg C m⁻² d⁻¹) compared to main-stem river sites both in the lowland river (129 μg C m⁻² d⁻¹) and upland river which displayed an uptake of CH₄ (-1210 μg C m⁻² d⁻¹). The main driver of spatial variability in CH₄ production rates was the quality of the sediment, as production was higher in fine grained sediments rich in carbon compared to sand and gravels with a low carbon content. CH₄ production also varied seasonally, with temperature and seasonal variation in sediment quality as the predominant driving factors.

CO₂ emission densities across the freshwater catchment ranged from 517 to 2550 mg C m⁻² d⁻¹ with a catchment mean flux density of 1500 mg C m⁻² d⁻¹. Flux densities on the whole increased along the main river stem from upland sites to lowland sites, with higher fluxes in lowland tributaries. Seasonally, CO₂ flux density was highest in late summer and autumn and lowest in winter at most sites, highlighting the importance in seasonal environmental controls such as temperature, light, and substrate availability. Production rates in the sediment increased from upland to lowland sites with highest production rates evident in the lowland tributaries, and in autumn sediment samples.

N₂O emission density also showed considerable spatial and seasonal variation across the catchment with flux densities ranging from 176 to 1850 µg N m⁻² d⁻¹ with a mean flux of 780 µg N m⁻² d⁻¹. Mean fluxes were highest in the lowland tributaries and lowest in the upland river with sediment experiments finding similar spatial variation in N₂O production. On the whole, in-stream N₂O production and emission across the freshwater catchment was driven by increases in nutrient concentration (NO₃⁻, NH₄⁺) which in turn was related to the proportion of agricultural landuse.

The saturation and emission of GHGs also varied substantially both spatially and temporally in the River Tay estuary, with a mean emission density of 2790 µg CH₄-C m⁻² d⁻¹, 990 mg CO₂-C m⁻² d⁻¹ and 162 µg N₂O-N m⁻² d⁻¹. The spatial variability of GHG concentrations and emission densities in the estuary were predominantly controlled by the balance between lateral inputs (from tidal flushing of surrounding intertidal areas), *in-situ* microbial production/consumption (both in the water column and bed sediments) and physical mixing/loss processes. Although emission densities of CH₄, CO₂ and N₂O appear low compared to the freshwater river, this is because the estuary is emitting large quantities of gas in the middle and outer estuary, for example net annual emission of N₂O increased from 84.7 kg N₂O-N yr⁻¹ in the upper freshwater section of the estuary to 888 kg N₂O-N yr⁻¹ in the middle estuary section, then decreased to 309 kg N₂O-N yr⁻¹ in the saltwater lower estuary.

Overall, this study has shown that both dissolved and aerial fluxes of GHGs vary markedly both spatially and temporal from source to sea in a temperate river catchment, with hotspots of in-stream production and emission across the river catchment. The catchment (river, lake and estuary) was a smaller source of CO₂, CH₄ and N₂O emission (total emission and by area) compared to other highly polluted aquatic systems both in the UK and globally.

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1 Introduction

1.1. Research rationale and aims

The cycling of nitrogen and especially carbon in aquatic ecosystems has attracted renewed interest over the last few years; especially in estuaries (Barnes and Upstill-Goddard, 2011; Borges and Abril, 2011); and across river catchments (Battin et al., 2008, 2009; Cole et al., 2007; Garnier et al., 2009; Tranvik et al., 2009). In the recent past, it was thought that the only significant contributions to the global carbon cycle were from the flux and storage of carbon between the atmosphere, terrestrial and oceanic pools (Trimmer et al., 2012) but it is now thought that freshwater ecosystems significantly contribute to the global carbon cycle (Battin et al., 2008, 2009; Cole et al., 2007; Tranvik et al., 2009). Moreover, it was believed that aquatic environments merely acted as a conduit for carbon, 'piping' it to the oceans, with the cycling of carbon in freshwaters largely ignored by the global models used by the Inter-governmental Panel on Climate Change (IPCC) (IPCC, Solomon Working Group, 2007). However recently it has been estimated that a significant portion of global terrestrial C inputs is transformed in freshwaters, with 44 % entering the atmosphere as gaseous C, with a further 22% buried in sediments and the remainder transported to the sea (Battin et al., 2009; Aufdenkampe et al., 2011) where it may undergo further substantial processing in the mixing zone between fresh and saltwater in estuaries (Borges and Abril, 2011).

On the whole this indicates that there is substantial need for better understanding of biogeochemical cycling of carbon in catchments. However, the carbon cycle is not processed in isolation in aquatic environments, being interconnected with the cycling of nitrogen (N) and phosphorus (P) with primary production requiring a balance of C, N and P.

Whilst the cycling of N has received a wealth of attention in the literature, the majority of studies have focussed on the potential removal of anthropogenic nitrate (NO_3^-) via the microbial process of denitrification, rather than focusing on the whole N cycle (Burgin and Hamilton, 2007). For example, it has been estimated recently that rivers denitrify up to 30% of the N they receive, and overall freshwaters (including groundwaters) account for up to 20% of global denitrification (Wollheim et al., 2008). Thus like with carbon, aquatic ecosystems do not just 'pipe' N through the landscape, but facilitate substantial transformation (Battin et al., 2009; Davidson and Seitzinger 2006; Garnier et al., 2009;

Groffman et al., 2006; Mulholland et al., 2008). Moreover, it is known that the processes that are responsible for N transformation require a labile source of carbon, yet the vast majority of studies focusing on these macronutrients study them in isolation or over small spatial scales (such as an upland river, a lake or an estuary). To obtain a more complete understanding of global C and N cycling it is important to understand fluxes and transformations in connected inland waters and estuaries across whole landscapes.

Moreover, the integration of macronutrient cycles is now a key science area within the UK, with the NERC Macronutrient Cycles Programme established to understand the interactions between N, P and C cycles (Whitehead and Crossman 2012).

Currently the key uncertainties in the cycling of C and N in aquatic environments revolve around the progressive downstream biotic and abiotic transformation of both nutrients. It is now known that there are a number of processes that transform and remove a portion of C and N from the inland aquatic environment before they reach the sea but it is still not clear how these processes differ either in one component or between separate components of the aquatic environment and how much the cycling of C and N are connected. As mentioned previously, these processes can transform C and N into different species but importantly, can produce the greenhouse gases (GHGs) CO₂, methane (CH₄) and nitrous oxide (N₂O) which are subsequently released into the atmosphere. Concentrations of GHGs in the atmosphere have increased dramatically over the last 100 years (IPCC 2007). For example, atmospheric concentrations of CO₂, CH₄ and N₂O have increased from pre-industrial concentrations of 280 ppm, 700 ppb, and 270 ppb, to the current concentrations of 389 ppm, 1808 ppb and 324 ppb respectively (WMO 2011). Although the research in this area is abundant, there is still uncertainty relating to specific emission sources, especially those from aquatic environments.

The production of GHGs in aquatic environments has been mainly attributed to microbial processing of C and N in sediments and on particles suspended in the water column. However specific knowledge on these processes is currently limited and there is a wide range of studies that suggest that the interaction between processes both in the water column and in the sediments are inherently complex, and subject to a wide range of biotic and abiotic controls. As yet it is not known how the emission of the three GHGs differs between aquatic environments, and whether subtleties related to individual river catchments play an important role in overall flux rates to the atmosphere.

Of the three GHGs, the emissions of CH₄ from aquatic environments (especially rivers) is the least studied and poorly defined but freshwaters on the whole are thought to significantly contribute to global CH₄ budgets (Bastviken et al., 2011; Tranvik et al., 2009). A recent estimation of freshwater CH₄ emission (0.65 Pg C yr⁻¹ as CO₂ eq; Bastviken et al., 2011) combined with that for CO₂ (1.4 Pg C yr⁻¹ as CO₂ eq; Tranvik et al., 2009) corresponds to 79% of the estimated land GHG sink, confirming that freshwater are important in the global GHG balance. Moreover, this indicates that the terrestrial GHG sink could be overestimated (Bastviken et al., 2011) and that new data on GHG release from freshwaters are required in future revisions of net GHG fluxes.

Overall, whilst it is not true to say the biogeochemical cycling of C and N in freshwater ecosystems has not been recognised and studied extensively there is still a significant need to quantify and understand the processing, especially the production of GHGs, along a river continuum, from source to sea.

This thesis therefore uses the whole catchment approach to study C and N cycling with specific objectives to:

1. To quantify and understand spatial and temporal GHG dissolved concentrations and atmospheric fluxes in the River Tay estuary (Paper I)
2. To quantify and understand spatial and seasonal GHG dissolved concentrations and atmospheric fluxes in the freshwater River Tay catchment from uplands to lowland (Paper II).
3. To investigate the production of GHG from loch sediments (III)
4. To investigate the spatial and seasonal production of GHG from river sediments (IV)

The overall aim is to quantify and compare the spatial and temporal fluxes and production of GHGs from source to sea in the River Tay Catchment.

1.2. Scientific Background

Fluvial networks link diverse components of the landscape, such as vegetation, soils and groundwater, with the atmosphere and oceans resulting in a conduit for nutrient transformation, transportation and storage. Importantly, streams and rivers act as a 'highway' for fast return of nutrients from various long term and short term stores, where a suite of processes (both physical and biological) work to transform and transport nutrients along a continuum of aquatic environments, ultimately redistributing terrestrially derived nutrients to the oceans and the atmosphere.

1.2.1 Carbon

The global cycles of the carbon (C) is comprised of three main compartments: land, ocean and atmospheric pools. Whilst a large proportion of carbon is found either in bedrock or sediments, the fluxes from these pools are small compared to the fluxes between the atmosphere, biosphere, soils, freshwaters, and ocean (Battin et al., 2009). The cycling of global carbon, over geologic timescales is mainly controlled by volcanic CO₂ outgassing, contact metamorphism, tectonic uplift, erosion and silicate weathering (Scaller et al., 2011; Berner & Kothavala, 2001). Over shorter timescales, carbon mainly cycles through the biosphere transforming inorganic carbon to organic carbon (Reich, 2010), some of which is re-mineralized back to inorganic carbon, but with considerable amounts stored in various long term and short term stores, such as in soil organic matter (peatlands, mineral soils etc) and in river, lake and ocean sediments (Schlesinger, 1997).

Present day carbon cycling is therefore dominated by biological transformations, performed by either autotrophic or heterotrophic organisms. The former synthesises chemical energy from either sunlight (photoautrophic) or from the reduction of chemical compounds (chemoautrophic), while heterotrophic organisms utilises external organic compounds for an energy source. Together these two groups of organisms perform equally fundamental roles in carbon cycling: photosynthesis converting CO₂ and water to chemical energy and oxygen; and respiration where, in the reverse of photosynthesis, organic matter is converted back to CO₂, water and energy whilst consuming oxygen.

The difference between photosynthesis and respiration on an organismal level is known as net primary production (NPP; Chapin et al., 2006) and overall is a close approximation of biomass growth. Some of this biomass is lost to heterotrophic organisms through respiration, or through necrosis. Overall the difference between

ecosystem gross primary production, GPP, and total ecosystem respiration (ER) is known as net ecosystem production (NEP) and it is often the case that NEP indicates if a system is a sink or source of carbon, especially in terrestrial systems. However in aquatic systems, export of carbon from the terrestrial environment plays a significant role in carbon balancing (Chapin et al., 2006).

Carbon transportation (total and dissolved; organic and inorganic) through these aquatic networks represents a key link in the global carbon cycle (Brunet et al., 2005; Richey 2005; Battin et al., 2009; Aufdenkampe et al., 2011). Carbon is found in aquatic environments in various forms and is sourced accordingly; total organic carbon (TOC), made up of both dissolved and particulate organic carbon (DOC and POC) is produced through the mobilisation of natural and anthropogenic organic matter which is transferred to aquatic environments. TOC can be derived from allochthonous sources such as from soil organic matter which is solubilised by successive oxidation (Kleber & Johnson, 2010) and both solubilised and particulate carbon is transported to groundwaters and other aquatic environments (Aitkenhead-Peterson et al., 2003). Also from autochthonous sources (derived from in-stream generation of organic material) and from anthropogenic sources (such as effluents and industrial discharges). Total inorganic carbon (TIC), comprised of dissolved and particulate inorganic carbon (DIC and PIC) in freshwaters are found in the forms of HCO_3^- , CO_3^{2-} and dissolved gaseous free CO_2 which are proportionally available, dependent on pH. The concentration of DIC in aquatic systems is determined by four main processes including (1) inputs of gas-laden ground and soil waters; (2) in-stream respiration both by aquatic plants and through heterotrophic respiration of organic matter; (3) weathering of carbonate and silicate rocks within the catchment in soils and underlying bedrock; and (4) atmospheric exchange through outgassing and drawdown depending on the water/atmospheric equilibrium. The importance of each of these DIC sources is subsequently dependent on a number of factors associated with the individual catchment characteristics, including the surrounding bedrock geology, catchment soils, topography, hydrology and position within the river network (Hope et al., 1994; Jones and Mulholland 1998b).

Concentrations of CH_4 in freshwaters are mainly determined by the activity of obligate anaerobic bacteria in riparian soils and stream sediments (Jones and Mulholland, 1998c from Hope 1994) and are, therefore, sourced from both terrestrial soils, especially upland areas of peat, and within-stream production.

Generally freshwater aquatic systems act as net sources of CO₂ to the atmosphere. This trend of CO₂ super-saturation has been recorded in streams (Hope et al., 2001; Billett et al., 2004) lakes (Kortelainen et al., 2006), rivers (Jones and Mulholland 1998; Abril et al., 2000; Cole and Caraco, 2002) and estuaries (Abril and Borges, 2004; 2011). The flux of CO₂ through and out of aquatic systems is directly linked with the respiration of organic carbon in the sediments and on particles suspended in the water column by microbial respiration and photochemistry or through the addition of super-saturated soil and groundwater (Abril and Borges 2004; Dawson et al. 2001).

Organic carbon (either transported as particulate or dissolved) is key to in-stream processing and loss of C to the atmosphere. Particulate organic material, originates predominantly from allochthonous sources such as soils, and plant materials and is transported downstream and deposited in low energy sites in fine sediment patches. This sediment OM is resuspended during floods, and re-deposited through the river continuum where it may be buried or further decomposed and physically broken down by biota (producing DOC) (Evans and Warburton 2001, 2005; Monaghan et al., 2001). In rivers rich in organic C, decomposition of dissolved organic material by heterotrophic organisms creates a net heterotrophic system (respiration > photosynthesis) and thus acts as a source of CO₂ to the atmosphere (Dawson et al., 2001, 2004).

In the case of CO₂ additions from soil waters, this source is primarily controlled by soil properties such as porosity and moisture levels (Skiba and Cresser 1991; Hope et al., 2004). For example, in free draining, well aerated mineral soils, CO₂ is mainly lost to the atmosphere, but in organic rich, water saturated peaty soils, CO₂ (and CH₄) dissolve into soil pore waters and is more readily transported to surface waters (Billett et al., 2004). This elevated gas flux has been shown to be rapidly out-gassed once in the main stream channel, through atmospheric equilibration processes; the speed and effectiveness of which is dominated by the water turbulence within the receiving stream (Billett et al., 2004).

Methane is found in super-saturation in most freshwaters including streams, rivers, lakes and estuaries and recently Bastviken et al. (2011) estimated that freshwaters emit up to 103 Tg CH₄ yr⁻¹, corresponding to 0.65 Pg C as CO₂ equivalent (100 year period). Like CO₂ it is formed through the decomposition of organic carbon, but it is an anaerobic process occurring in oxygen-depleted zones, both in terrestrial and aquatic systems. Methane produced in terrestrial soils can be discharged into streams as super-saturated

soil and groundwaters, which like CO₂ is rapidly out-gassed (Billett et al., 2001, 2004). In aquatic environments CH₄ production is mainly confined to oxygen deprived layers in sediments (dependent on the oxygen saturation in the water column) and is produced by anaerobic methanogenic archaea, with activity determined by the supply of organic matter, availability of electron acceptors and temperature (Kiene 1991). Even after the production of CH₄ in-stream, there is no guarantee of emission from the water to the atmosphere with many studies reporting the oxidation of a large percentage of CH₄ in aerobic sediment layers and the water column, particularly in lakes (Huttenen et al., 2003) and estuaries (Abril and Iversen 2002) but more recently in river gravels (Trimmer et al., 2009).

So far, research into CH₄ production and emission in aquatic systems has been limited with the majority of studies focusing on wetlands and lake systems. Streams, rivers and estuaries lag behind in terms of the amount of available studies but emerging slowly is a picture of great diversity between the controls on production and emission in each of these distinct aquatic environments. For instance, a number of studies have been conducted in upland streams to constrain the cycling of carbon through these systems and have found super-saturated soil water to be the main source of CH₄ within the systems with limited in-stream production (Hope et al., 2004).

In rivers, the well oxygenated bed consisting of gravels has been thought to be too oxic to sustain methanogenesis, however ingress of fine particulate material can effectively restrict flow and oxygen delivery creating anoxic conditions (Jones and Mulholland 1998b). Moreover, many lowland rivers contain substantial areas of fine sediment accumulation providing ideal conditions for anoxic conditions to develop. Sanders et al., (2007) reported high CH₄ emissions from an English chalk stream with emissions ranging from 0.02 to 45 μmol CH₄ m⁻² h⁻¹. However much of the CH₄, that is not directly transported to the atmosphere by ebullition or plant stem transport, is oxidised in areas of oxic gravels. Rivers beds are predominantly a mosaic of substrata, and it has been found that highest concentrations of CH₄ were associated with fine sediment deposits, and in gravel zones were in general deplete of CH₄ relative to the water column (Sanders et al., 2007; Trimmer et al., 2009; Trimmer et al., 2010). On the whole this indicates that rivers beds act as a complex mix of sinks and sources of CH₄.

Other important sources of CH₄ in river catchments are lakes and reservoirs with these sites classified as CH₄ “hotspots” where high inputs of organic matter occur. This has

been highlighted in various studies finding a direct relationship between organic matter in lake sediments (often linked to increasing lake trophy; Casper 1992) and CH₄ production (Huttenen 2001, 2002, 2003; Casper et al., 2000). Despite corresponding high levels of CH₄ oxidation in upper sediments these lake systems still proved to emit considerable amounts of CH₄ to the atmosphere and are recognised as a significant source in global methane budgets (Bastviken et al., 2004). Moreover, despite only covering 0.9 % of the earth's surface (Downing et al., 2006), 6 – 16 % of the total natural emission of CH₄ may be sourced from lakes (Bastviken et al., 2004).

In shallow lake systems and in shallow sediments, much of the methane produced is exported by ebullition directly to the atmosphere, however in deeper sediments the major export is by diffusive flux and water column mixing (Baskviken et al., 2004; Juutinen et al., 2009) and once there is readily oxidised in either oxygen rich upper sediments or the water column (Whalen, 2005; Kankaala et al., 2006).

The CH₄ produced in terrestrial environments, lakes and rivers is exported downstream, and the portion of which is not evaded to the atmosphere or oxidised to CO₂ is transferred to the estuarine and coastal aquatic environments. According to Abril and Borges (2004) estuarine environments have much higher gas transfer rates due to increased surface area and increases in wind exposure. This has been shown to result in the physical ventilation of river CH₄ in the upper estuary and in plumes at sea (Upstill-Goddard et al., 2000; Bange et al., 1994). The rate and amount of CH₄ exported from an estuary has been found to correlate with the residence time of the estuary, where those with shorter residence times have been shown to export CH₄ some distance out to the sea (Scranton and McShane 1991) but in systems with longer residence times almost no CH₄ is exported beyond the estuary mouth and concentrations are thought to be controlled by evasion and oxidation of the CH₄ (Middleburg et al., 2002). The diversity of the habitats within estuaries can play an important role in CH₄ concentrations with mud-flats and marshes importance sources of CH₄ production and advection to the main estuarine channel. This has been linked to increased organic matter, longer water residence times and sediments with anoxic zones (Middelburg 1996, Van der Nat & Middelburg 2000).

2.2 Nitrogen in aquatic systems:

Currently nitrogen is being exported on a large scale from terrestrial habitats to the

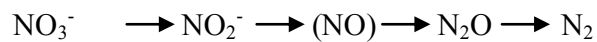
oceans via river export estimated to be between 48 and 59 Tg N yr⁻¹ (Boyer et al., 2006 from Clough et al., 2007). It has been estimated that the N flux to the North Sea has increased approximately 10-fold over the last 4 decades (Howarth et al., 2002). This flux increase has been attributed to the general increase in intensive agriculture and is on par with an overall increase in nutrient concentrations in surface, groundwater and coastal waters (Heathwaite et al., 2000) which is influenced additionally by inputs associated with effluents and atmospheric deposition. The latter input highlights the changing importance of nutrient sources along the river continuum, where it is atmospheric deposition in upland environments (characterised by high precipitation leading to dilution) that provides the bulk of N in surface waters coupled with lower mineralization and absence of intensive-agriculture (Wade et al., 2005). In contrast, Wade et al. (2005) state that NO₃⁻ concentrations increase downstream related to the predominance of agriculture and livestock production, proximity to urban/industrial areas, and greater evaporation rates in lowland areas. Nitrogen is found in various forms within aquatic systems such as dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN) with the latter comprised of ammonium (NH₄⁺), nitrite (NO₂⁻) and nitrate (NO₃⁻). Together with particulate organic nitrogen (PON) these N species are cumulatively reported as total N (TN). Nitrate has been reported by many workers as the pre-dominant soluble form found in most surface waters (Neal and Robson, 2000; Neal and Davies, 2003), with the exception of upland or wetland dominated catchments where organic-N may form a substantial portion of the total (Chapman et al., 1999, 2001; Neal et al., 2003a). Nitrogen is also found in gaseous form as N₂O in aquatic systems, and is both derived from allochthonous sources from groundwaters and soil waters, as well as in-stream production in benthic sediments.

The production of N₂O is linked to the turnover of inorganic nitrogen in sediments by the microbial processes of nitrification and denitrification (Poth and Focht, 1985; Wrage et al., 2001). Nitrification (the conversion of ammonium (NH₄⁺) to NO₃⁻) occurs in oxic sediment conditions where it is believed to play a major role in the sediment-water exchange of nutrients and also produces N₂O (Barnes and Owens 1998). However, it is denitrification that is often viewed to be crucial in terms of production and emission of N₂O from aquatic environments (Barnes and Owens 1998). Denitrification is the process by which NO₃⁻ is reduced to gaseous forms (N₂O and N₂) in anoxic conditions and represents the major pathway for the removal of nitrogen in aquatic systems with

Seitzinger (1990) reporting 70% of nitrogen loadings being removed by denitrification in aquatic sediments. Denitrification is carried out by heterotrophic bacteria which use NO_3^- preferentially as the terminal electron acceptor in place of O_2 whilst carrying out the oxidation of organic compounds producing CO_2 (Germon, 1985) characterised by the stoichiometric equation following:



The reduction of NO_3^- follows, as shown in the next equation, in a set sequence that reduces the oxidation status of the N species:



Denitrification involves several steps involving many functional types of bacteria. It, therefore, takes entire bio-communities to complete the process with end products of either N_2O and N_2 . However the imbalanced activity of NO_3^- reductase can result in an accumulation of intermediate species (Martienssen and Schops, 1999). For example, the intermediates NO may be produced but notably in minor quantities due to its reactive nature, whilst N_2O evolution may be substantial. However in comparison, N_2 which is the final reduced product of denitrification is found in far greater concentrations.

Denitrification and nitrification are co-existing processes separated only by O_2 availability in the sediment. A study by Revsbech, Jacobsen and Nielsen (2005) found through examining river sediments using micro-sensor analysis that nitrification took place in the aerobic surface zone over a few millimetres thickness of sediment and likewise denitrification took place in the anoxic zone over a similar thickness of sediment. However previous studies by Oremland et al. (1984) found that denitrification occurred endogenously up to 3 cm in depth, but noted that the potential for denitrification existed at all depths to 15cm dependant on NO_3^- availability.

Revsbech et al., (2005) also find evidence that nitrification in the oxic layers provides some of the nitrogen source (NO_3^-) for denitrification and that the oxic layers act as a diffusive barrier for NO_3^- in the water column, with denitrification rates subsequently a

product of nitrate concentrations in the water column and diffusive rate. The depth of the anoxic layer and subsequently the denitrification zone is determined by the slow diffusive property of dissolved oxygen (DO) into the sediments as well as the contribution played by bio-turbating animals, benthic microalgae and plants which can both increase or reduce the oxygen availability (Revsbech et al., 2005). The diffusive rate of NO_3^- into the sediments is related to the oxic layer thickness suggesting at times, where oxygen penetration is higher, that denitrification is potentially limited by NO_3^- availability due to the increased depth of the denitrifying zone.

These are not the only controls of denitrification in aquatic sediments with Pfenning and McMahon (1996) finding that temperature also has a significant impact, stating that NO_3^- concentrations were higher in the winter in connection with a drop in denitrification rates related to the lower temperatures.

It has, therefore, emerged that there are several major controls on denitrification rates (NO_3^- concentration, oxygen availability, organic matter availability, temperature) all of which are interconnected with further external controls. These exist as a host of abiotic factors related to the specific surrounding environment, which gives rise to the potential of significant variations between different functional aquatic environments. For example, the rate of denitrification is related to sediment-water interactions where physical factors associated with hydrology (water residence time, depth, flow, and channel morphology) affect the concentrations of NO_3^- and OC; as well as temperature and anoxic conditions, on top of controlling substrate availability. Seasonality also has a part to play controlling temperature changes that affect microbial activity and nutrient concentrations (related to growing season and nutrient release), oxygen availability (biological activity; lake stratification), fertiliser application time (related to NO_3^- concentrations) and seasonal controls of OC concentrations (related to flushing of DOC and organic material in the autumn).

The presence of aquatic plants can alter dissolved nutrient concentration through direct uptake by the roots. Coupled nitrification-denitrification can occur through an increase in the aerobic-anaerobic interface in the sediment (Knowles, 1982). Additionally the presence of benthic macro-fauna and meio-fauna has also been shown to increase denitrification in studies by Svensson and Leonardson (1996) and Tuominen et al., (1999) directly related to the increase of NO_3^- diffusion and coupled nitrification-denitrification sites through animal burrows.

1.3. Overview of Thesis

This study was based on the River Tay catchment. The main study aims were a) to quantify the spatial and temporal variability of GHG concentrations and fluxes across an entire catchment – from source to sea, and b) to understand what controls and drives the fluxes in the catchment. This included both detailed study of monthly fluxes of GHGs in the freshwater catchment over a two year period as well as detailed seasonal study of GHGs in the River Tay estuary.

The thesis is composed of four individual papers. Paper I describes the spatial and seasonal variability in N_2O , CO_2 and CH_4 in the macrotidal River Tay estuary. Seven seasonal transects of the estuary were conducted along 10 sites to try and form an understanding both of the magnitude of seasonal fluxes and the controlling mechanisms.

This is followed by Paper II which examines the spatial and temporal variability in N_2O , CO_2 and CH_4 across the freshwater catchment at nine sites, sampled monthly over two years. These sites ranged from upland river sites, the inflow and outflow of a small loch on a sub-tributary, and the main lowland river and tributaries.

The final papers investigate the importance of sediments to the in-stream production of GHG in freshwater lakes (Paper III) and in the River Tay (Paper IV). Studies were conducted on a number of specific sites within the catchment to ascertain the production within these aquatic environments. The thesis concludes with a discussion of the fluxes of GHG and controlling mechanisms across the catchment from source to sea collating the finding from the 4 main papers.

2. Materials and Methods

2.1 Study Sites

The River Tay catchment includes the Rivers Tay, Tummel (Site 3) and Isla (Site 7), as shown in Figure 1a. The majority of rivers rise in the northern and western parts of the catchment where there is significant relief, including the Ben Lawers mountain range (up to 1214 m above sea level) (Figure 1b). Land use in the catchment is generally agricultural, although the upper parts are predominantly heather moorland and coniferous plantation whilst the lower parts are much more fertile and mainly used for arable farming (Figure 1b).

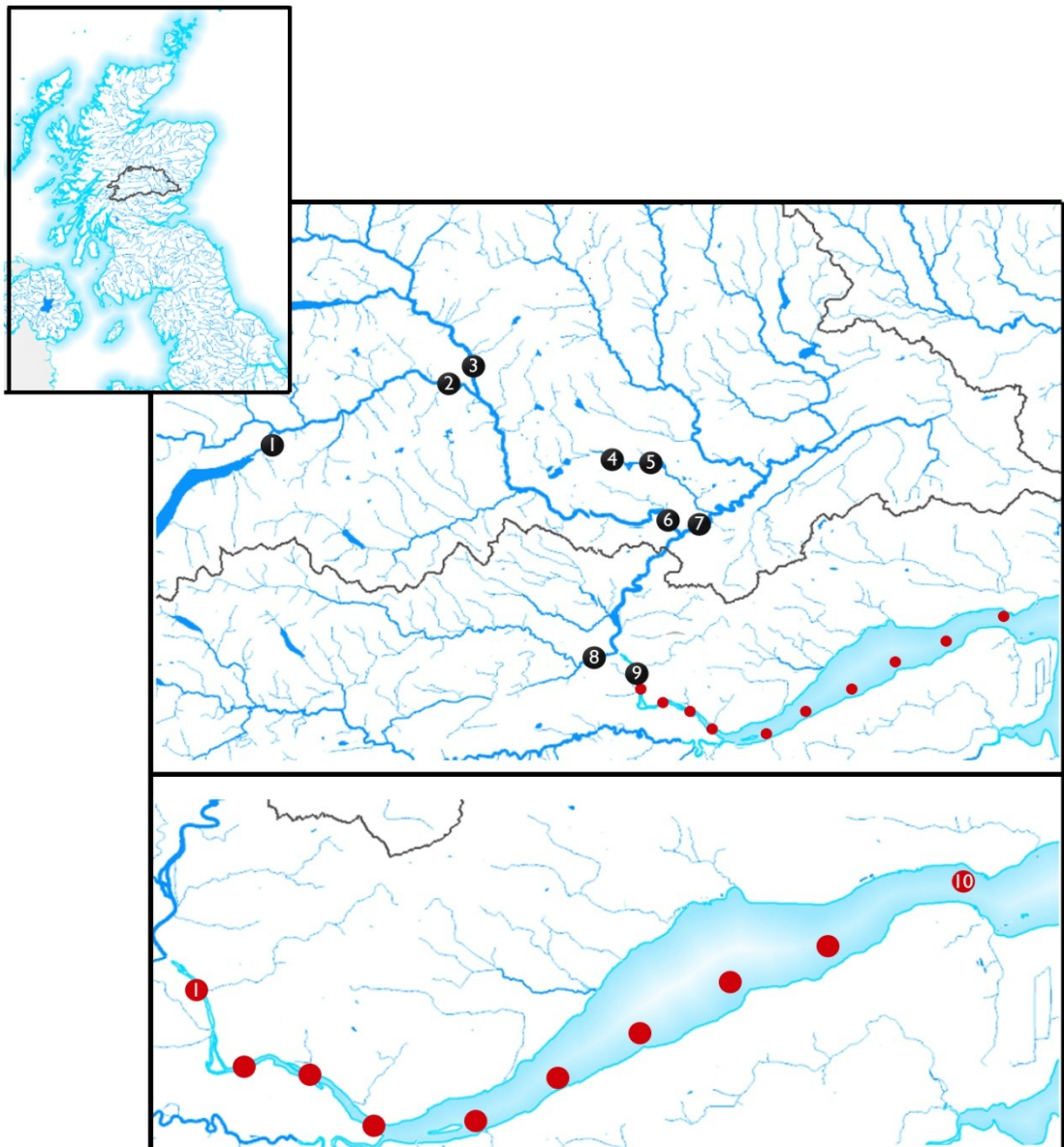


Figure (1a) River Tay Catchment diagram including sampling sites

There are several small settlements throughout the area, and the city of Perth (population in 2001 census 43,450) is located at the bottom of the catchment. There are a number of protected areas in the Tay catchment, including Special Areas for Conservation (SACs), Special Protection Area (SPAs) and Sites of Scientific Interest covering mountainous vegetation, the Forest of Clunie in the east and Rannoch Moor in the west, which contains the most extensive area of western blanket and valley mire in Britain. The south-eastern fertile and flat part of the catchment is extensively farmed and is designated under the Nitrates Directive for groundwater. The geology of the area comprises bands with psammite to the northwest, psammite and semi-pelite in the mid parts and sandstone to the east. Till deposits are scattered throughout the catchment, with glacial sands and gravels and alluvium in the river corridors. River quality for the majority of the catchment is classified by SEPA as excellent.

The Tay estuary is situated between Perth and Dundee on the east coast of Scotland and drains into the North Sea (Fig. 1a/b). The estuary has an area of 122 km² (OSPAR, 2006) with a tidal range of 3.5 m at Dundee and 2.5 m at Perth and Bridge of Earn (Nassehi and Williams, 1987), creating an intertidal area of 75 km² which is mainly on the northern shore of the lower estuary. Overall the estuary is shallow and classified as macrotidal (mean tidal range >2 m), with partial to well-mixed waters (OSPAR, 2006). The estuary receives a significant input of freshwater from the Rivers Earn and Tay, with the latter being the UK's largest river in terms of discharge with an annual mean flow of 167 m³ s⁻¹ at the tidal limit upstream of Perth (Williams and West, 1975), and with an estuarine water residence time between 2 and 14 days (Balls, 1992). For the purposes of this paper, the Tay estuary is defined as extending from the city of Perth in the west to the mouth which is bounded by Tayport to the south and Broughty Ferry to the north. It includes a section of predominantly freshwater intertidal river, downstream from Perth to Newburgh (between points 1 and 5 in Fig.1a/b).

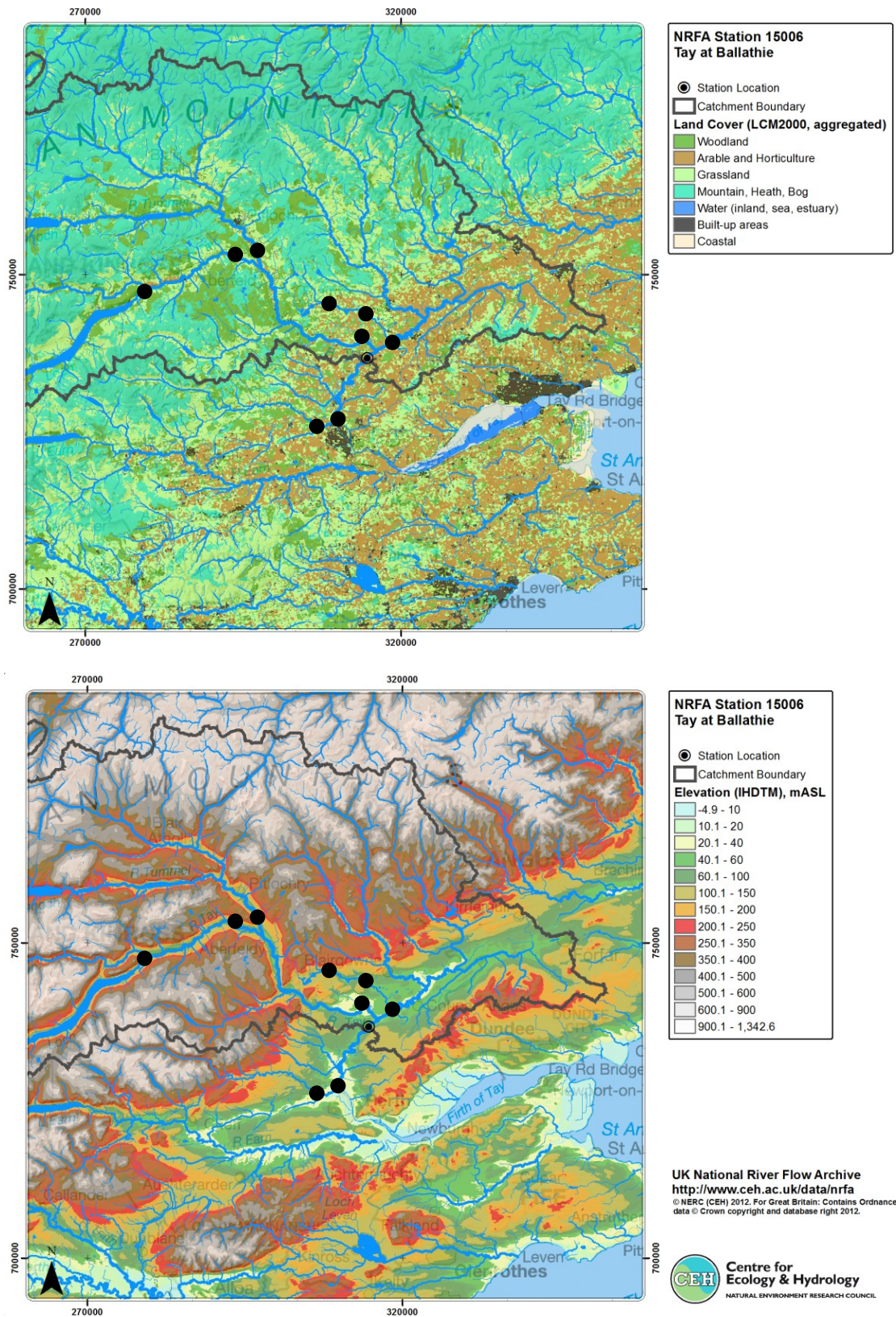


Figure 1b. Landuse and elevation maps of the River Tay catchment (NRFA 2012) black circles equal sampling locations in the freshwater catchment.

Table 2: Variables measured in the four papers

Variable	Field Method	Laboratory Method	Paper			
			I	II	III	IV
Dissolved CH ₄ , CO ₂ and N ₂ O	Headspace Sampling ¹	Gas Chromatography	X	X		
TDC, DOC and DIC	Water Sample	TOC Analyser		X		
TdN, DON, DIN (NO ₃ ⁻ and NH ₄ ⁺)	Water Sample	Colorimeter	X	X	X	X
Water Temperature, pH and Dissolved oxygen	Hach Multi Probe	-----	X	X	X	X
Sediment Production of GHGs	Sediment Sample	Sediment Incubation			X	X
Sediment Dry Weight	Sediment Sample	Oven Drying			X	X
Sediment Organic Matter Content		Loss-on-Ignition (method)			X	X

¹ Method described in more detail in following text

2.2. Methods

A variety of methods were employed in this wide-ranging study, which included those employed to calculate both dissolved and areal fluxes of GHGs, alongside a series of auxiliary measurements intended to help explain variability across the estuary (Paper I) and river catchment (paper II). Sediment experiments were used both for the loch sites (Paper III) and across the river catchment (Paper IV). Whilst specific details of individual methods are given in each of the papers, an additional summary of variables studied and methods used throughout the thesis are given in Table (2). The key methods of particular importance to this study are described in greater detail below.

2.2.1 Head Space Method

The headspace method is a widely used and cited direct method to calculate the concentration of gas dissolved in a liquid (Kling et al., 1991 Billett et al., 2004; Hope et al., 2004; Billett et al., 2008). An exact volume of water was equilibrated with an exact volume of ambient atmosphere in a luer-lock syringe with attached 3-way valve. In both the estuary and main river, 40 ml of water was equilibrated with 20 ml of headspace. The syringe was shaken underwater, vigorously for one minute, to ensure that the water was fully equilibrated with gas headspace at the temperature of the ambient surrounding water. The headspace was then immediately injected into pre-evacuated 12 ml gas tight vials for later analysis using gas chromatography. Grease was applied directly to the rubber septa of the vial to ensure vials remained gas tight until analysis. A further sample of ambient air was injected into a vial, and water temperature recorded. The concentration of CO₂, CH₄ and N₂O were then used to calculate the original water concentration based on Henry's law and standard calculations; see Equation (1) below (Dawson, 2000) and solubility coefficients are provided in appendix A (see page).

[GAS](aq) is the gas concentration originally in the water sample (mg l⁻¹), P_f is the final partial pressure of the gas in the syringe (atm) (Equation 2), P_i is the initial partial pressure of the equilibration gas (atm) (Equation 3), P_{wa} is the total ambient pressure (atm) at the sampling depth, Y_f is the concentration in the equilibrated headspace (ppmv), Y_i is the concentration of the headspace prior to equilibration (ppmv), K_H is the Henry's law constant (also known as the solubility coefficient), V_{hs} is the headspace volume (L), V_w is the water volume (L), r is the universal gas constant (0.082057) and T

is the stream temperature (°K). The solubility of gases in water is dependent both on water temperature as well as salinity, and the values of K_H included adjustment for temperature and salinity and the methods used to calculate K_H are detailed in Appendix A along with tables detailing the range of solubility coefficients calculated for each gas.

$$[GAS]_{(aq)} = [P_f \times (K_H + (V_{hs} \div V_w) \times (1 \div r) \times T)] - [P_i \times ((V_{hs} \div V_w)) \times (1 \div r) \times T] \quad (1)$$

$$P_f = Y_f \times P_{wa} \quad (2)$$

$$P_i = Y_i \times P_{wa} \quad (3)$$

2.2.2 Calculating Estuarine Fluxes:

Gas fluxes (F) across the air-water interface depend on the concentration gradient and the gas transfer velocity (k) and were calculated using Eq. (4):

$$F = k_i (Gas_d - Gas_{eq}) \quad (4)$$

where k_i ($m\ h^{-1}$) is the gas transfer velocity, Gas_d ($\mu g\ m^{-3}$) is the dissolved gas concentration and Gas_{eq} ($\mu g\ m^{-3}$) is the dissolved concentration at equilibrium with the relevant atmospheric concentration.

Gas exchange between surface waters and the atmosphere predominantly varies according to saturation of the relevant gas and the gas transfer velocity. However, currently there are no theoretical models available that accurately estimate gas transfer rate (Demars and Manson 2012); with transfer rates often estimated using empirical equations or direct measurements in individual systems. The latter can obtain accurate results (10 % standard error) but are challenging to undertake in large aquatic environments such as lowland rivers and estuaries (Richey et al., 2002). They also only cover discrete moments both in time and space. This has driven the need for empirical relationships that involve predictor variables such as depth, slor and velocity (e.g Raymond et al., 2012).

For CH₄, CO₂ and N₂O, which are sparingly soluble gases, the gas transfer velocity is predominantly driven by turbulence at the water-air boundary (Liss and Slater, 1974) which, in lakes and open oceans, tends to be mainly created by wind stress, with k usually parameterized as a function of the wind speed (e.g., Liss and Merlivat, 1986; Wanninkhof, 1992). In estuaries determination of k is more complex because turbulence is created through the combination of tidal velocity, bottom friction and dynamic bed topography. In general there is a lack of agreement in the literature on relationships between k and the factors affecting turbulence in estuaries, a problem compounded by uncertainty created through inherent differences in measurement techniques. In macrotidal estuaries, such as the Tay, previous studies have shown that tidal currents contribute significantly to gas transfer velocities (Borges et al., 2004a). Moreover, through the addition of a tidal velocity parameter, it is possible to explain the large variability in k -wind speed relationships across estuaries of differing size and hydraulic conditions. Overall, various authors have concluded that relationships between the factors affecting turbulence and k are often site specific, and the use of generic relationships to calculate k can lead to substantial errors (Kremer et al., 2003; Borges et al., 2004b).

Since k was not specifically measured in this study, gas fluxes were estimated using four parameterizations of k reported in the literature for relevant estuarine environments (Eq. 5-8). The parameterizations used were chosen either because of their widespread usage in the literature (Clark et al. (1995) and Raymond and Cole (2001) parameterizations) and/or their use in estuaries similar to the Tay, i.e. large, well mixed, macrotidal and dominated by their tributary river (such as the B04 Thames parameterization). The C95 parameterization (Eq. 5) is based on a tracer study of SF₆ release in the Hudson River Estuary and ²²²Rn mass balance in the San Francisco Bay (Clark et al., 1995). The RC01 parameterization (Eq. 6) is based on a compilation of published k values from various estuaries using a number of different methodologies (floating chamber, natural tracers and SF₆ tracers). Finally the B04 Scheldt and B04 Thames parameterizations (Eq. 7-8) are based on floating dome measurements in the Scheldt and Thames estuaries, respectively (Borges et al., 2004).

C95: Clark et al. (1995) function:
$$k_{600} = 2.0 + 0.24(u_{10}) \quad (5)$$

RC01: Raymond and Cole (2001) function:
$$k_{600} = 1.91 \times \exp^{0.35u_{10}} \quad (6)$$

B04 Scheldt: Borges et al. (2004b) function: $k_{600} = 4.045 + 2.580 \times u_{10}$ (7)

B04 Thames: Borges et al. (2004b) function: $k_{600} = 9.6 + 3.64 \times u_{10}$ (8)

where k_{600} is the gas transfer velocity of CO₂ normalised to 20°C (cm h⁻¹) and u_{10} is the monthly mean wind speed in m s⁻¹. Mean daily wind speed was obtained from the nearest meteorological station (Leuchars, approx. 8 km south of Tay estuary) data accessed from the British Atmospheric Data Centre (<http://badc.nerc.ac.uk>). This data was used to calculate mean monthly wind speeds ('month' defined as the 15 days before and 15 days after sampling date).

Gas transfer velocity is influenced by temperature and salinity with the degree of influence differing between gases. Following the method set out by Ferrón et al. (2007) k_{600} values from each of the parameterizations were used to calculate k_i for each gas at the recorded temperature and salinity in the field using Eq. 9:

$$k_i \div k_{600} = (Sc_i \div Sc_{600})^n \quad \text{or rearranged to } k_i = k_{600}(Sc_i \div 600)^{-0.5} \quad (9)$$

The Schmidt numbers, Sc_i expresses the effects of temperature and the specificity of each gas with respect to other gases on gas transfer properties; and therefore is the ratio of the kinematic viscosity of water over the diffusivity of the gas and is calculated through the formulations given by Wanninkhof (1992) for the individual temperature values and salinity values of 0 and 35, assuming Sc_i varies linearly with salinity and k_i has a dependency proportional to $Sc^{-0.5}$ (Ferrón et al., 2007).

The parameterizations used in this study to model emission fluxes of GHG from the Tay estuary do not take into account other emission pathways such as CH₄ ebullition, or emission from exposed tidal sediments. Since neither of these emission pathways were directly measured, the emission flux estimates in this thesis and following papers only account for the portion of GHG emitted directly from water surfaces.

2.2.3 Calculating River Emission Fluxes:

Gas transfer velocity in rivers is mainly driven by turbulence at the water-air boundary which is predominantly driven by river depth and velocity (Liss and Slater, 1974) and a number of different parameterizations have been adopted to model turbulence and gas transfer rate. We followed the method set out by Garnier et al. (2009) used to calculate fluxes in the River Seine, which is adapted from Wanninkhof (1992) and Borges et al. (2004):

$$k_{600} = 1.719 \times v^{0.5} \times d^{-0.5} \quad (10)$$

where k_{600} is the gas transfer velocity (cm h^{-1}), v is the water velocity (cm s^{-1}) and d is the water depth (m).

Water velocity was estimated for each site on each sampling occasion using the equation $Q = V \times A$ and data from SEPA for flow ($Q \text{ m}^3 \text{ s}^{-1}$), stage height (m) and channel width (m) at the main gauging stations within the Tay catchment. This equation calculates the mean water velocity for all depths at each site and is the best approximation available for stream velocity. Gas transfer velocity is influenced by temperature and salinity with the degree of influence being different for each gas and equation (9) was used to convert k_{600} to either $k_{\text{N}_2\text{O}}$ or k_{CH_4} .

2.2.4 Dissolved Fluxes

Dissolved fluxes or ‘export’ was determined using two methods. Daily fluxes were estimated based on the dissolved concentration (on sampling day) multiplied by the mean river discharge (on sampling day). Over longer timescales, in order to interpolate between widely spaced measurements of dissolved concentrations, and to calculate annual loads of both dissolved GHGs and nutrients, ‘Method 5’ of Walling and Webb (1985, described in Hope et al., 1997b) was used. Annual fluxes are estimated based on the instantaneous discharge rates, corresponding concentration measurements, and the mean of the continuous discharge data for the sampling site. Dissolved fluxes were only calculated for sites close to SEPA flow monitoring stations or for which flow could be easily estimated from the difference between discharge stations (such as for Site 7).

‘Method 5’ is described in the following equation (12), where K is used to scale units to annual catchment values, C_i is the instantaneous concentration which is associated to instantaneous discharge Q_i , Q_r is the mean discharge for the full study period, and n is the number of instantaneous samples measured.

$$\text{Load} = K \times Q_r \times \sum_{i=1}^{n-1} [C_i \times Q_i] / \sum_{i=1}^{n-1} Q_i \quad (12)$$

The standard error of these flux estimates was calculated using equation 13 (Hope et al., 1997b), where F is the total annual discharge, and C_f is the flow weighted mean concentration

$$SE = F \times \text{var}(C_f) \quad (13)$$

The variance ($\text{var}C_f$) is estimated in the following equation (Hope et al., 1997b), where Q_n is the sum of all individual Q_i values.

$$\text{Var}(C_f) = [\sum (C_i - C_f)^2 \times Q_i / Q_n] \times \sum Q_i^2 / Q_n^2 \quad (14)$$

3. Paper I

Spatial and Temporal fluxes of the greenhouse gases N_2O , CO_2 and CH_4 in the macrotidal Tay Estuary

J. F. Harley, B. Dudley, L. Carvalho, K.V. Heal, R.M. Rees, U. Skiba

Spatial and Temporal fluxes of the greenhouse gases N₂O, CO₂ and CH₄ in the macrotidal Tay Estuary

J. F. Harley, B. Dudley, L. Carvalho, K.V. Heal, R.M. Rees, U. Skiba

Abstract

Spatial and temporal dynamics of CH₄, CO₂ and N₂O in the Tay Estuary, Scotland, provide a representative picture of greenhouse gas fluxes from one of the least polluted macrotidal estuaries in Europe. Measurements of dissolved gas saturations and ancillary water chemistry data are reported for seven sampling surveys conducted between April 2009 and June 2010. Mean gas saturations for CH₄, CO₂ and N₂O were 2602 %, 262 % and 118 % respectively. The Tay shows similar spatial and temporal patterns in gas saturations compared with other European estuaries, reflecting common controls of production and loss processes. Methane showed the greatest temporal and spatial variability with saturations greatest in summer transects, with peaks in saturation occurring in the freshwater upper estuary and sharply decreasing in the mid-estuary mixing zone. Concentrations of CO₂ and N₂O were also generally higher in the upper to middle estuary in summer, although seasonality was less pronounced. Gas fluxes were all positive, with the one exception of N₂O in the winter. This demonstrates that, even a relatively unpolluted estuary like the Tay, is an important source of greenhouse gas fluxes, transferring substantial quantities of C and N derived from the freshwater catchment into the atmosphere. Current literature estimates of estuarine inputs to global greenhouse gas budgets are reported to be over-estimates due to the bias of studies of nutrient impacted estuaries. This study of the Tay, provides much needed estimates to represent budgets for relatively unpolluted estuaries.

Introduction

Carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) are important greenhouse gases that contribute to global warming (IPCC, 2007). Although CH₄ and N₂O are found in much lower concentrations in the atmosphere than CO₂ they still contribute significantly to global warming through their ability to absorb infrared radiation much more intensely than CO₂ (Forster et al., 2007). Methane and N₂O play an important part in chemical reactions in the troposphere and stratosphere. In the troposphere, CH₄ reacts photo-chemically affecting O₃ and OH radical concentrations

and in the stratosphere the extent of O₃ destruction (Crutzen, 1991). Nitrous oxide on the other hand, is the major source of NO radicals in the stratosphere which are involved in the depletion of stratospheric ozone (Crutzen and Schmailzl, 1983), and, since the decline of CFCs, is now the most important compound leading to O₃ depletion (Ravinshankara et al., 2009).

Importantly, the concentrations of CH₄, CO₂ and N₂O in the atmosphere have been increasing significantly since pre-industrial times (Forster et al. 2007), which indicates a global imbalance between sources and sinks. For example, atmospheric concentrations of CO₂, CH₄ and N₂O have increased from pre-industrial concentrations of 280 ppm, 700 ppb, and 270 ppb, to the current concentrations of 389 ppm, 1808 ppb and 324 ppb respectively (WMO 2011). Over the last decade CH₄ concentrations had stabilised to a degree (~ 1.8 ppmv from 1999 to 2005) but unexpectedly increased again in 2007 (Rigby et al. 2008) highlighting the sources and sinks are both dynamic, evolving and not fully understood. To better understand the potential imbalances in greenhouse gas budgets it is necessary to quantify all known sources and sinks. The contributions of aquatic environments to the global budgets of CH₄, CO₂ and N₂O are still uncertain. This is especially the case with the world's oceans, and particularly coastal regions such as estuaries (Bange, 2006) with uncertainty linked in part to the limited number of studies available and seasonal variability.

The global oceans are a large contributor to tropospheric N₂O, ~3.8–5.8 Tg N yr⁻¹ which represents 10–33% of the global source total (Denman et al., 2007; Jiang et al. 2007), and up to 5% (Denman et al., 2007) of the CH₄ emission. However, emissions are not spatially homogeneous across the oceans, with most sourced from biologically productive regions, such as estuaries and near-coastal areas. As much as 33–60% of the total global oceanic N₂O emissions (Bange et al., 1996; Seitzinger et al., 2000) and 75% of the CH₄ emissions (Middleburg et al., 2002; Abril and Borges, 2004; Bange, 2006) are estimated to be from estuaries and coastal areas, despite estuaries only representing ~0.4% of the global ocean area. Estuarine waters are thought to be strong sources of atmospheric CO₂ whereas the oceans are net sinks (Frankignoulle et al., 1998; Raymond et al., 2000; Abril and Borges, 2004).

Estuarine systems are of further interest because they are often the final repository for carbon and nitrogen derived from the terrestrial environment and transported by rivers, streams and groundwater into the marine environment. Human activities within catchments (e.g. agricultural fertiliser usage and discharge of sewage effluent) have

increased the nutrient loads, especially inorganic nitrogen, to estuaries (Howarth et al., 1996) which in turn has enhanced net ecosystem production. One important consequence of increased nitrogen loads to estuaries is the enhanced microbial production and emission of N_2O through denitrification and nitrification in suspended particles and bed sediments (Barnes and Owens, 1998; De Wilde and de Bie, 2000; Garnier et al., 2006). Estuarine processes for transforming nitrogen may greatly affect the final coastal loading as denitrification is a potential sink for nitrate (NO_3^-) and nitrification a potential source for NO_3^- .

In this paper, we present the spatial and temporal variations of dissolved CH_4 , CO_2 and N_2O concentrations and saturations in the Tay estuary. These are used to estimate atmospheric fluxes for both individual compartments within the estuary and total estuarine area which and compared and contrasted to other estuaries in the UK and Europe. The Tay estuary is particularly important as it is the UK's largest river in terms of discharge to the sea. Emissions from the Tay estuary can also be considered representative of many northern European rivers draining large areas of peatland and agricultural land, but with relatively low levels of point source pollution from urban areas.

Study site

The Tay estuary is situated between Perth and Dundee on the east coast of Scotland and drains into the North Sea (Fig. 1). The estuary has an area of 122 km² (OSPAR, 2006) with a tidal range of 3.5 m at Dundee and 2.5 m at Perth and Bridge of Earn (Nassehi and Williams, 1987), creating an intertidal area of 75 km² which is mainly on the northern shore of the lower estuary. Overall the estuary is shallow and classified as macrotidal (mean tidal range >2 m), with partial to well-mixed waters (OSPAR, 2006). The estuary receives a significant input of freshwater from the Rivers Earn and Tay, with the latter being the UK's largest river in terms of discharge with an annual mean flow of 167 m³ s⁻¹ at the tidal limit upstream of Perth (Williams and West, 1975), and with an estuarine water residence time between 2 and 14 days (Balls, 1992). For the purposes of this paper, the Tay estuary is defined as extending from the city of Perth in the west to the mouth which is bounded by Tayport to the south and Broughty Ferry to the north. It includes a section of predominantly freshwater intertidal river, downstream from Perth to Newburgh (between points 1 and 5 in Fig.1).

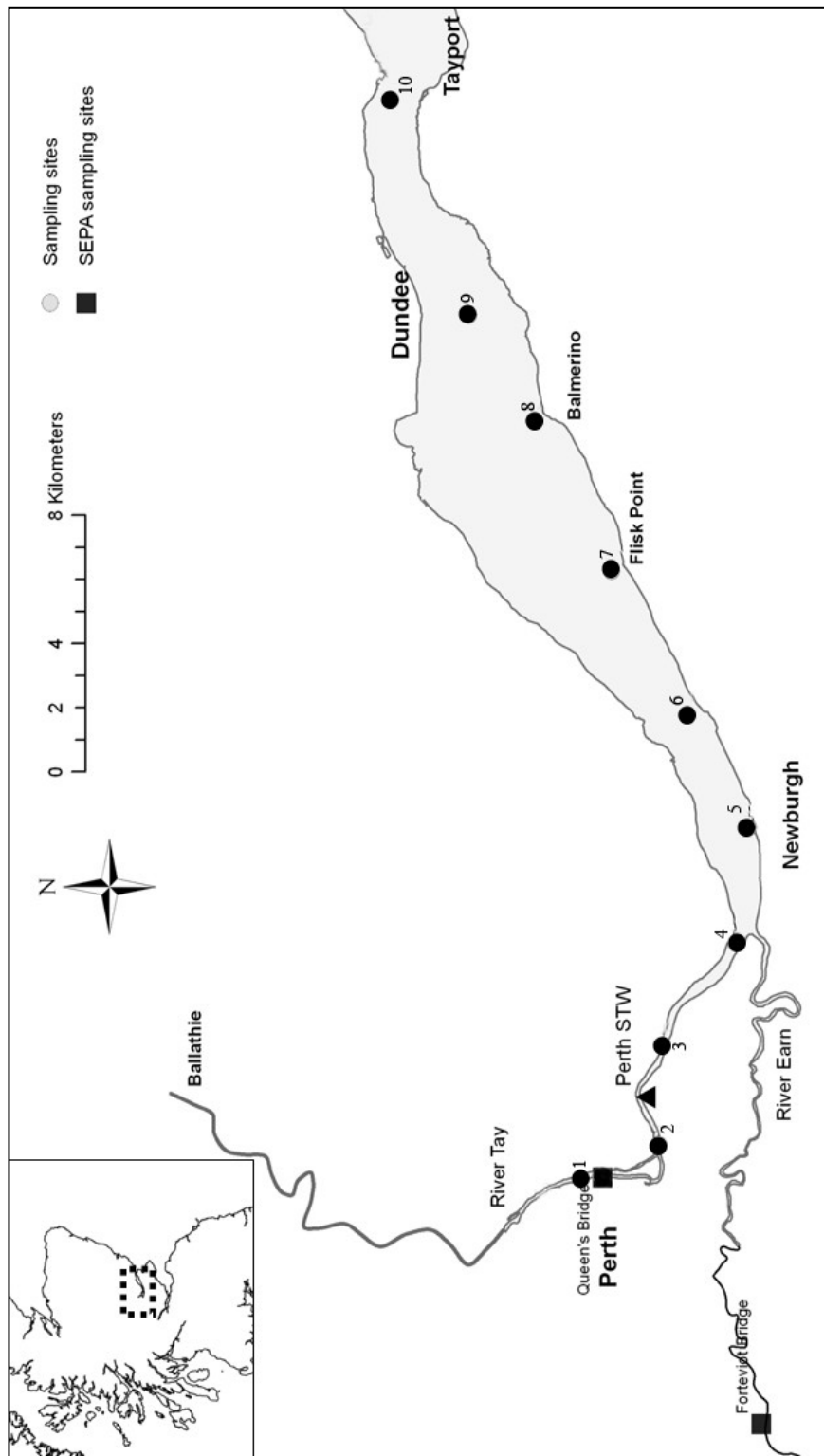


Figure 1: Map of estuary showing main sampling locations, and main features of estuary.

Methods

Water sampling and analysis

Water from the Tay estuary was sampled for chemical analysis and determination of GHG (green house gases) concentrations from a rigid inflatable boat on a transect of 10 sites on 7 occasions from April 2009 to June 2010. The locations of sampling sites were determined beforehand, based on an existing sampling site of the Scottish Environment Protection Agency (SEPA) (Site 1), the wish to sample upstream and downstream of known sources (Sites 2 and 3, above and below the Perth Waste Water Treatment Works, and Sites 4 and 5, above and below the confluence with the River Earn), and the desirability of equally-spaced sampling points along the lower estuary (Sites 6 to 10). GPS was used to locate the sites when sampling. On all occasions sampling started upstream in the morning at high tide, and continued travelling downstream on the outgoing tide, with the aim of minimising temporal variation in the water quality parameters. On one occasion (August 2010) sampling was conducted over an 8 hour period, with samples take every 2 hours, at one site (site 5) to assess the temporal variation over a half tidal cycle; from high tide to low tide.

Temperature and salinity were measured *in-situ* at 10 cm below the water surface using a Hydrolab Quanta Probe (Hach) at each sample location, and replicate surface water samples were collected using 3 separate 10 litre plastic buckets. From each bucket, a well-mixed, known volume of water (between 200 and 800 ml), was filtered through a pre-combusted (500°C for 10 h) and pre-weighed Whatman GF/F 0.7 µm, 47 mm diameter filter paper, using a Nalgene filtration unit and an electric vacuum pump, to obtain sub-samples for analysis of NO₃⁻ and NH₄⁺. The filtered sub-samples were transferred to sample tubes (Sterilin 50 ml; high clarity polypropylene) and sealed and placed in an ice box for transportation to the laboratory freezer where they were stored until analysis. Samples were analysed colorimetrically using a SEAL AQ2 discrete analyzer by the sulphanilamide/NEDD (N-1-naphthylethyene diamine dihydrochloride) reaction for NO₃⁻ and idophenol blue chemistry for NH₄. Immediately after filtration in the field, all filter papers were placed in foil packets and stored in liquid nitrogen, then transferred directly to a freezer (-18 °C) on return to the laboratory for determination of total suspended solids (TSS) by oven-drying at 105°C for 4 hours and re-weighing.

Measurement of dissolved CH₄, CO₂ and N₂O

The widely used headspace technique (Kling et al., 1991; Billett et al., 2004; Hope et al., 2004; Billett et al., 2008), in which a known volume of water is equilibrated with a known volume of ambient atmosphere, was used to determine the concentrations of dissolved CH₄, CO₂ and N₂O. Gas concentrations were determined in surface waters sampled in triplicate from each site. For each sample, a 60 ml syringe (with 3 way valve) was used to extract 40 ml of estuary water from 10 cm below the surface, after which 20 ml of ambient air was added. The syringe was then shaken vigorously underwater for 1 min, equilibrating the headspace and water at water temperature. The headspace gas was then immediately injected into pre-evacuated 12 ml gas tight vials for later analysis using gas chromatography. A further sample of ambient atmosphere was injected into a 12 ml gas tight vial, and water temperature and atmospheric pressure were recorded. N₂O and CH₄ were analysed on a HP5890 Series II gas chromatograph (Hewlett Packard (AgilentTechnologies) UK Ltd., Stockport, UK) with an electron capture detector (ECD) and flame ionisation detector (FID) with accuracy of 30 ppb for N₂O and 70 ppb for CH₄, respectively. CO₂ was measured simultaneously on another HP5890 Series II gas chromatograph fitted with a methaniser, facilitating the dual analysis of CH₄ and CO₂ by FID. The concentrations of dissolved CO₂, CH₄ and N₂O were calculated from the concentrations measured from the headspace using the functions for the Bunsen solubility given by Wiesenburg and Guinasso (1979) for CH₄, and Weiss and Price (1980) for CO₂ and N₂O. Saturation values for the gases were calculated as the ratio of the concentration of the dissolved gas and the expected equilibrium water concentration.

Estimating CH₄, CO₂ and N₂O gas fluxes from the estuary

Gas fluxes (F) across the air-water interface depend on the concentration gradient and the gas transfer velocity (k) and were calculated using Eq. (1):

$$F = k_i(Gas_d - Gas_{eq}) \quad (1)$$

where k_i (m h⁻¹) is the gas transfer velocity, Gas_d (μmol m⁻³) is the dissolved gas concentration and Gas_{eq} (μmol m⁻³) is the dissolved concentration at equilibrium with the relevant atmospheric concentration.

Gas exchange between surface waters and the atmosphere varies according to saturation

of the relevant gas and the gas transfer velocity. For CH₄, CO₂ and N₂O, which are sparingly soluble gases, the gas transfer velocity is driven by turbulence at the water-air boundary (Liss and Slater, 1974) which, in lakes and open oceans, tends to be mainly created by wind stress, with k usually parameterized as a function of the wind speed (e.g., Liss and Merlivat, 1986; Wanninkhof, 1992). In estuaries, determination of k is more complex because turbulence is created through the combination of tidal velocity, bottom friction and dynamic bed topography. In general there is a lack of agreement in the literature on relationships between k and the factors affecting turbulence in estuaries, a problem compounded by uncertainty created through inherent differences in measurement techniques. In macrotidal estuaries, such as the Tay, previous studies have shown that tidal currents contribute significantly to gas transfer velocities (Borges et al., 2004a). Moreover, through the addition of a tidal velocity parameter, it is possible to explain the large variability in k -wind speed relationships across estuaries of differing size and hydraulic conditions. Overall, various authors have concluded that relationships between the factors affecting turbulence and k are often site specific, and the use of generic relationships to calculate k can lead to substantial errors (Kremer et al., 2003; Borges et al., 2004b). Therefore, parameterizations developed for a specific estuary or range of estuaries could over or underestimate k based on the specific nature of the estuary, i.e. the relative magnitude of turbulence driving factors such as wind, tidal velocity and bed topography.

Since k_i was not specifically measured in this study, gas fluxes were estimated using four parameterizations of k reported in the literature for relevant estuarine environments (Eq. 2-5). The parameterizations used were chosen either because of their widespread usage in the literature (Clark et al. (1995) and Raymond and Cole (2001) parameterizations) and/or their use in estuaries similar to the Tay, i.e. large, well mixed, macrotidal and dominated by their tributary river (such as the B04 Thames parameterization). The C95 parameterization (Eq. 2) is based on a tracer study of SF₆ release in the Hudson River Estuary and ²²²Rn mass balance in the San Francisco Bay (Clark et al., 1995). The RC01 parameterization (Eq. 3) is based on a compilation of published k values from various estuaries using a number of different methodologies (floating chamber, natural tracers and SF₆ tracers). Finally the B04 Scheldt and B04 Thames parameterizations (Eq. 4-5) are based on floating dome measurements in the Scheldt and Thames estuaries, respectively (Borges et al., 2004).

$$\text{C95: Clark et al. (1995) function: } k_{600} = 2.0 + 0.24(u_{10}) \quad (2)$$

$$\text{RC01: Raymond and Cole (2001) function: } k_{600} = 1.91 \times \exp^{0.35u_{10}} \quad (3)$$

$$\text{B04 Scheldt: Borges et al. (2004b) function: } k_{600} = 4.045 + 2.580 \times u_{10} \quad (4)$$

$$\text{B04 Thames: Borges et al. (2004b) function: } k_{600} = 9.6 + 3.64 \times u_{10} \quad (5)$$

where k_{600} is the gas transfer velocity of CO₂ normalised to 20°C (cm h⁻¹) and u_{10} is the monthly mean wind speed in m s⁻¹. Mean daily wind speed was obtained from the nearest meteorological station (Leuchars, approx. 5 miles south of Tay estuary) data accessed from the British Atmospheric Data Centre (<http://badc.nerc.ac.uk>). This data was used to calculate mean monthly wind speeds ('month' defined as the 15 days before and 15 days after sampling date).

Gas transfer velocity is influenced by temperature and salinity with the degree of influence differing between gases. Following the method set out by Ferrón et al. (2007) k_{600} values from each of the parameterizations were used to calculate k_i for each gas at the recorded temperature and salinity in the field using Eq. 6:

$$k_i \div k_{600} = (Sc_i \div Sc_{600})^n \quad \text{or rearranged to } k_i = k_{600}(Sc_i \div 600)^{-0.5} \quad (6)$$

The Schmidt numbers, Sc_i , is the ratio of the kinematic viscosity of water over the diffusivity of the gas and is calculated through the formulations given by Wanninkhof (1992) for salinity values of 0 and 35, assuming Sc_i varies linearly with salinity and k_i has a dependency proportional to $Sc^{-0.5}$ (Ferrón et al., 2007).

Using these calculated k_i values the gas flux density was calculated (Eq. 1) and was up scaled to total estuarine area using two methods. The first method (Method A), multiples the mean flux density by the total estuary area. The second method (Method B) subdivides the estuary into 10 compartments, one for each sampling site, with compartment boundaries at equidistant points between the sites. Each compartment area is multiplied by the flux density at the representative site, giving an emission value for every site/compartment in each of the seasonal surveys. The mean yearly emission for the estuary is calculated from the average of these compartment emissions. Since,

estuary sampling was conducted on or near high tide throughout the estuary, it is assumed that total water area within the estuary covers the entire estuary area. Therefore the flux estimates represent emissions at high/full tide, and do not represent estuarine emissions at low tide, which would also have to account for emissions sourced from intertidal mud and sand banks.

Fluxes were also calculated as CO₂ equivalents (CO_{2eq}) by multiplying the emissions by their global warming potentials (GWP): 1 for CO₂, 23 for CH₄ and 298 for N₂O (100 yr time horizon) (IPPC, 2007).

Data analysis

Pearson's correlation coefficients were calculated using Minitab v.16 to examine associations between the measured gas saturations and other determinants. The significance level used was $p < 0.05$. A seasonality index was produced by splitting the data into two groups: winter/spring (February 2010, April 2009 & 2010) and summer/autumn (July 2009, June 2009 & 2010, September 2009). The summer/autumn means were divided by the winter/spring means to derive a seasonality index both for the estuary and for each sampling site, with a value close to 1 indicating little seasonal difference and values above 1 indicating higher gas saturations in summer/autumn than winter. Statistical significance between winter/spring saturations vs. summer saturations at each site were tested with a two sample t-test with a significance level of $p < 0.05$.

The complete dataset was also analysed using a combined ordination approach, through a combination of principal component analysis (PCA) and cluster analysis. PCA is frequently used to analyse large, multivariate environmental datasets (Clarke and Warwick, 2001), in the aid of simplifying data and allowing for the recognition of patterns and sample similarity. Combined with k-means cluster analysis, it was used here to define the controls on gas saturation over temporal and spatial scales, indentifying distinct environmental conditions. The data included in the PCA was not normalised, standardized or transformed before analysis, therefore correlation instead of covariance analysis was used.

Results and Discussion

Hydrographical Setting

The 7 survey transects of the Tay estuary were designed to encompass the seasonal changes found within the estuary. The mean, minimum and maximum CO₂, CH₄ and N₂O saturations for all 10 sites for each survey are displayed in Table 1, alongside other measurements of water chemistry. The 7 sampling surveys achieved temporal coverage of the main seasonal changes as highlighted by the surface temperature which ranged from 2.2°C to 19.6°C. There was, however, greater replication of summer months, as this season was expected to have the highest and most variable flux measurements.

Table 1. Mean (range) gas saturations and water chemistry for the 7 sampling transects (n=69)

	NO₃-N	NH₄-N	DO	DO-SAT	pH	Conductivity
	mg l⁻¹	mg l⁻¹	mg l⁻¹	%		mS cm⁻¹
Mean	0.59(0.22 - 1.37)	0.04(0.02 - 0.08)	10.1(7.6 - 12.4)	93.3(82.7 - 99.8)	7.5(6.3 - 9.0)	6.7(0.1 - 37.5)
Apr 09	0.50(0.46 - 0.56)	0.04(0.02 - 0.06)	11.1(10.9 - 11.5)	96.9(95.9 - 98.3)	7.5(7.3 - 7.6)	0.9(0.07 - 6.61)
Jun 09	0.60(0.29 - 0.91)	0.05(0.04 - 0.06)	9.8(9.3 - 10.1)	(0.0 - 0)	7.3(6.3 - 9.0)	9.7(0.08 - 37.5)
Jul 09	0.46(0.34 - 0.61)	0.04(0.02 - 0.06)	8.3(7.6 - 9.0)	90.1(84.1 - 95.6)	7.5(7.1 - 8.0)	7.8(0.08 - 33.5)
Sep 09	0.48(0.30 - 0.64)	0.04(0.03 - 0.07)	10.4(9.5 - 11.0)	98.8(96.0 - 99.8)	7.2(6.8 - 7.8)	6.1(0.07 - 28.3)
Feb 10	1.06(0.73 - 1.37)	0.06(0.04 - 0.08)	12.2(11.9 - 12.4)	91.9(90.6 - 92.8)	7.7(7.3 - 8.4)	4.4(0.11 - 24.8)
Apr 10	0.59(0.44 - 0.83)	0.03(0.03 - 0.06)	11.5(11.1 - 12.2)	95.9(93.5 - 98.3)	7.6(7.2 - 8.1)	4.4(0.08 - 24.4)
Jun 10	0.30(0.22 - 0.46)	0.04(0.02 - 0.06)	8.4(7.9 - 8.9)	87.2(82.7 - 90.9)	7.3(6.3 - 9.0)	11.4(0.09 - 37.5)
	TSS	TSS-LOI	Temp.	CO₂-SAT	CH₄-SAT	N₂O-SAT
	mg l⁻¹	%	°C	%	%	%
Mean	40(0 - 300)	1.5(0.19 - 3.66)	12.6(2.2 - 19.6)	262 (65.7 - 644)	2602 (100 - 13135)	118(69 - 188)
Apr 09	-	-	9.3(8.6 - 10.6)	283 (199 - 394)	1466 (777- 2424)	114(110 - 161)
Jun 09	18(2.9- 42.3)	1.5(0.76 - 3.34)	14.5(13.3 - 15.6)	291 (129 - 644)	2192 (318- 3776)	124(104 - 163)
Jul 09	27(2.0- 106)	1.5(0.66 - 2.31)	18.6(17.6 - 19.6)	219 (133 - 337)	528 (664- 13135)	119(69 - 188)
Sep 09	46(0.7- 134)	1.5(0.53 - 2.44)	12.7(12.0 - 13.0)	321 (133 - 628)	-	128(87 - 176)
Feb 10	85(0.0- 300)	1.6(0.19 - 3.66)	2.9(2.2 - 3.6)	184 (66 - 320)	2043 (851- 3541)	96. (80 - 113)
Apr 10	36(0.2- 131)	1.2(0.28 - 2.86)	8.1(6.7 - 9.9)	285 (143 - 446)	878 (100- 2585)	105(92 - 129)
Jun 10	28(0.8- 52.5)	1.4(0.62 - 2.16)	17.9(15.7 - 18.7)	302 (200 - 387)	4708 (281- 10976)	138(108 - 177)

Spatial, temporal and seasonal patterns in CO₂ saturation and controlling factors

Estuaries in general are heterotrophic ecosystems and are characterised by a wide range of CO₂ saturations, with significant spatial, seasonal and cross-system variability, and are subsequently net sources of CO₂ to the atmosphere (Frankignoulle et al., 1998; Abril and Borges, 2004; Borges and Abril, 2011). A number of sources and sinks controlled by biotic and abiotic factors have been found to explain temporal and spatial patterns of CO₂-sat observed in other estuaries. In brief, these include the balance between heterotrophy and autotrophy, calcification/dissolution of calcium carbonate, physical mixing of source waters (rivers, groundwater), advection from intertidal areas and physical outgassing (Frankignoulle et al., 1998; Abril and Borges, 2004; Borges and Abril, 2011).

The distribution of CO₂-sat during estuarine transit over the 7 sampling dates is shown in Fig. 2. The CO₂ saturations in the estuary displayed pronounced seasonal, spatial and temporal variability with several notable features emerging from the data. In all cases, CO₂ saturation was higher in the freshwater zone of the estuary and decreased as salinity increased. Likewise, CO₂ saturation in river water entering the estuary (Site 1) was generally greater than the saturations leaving the estuary (Site 10). However, maximum estuarine saturations were more often than not found towards the middle estuary transition zone. Over the 7 sampling dates the estuary was dominated by supersaturated CO₂ conditions with 66 out of 69 measurements supersaturated. Dissolved CO₂ concentrations ranged from 0.17 to 1.27 mg l⁻¹ corresponding to saturations of 66 to 644 %. In terms of temporal variability, Fig. 3 highlights the large variability found over a half tidal cycle at one location near the middle estuary (site 5) in all three gases and measured water chemistry. CO₂-sat in general increased with the falling tide as conductivity (representing salinity) decreases, rising from 294 % to 348 %.

The patterns of CO₂-sat observed in the Tay are similar to those found in other estuaries reported in the literature (Table 5). For example, CO₂-sat, across all sampling transects, is higher in the upper freshwater estuary decreasing downstream with increasing salinity. Like many other estuaries, the Tay estuary receives freshwater that is generally supersaturated in CO₂ due to organic carbon mineralization in soils, river waters and sediments (Jones and Mulholland, 1998; Neal et al., 1998; Cole and Caraco, 2001).

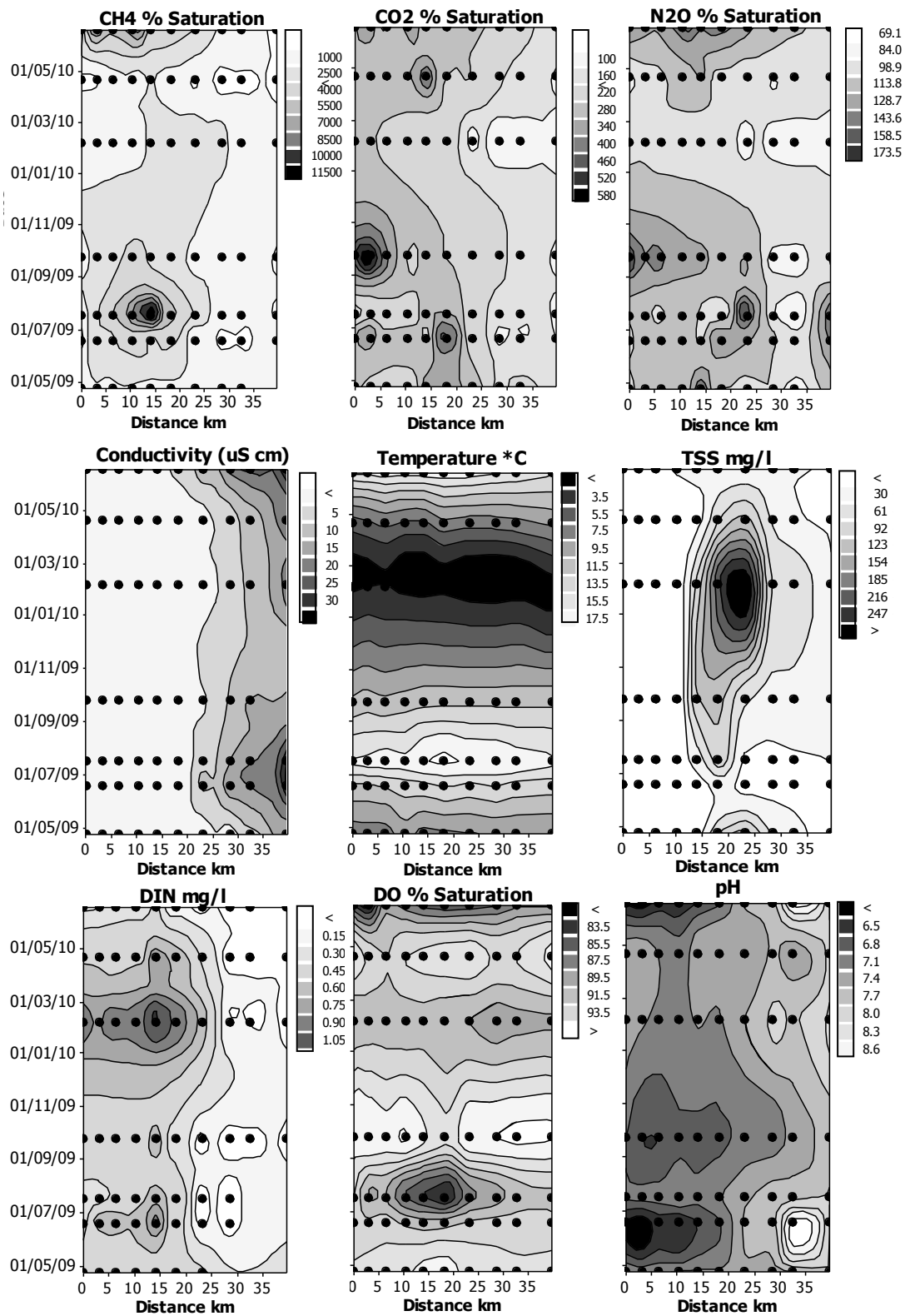


Figure 2: Surface contour plots showing temporal and spatial variability of CH₄, CO₂ and N₂O saturation and key water chemistry constituents over the 7 transects between April 2009 and June 2010. The x-axis represents the distance along the estuary and the y axis represents the sampling date which is highlighted by the black circles.

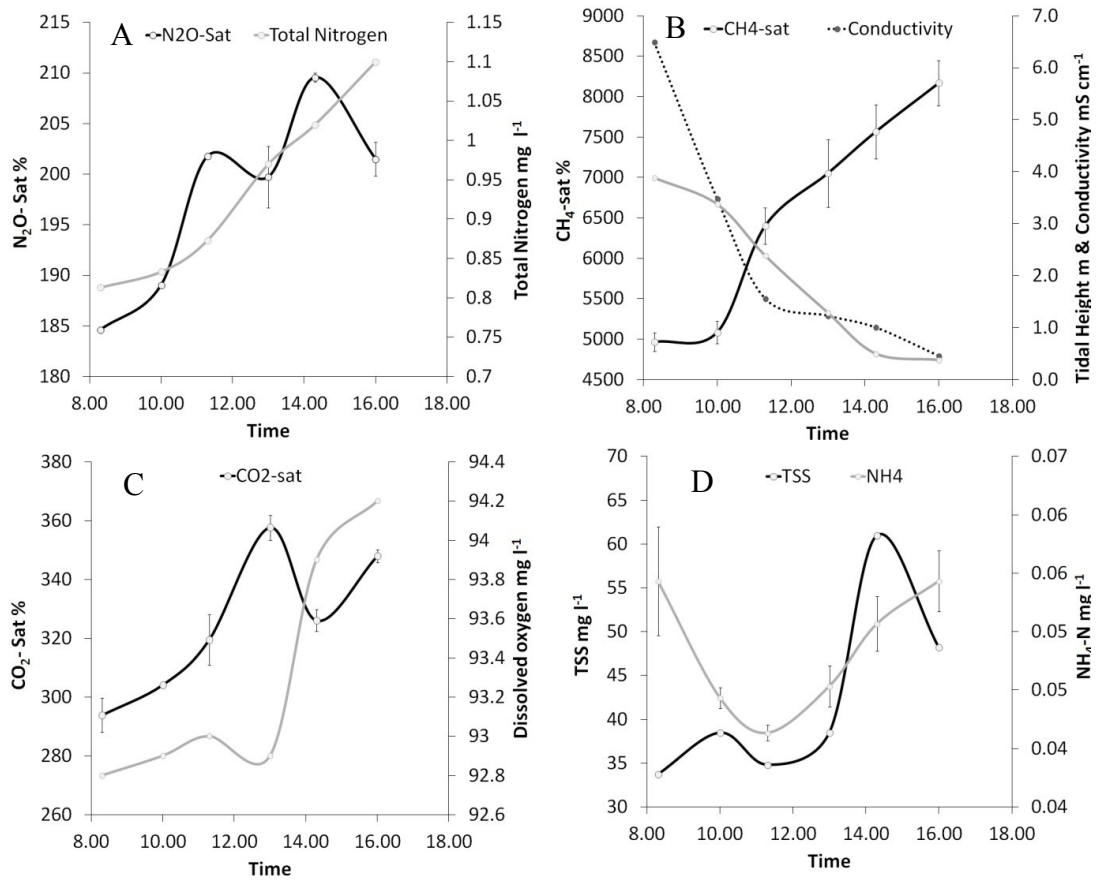


Figure 3. Mean gas saturations and water chemistry across a half tidal cycle at site 5, Newburgh (18.08.2010). Error bars equal the standard error of the mean (n=18).

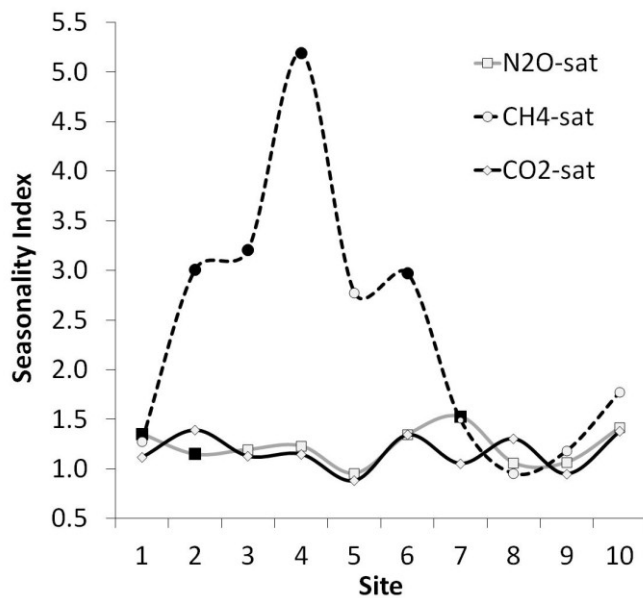


Figure 4. Seasonality Index (mean summer gas saturation divided by mean winter/spring saturations) along the estuary. Values above a seasonality index of 1 indicate higher summer saturations. Data points shaded black indicate significant differences between the seasonal index for each gas at each site ($p < 0.05$ (t-test)).

Furthermore, like many other estuaries, CO₂-sat shows marked variability in the upper freshwater zone with notable ‘peaks’ and ‘troughs’ forming spatial patterns with significant variability over the 7 sampling dates. Outside the influence of the MTZ, pelagic maximum CO₂-sat peaks occurred in late April (2009 +2010), June (2009 + 2010) and July (2009) coinciding with higher temperatures, spatial DO-sat minima and spatial TSS maxima. CO₂-sat was found to be correlated to a number of measured water chemistry parameters (Table 2).

Table 2. Pearson’s correlation coefficients for pooled data (n=69) and by sampling occasion (n=10)

	N ₂ O-Sat.	CH ₄ -Sat.	CO ₂ -Sat.	Temp °C	NO ₃ mg N l ⁻¹	NH ₄ mg N l ⁻¹	pH	DO mg l ⁻¹	DO sat.	Cond. mS cm	TSS mg l ⁻¹
All data†											
N ₂ O-sat	1.00	0.55	0.69*	0.85**	-0.83**	-0.51	-0.91***	-0.80**	-0.25	0.59	-0.69
CH ₄ -sat	0.55	1.00	-0.17	0.78**	-0.45	-0.23	-0.29	-0.88***	-0.77*	0.67*	-0.34
CO ₂ -sat	0.69*	-0.17	1.00	0.38	-0.71*	-0.61	-0.76**	-0.23	0.39	0.08	-0.60
Jun-10											
N ₂ O-sat	1.00	0.74**	0.80***	0.71**	-0.10	-0.34	-0.60*	-0.78***	-0.20	-0.71**	0.18
CH ₄ -sat	0.74**	1.00	0.78***	0.63**	-0.10	0.07	-0.63*	-0.77***	-0.22	-0.69**	0.00
CO ₂ -sat	0.80***	0.78***	1.00	0.65**	-0.41	-0.24	-0.72**	-0.98***	-0.65**	-0.79***	-0.17
Apr-10											
N ₂ O-sat	1.00	0.83***	0.74**	-0.41	0.40	0.54*	-0.32	-0.02	-0.23	-0.28	-0.32
CH ₄ -sat	0.83***	1.00	0.91***	-0.53*	0.69*	0.55*	-0.38	-0.10	-0.26	-0.58*	-0.20
CO ₂ -sat	0.74**	0.91***	1.00	-0.80***	0.43	0.35	-0.59*	-0.19	-0.41	-0.79***	-0.27
Feb-10											
N ₂ O-sat	1.00	-0.18	0.87***	0.61*	0.09	-0.52	-0.68**	-0.42	0.61*	-0.71**	-0.43
CH ₄ -sat	-0.18	1.00	-0.31	-0.55*	-0.09	0.44	-0.10	0.63**	-0.20	-0.41	0.77***
CO ₂ -sat	0.87***	-0.31	1.00	0.83***	0.33	-0.34	-0.75**	-0.61*	0.78***	-0.63**	-0.34
Sep-09											
N ₂ O-sat	1.00	0.49	0.76**	-0.82***	0.26	-0.76***	-0.71**	0.75**	-0.29	-0.85***	-0.36
CH ₄ -sat	0.49	1.00	0.32	-0.31	0.21	-0.69**	-0.73**	0.61*	-0.28	-0.70**	-0.01
CO ₂ -sat	0.76**	0.32	1.00	-0.70**	0.20	-0.65**	-0.76***	0.79***	-0.16	-0.60*	-0.43
Jul-09											
N ₂ O-sat	1.00	-0.02	-0.25	-0.20	-0.40	-0.06	0.06	0.13	0.13	0.09	-0.30
CH ₄ -sat	-0.02	1.00	0.66**	0.32	0.86**	-0.22	-0.54*	-0.75**	-0.80***	-0.61*	0.28
CO ₂ -sat	-0.25	0.66**	1.00	0.64**	0.75*	-0.24	-0.56*	-0.76**	-0.80***	-0.64**	0.63**
Jun-09											
N ₂ O-sat	1.00	0.33	0.51	0.33	-0.24	0.13	0.04	-0.28	-	0.21	0.66**
CH ₄ -sat	0.33	1.00	0.62*	-0.11	0.67	0.28	-0.65**	-0.62**	-	-0.57*	-0.12
CO ₂ -sat	0.51	0.62*	1.00	-0.03	-0.53	-0.12	-0.60*	-0.50*	-	-0.65**	0.14
Apr-09											
N ₂ O-sat	1.00	0.58*	0.20	-0.23	-0.01	0.41	-0.56*	0.60*	0.50	-0.58*	-
CH ₄ -sat	0.58*	1.00	-0.04	-0.15	-0.20	-0.40	-0.83***	0.24	0.09	-0.60*	-
CO ₂ -sat	0.20	-0.04	1.00	-0.48	0.64	0.90***	-0.07	0.71*	0.79**	-0.25	-

*p < 0.1 ** p < 0.05 *** p < 0.01 significance

† = Means of monthly data used

For example a significant positive relationship was observed with N_2O -sat ($p < 0.1$) and significant negative relationships with NO_3^- ($p < 0.1$) and pH ($p < 0.05$).

The relationships between gas saturations and water chemistry however displayed marked temporal variability as highlighted by the change in relationships between sampling months in Table 2. For example, although no apparent relationship appeared in the pooled data between CO_2 -sat and DO/DO-sat, a strong seasonal pattern was apparent. In the summer sampling surveys (June 09, July 09, June 2010) a strong significant inverse relationship between CO_2 -sat and DO/DO% was clear. However, in the colder sampling periods this relationship either disappeared or changed completely. For example in April 09 and February 2010 a strong positive relationship appeared (0.79 $p < 0.05$; 0.78 $p < 0.01$ respectively) with no significant relationships observed in September 2009 and April 2010. On the whole, CO_2 -sat remained in general above atmospheric equilibrium across the estuary and between seasons with saturations across the estuary always higher in the summer than winter (Fig. 4) with the exception of site 5.

A consistent spatial pattern in DO-sat was apparent from the data in the summer/spring surveys (June 09, July 09, April 2010, and June 2010) in which values were close to atmospheric equilibrium in the upper estuary but decreased to a minimum in the middle estuary transition zone before recovering again towards the outer estuary. This pattern was broadly matched (although inversely) by CO_2 -sat and TSS with concentrations peaking at the DO minimum before decreasing towards the outer estuary. For example the peak CO_2 -sat in July 2009 (337 %) corresponded to a DO-sat minimum of 84 % and a peak TSS concentration of 106 mg l^{-1} .

This area of the estuary is known as the maximum turbidity zone (MTZ) where sediment is re-suspended in the water column and is known to be an area of intense heterotrophy, highlighted by the large O_2 depletions. It is also an area where autotrophy is severely limited, with the maxima of TSS causing steep light extinction inhibiting photosynthesis and promoting respiration of heterotrophic bacteria attached to the suspended particles (Crump et al., 1998; Goosen et al., 1999).

Outside the influence of the MTZ, pelagic phytoplankton blooms are a common occurrence in both the fresh and saltwater zones of estuaries corresponding to maximum light availability in the summer months (Borges and Abril, 2011). Gross Primary

Production (GPP) in the summer months can therefore create a sink of CO₂ and a source of O₂ which could significantly mask sources such as respiration. However, there is little evidence of pelagic phytoplankton blooms in the Tay estuary, with concurrent measurements of chlorophyll (Dudley et al., unpublished) always below a maximum of 30 µg/l, with no significant concentrations in the upper estuary. Instead, the peaks in chlorophyll consistently coincide with the MTZ. This is likely to be driven by either the resuspension of benthic algae or through a ‘trapping mechanism’ whereby the MTZ traps phytoplankton cells allowing a chlorophyll maximum to build up (Cloern et al., 1983). The presence of a significant benthic algae population throughout the estuary could however perform a similar function to that of pelagic phytoplankton blooms, and such processes could explain the variability observed both in CO₂.sat and DO-sat in the upper estuary. Variability in CO₂ saturations, especially in the upper estuary, could also arise from lateral transport of gas rich waters from tidal flats and marshes as reported by Cai et al. (1999). Intertidal marshes are generally a net sink of atmospheric CO₂ (Megonigal and Neubauer, 2009) due to high GPP. However, they also promote a net burial of organic matter into the sediments, from which large amounts of CO₂ are emitted to both the atmosphere and water column once submerged. It is possible that tidal flushing of GHGs from the fringe marshes and mudflats in the upper Tay estuary could account for the variability in CO₂.sat observed out with the MTZ between sites 1 – 4. However this remains unclear since production and emission rates were not directly measured from intertidal areas.

However, there are other possible driving factors that could play significant roles in controlling CO₂.sat in the Tay estuary. The ventilation of riverine CO₂ has been found to be a significant source of CO₂ emission in other estuaries, particularly estuaries with short freshwater residence times (Abril et al., 2000). The Tay estuary has a relatively short residence time (2 -11 days) compared to some other macro-tidal estuaries in the UK such as the Humber (30-60 days) (Barnes and Upstill-Goddard, 2011). It is likely that in the Tay, riverine CO₂ contributes significantly to overall CO₂.sat and emission within the estuary.

Spatial, temporal and seasonal patterns in CH₄ saturation and controlling factors

Processes controlling spatial and temporal CH₄ variability

The spatial and temporal distribution of CH₄-sat within estuarine environments can be influenced by a wide range of factors, but mainly result from the balance between lateral input (from rivers, marshes and mudflats), microbial production/oxidation and physical ventilation which are themselves subject to a wide range of controlling factors that result in large spatial and temporal heterogeneity in CH₄ distributions within estuaries.

Dissolved CH₄ concentrations ranged from 0.04 to 4.3 µg l⁻¹ corresponding to saturations of 100 % to 13100 % (Table 1). The distribution of CH₄-sat during estuarine transit for the 7 sampling surveys is shown in Fig. 2 and distributions over a tidal cycle at one site in Fig. 3. The mean (2602 %) and range of CH₄ saturations (100–13135 %) measured in the Tay estuary are within the range of values reported from other estuaries in the UK (Table 5) which generally fall between 100 % and 21000 %. Data on the Tay estuary and those reported in the literature clearly document the variability in dissolved CH₄ concentration and saturations found between and within estuaries, particularly in upper estuaries, as highlighted in our own results with strong seasonal and spatial changes in CH₄ saturation throughout the entire estuary.

Firstly, spatial variability in the upper estuary was highlighted by a pronounced increase in CH₄ saturation in the freshwater zone of the estuary which was stronger in the summer months (July 2009 % saturation values: 664-13135) and weaker in the winter/spring (April 2009% saturation values: 777-2424). This seasonal effect is highlighted in Fig. 4 with a pronounced spatial pattern in the seasonality of CH₄-sat, with a greater seasonality index score in the upper estuary peaking at site 4. Gas saturations were always higher in summer than in winter across all of the 10 sites (Fig. 4). The spatial increases in CH₄-sat in the upper estuary suggest that there are significant sources of CH₄ within this zone. Similar spatial CH₄ distributions have been reported in other European estuaries in which an increase in low salinity regions was linked to the dominance of CH₄ production over oxidation and degassing (Middelburg et al., 2002; Upstill-Goddard et al., 2000).

The inter-seasonal dynamics in CH₄ saturations in the upper estuary can be explained through the changes in temperature. The strong correlations (Table 2) between CH₄-sat

and temperature (0.78 $p < 0.05$) and CH₄-sat and DO (-0.88, $p < 0.01$) suggests that a microbial source of CH₄ is likely in the upper estuary.

Water residence time could also play a part. Freshwater input to the estuary varies seasonally, with lower flows in summer leading to an extension of the water residence time in the upper estuary and also higher organic matter sedimentation rates, fuelled by seasonal increases in GPP that could enhance microbial production of CH₄. Therefore the source of CH₄ could be from in-stream microbial cycling of carbon in bed sediments.

Another possible source is tidal flushing/pumping transporting CH₄ rich waters from fringe marshes and intertidal mudflats (Barnes et al., 2006; Bouillion et al., 2008; Grunwald et al., 2009) which are known to be areas of intense CH₄ production due to enhanced sedimentation rates and release of labile organic matter (Borges and Abril, 2011). Possible evidence of tidal flushing/pumping was observed in our own temporal data (Fig. 3b) with CH₄ concentrations clearly increasing as the tide ebbed. The temporal study was conducted over a half tidal cycle at one site near the middle estuary (site 5). Saturations nearly doubled over the 8 hours sampling period from 4964 % to 8168 % corresponding to a drop in tidal height of 3.9 m to 0.4 m and conductivity from 6.5 mS cm⁻¹ to 0.45 mS cm⁻¹. It is unclear whether this exclusively points to lateral pumping of inter tidal areas or is related to the mixing of river water from upstream as the tide ebbs. However similar findings have been found in a number of other studies, clearly showing temporal peaks in CH₄ concentrations at low tide, even in systems with no freshwater inputs (Bouillion et al., 2008; Grunwalk et al., 2009).

The second major pattern observed in the seasonal transects was a sudden decrease in CH₄ saturation generally in the mid-estuarine mixing zone (sites 4–8). This region is characterised by strong hydrodynamics, evidenced by the large increases of TSS and an increase in salinity as freshwater meets seawater (Fig. 2). In the summer months the sudden decreases coincide with the peak in TSS but occur before the increase in salinity (e.g. July 2009 and June 2010). However, in February 2010 the reduction in CH₄-sat coincided with the increase in salinity and not the increase in TSS. The timing of the sudden reduction in CH₄-sat was analysed further in Fig. 5 which plots CH₄-sat plotted with conductivity (representing salinity changes) and the gas transfer rate (scaled to total area of the compartment) with a line added to indicate the CH₄-sat under conservative mixing i.e. without any losses or gains. In July 09, April 2010 and June 2010 CH₄-sat

was considerably below the conservative mixing line indicating substantial sinks in CH₄. In February 2010, CH₄-sat generally behaved conservatively with a small increase in the lower salinity zone.

The rapid decrease observed in CH₄-sat from the upper to the lower estuary may be controlled by a number of factors. As for CO₂ one of the main sinks of CH₄ within estuaries is evasion to the atmosphere, which is likely to be most pronounced in the mid estuary mixing zone. Microbial aerobic oxidation of CH₄ to CO₂ by methanotrophs in aerobic conditions, using CH₄ as a sole carbon source (Hanson and Hanson, 1996), has also been shown to be an important sink in a number of estuaries (De Angelis and Scranton, 1993; Lilley et al., 1996; Sansone et al., 1999; Abril and Iversen, 2002; Abril et al., 2007). Salinity is an important control on CH₄ oxidation rates, with additions of salt inhibiting oxidation in their freshwater samples (De Angelis and Scranton 1993).

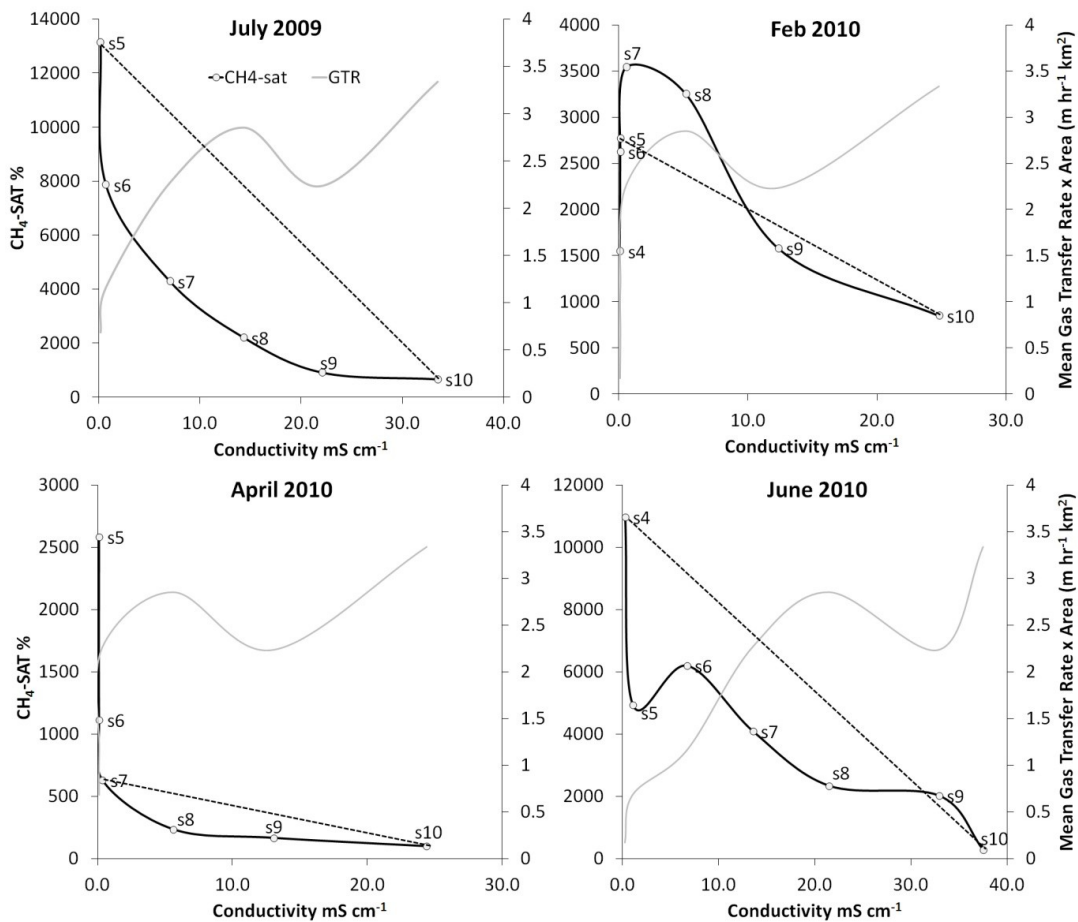


Figure 5. Conservative mixing diagrams for CH₄-sat plotted against conductivity (representing salinity) alongside the mean gas transfer rate (GTR) as a function of estuary compartment surface area for selected sampling occasions. The dotted straight line indicates the path that CH₄-sat should follow under conservative mixing.

Total suspended sediment has also been shown to be an important controlling factor in CH₄ oxidation, with significant negative correlation reported between suspended sediment and CH₄ concentration (Abril et al., 2007). The same correlation was not directly observed in our data, but this does not exclude the possibility of significant CH₄ oxidation in the Tay estuary. The conservative mixing plots (Fig. 5) show that there are substantial CH₄ losses at very low salinities in spring and summer 2010 that cannot be accounted for by a simple dilution mechanism. These may be largely attributed to gas evasion as the region of CH₄ loss corresponds closely to the MTZ (Fig. 2b) where the estuarine compartment area increases substantially as the area-scaled gas transfer rate shows in Fig. 5. However, this does not explain the seasonal heterogeneity in CH₄-sat patterns shown in Fig. 5; particularly why the sudden decrease in concentration was not observed in February 2009 when mixing appeared to be conservative and no apparent major change in gas transfer rate. Possible explanations included that CH₄ oxidation in the MTZ is strongly temperature dependent and thus was insignificant at the low water temperatures in the February survey or that there was a substantially greater source of CH₄ in the Estuary in February between site 6 and 8.

Spatial, temporal and seasonal patterns in N₂O saturation and controlling factors

Dissolved N₂O concentrations ranged from 0.18 to 0.56 N₂O-N µg l⁻¹ corresponding to saturations of 69 % to 188 % (Table 1; Figure 2). Saturations of N₂O in the Tay estuary are similar to those found in other low nutrient estuaries in the UK such as the Tweed, Tyne, Conwy, and Stour and substantially lower than reported from enriched estuaries such as the Humber, Thames, Colne and Deben (Table 5). Spatial variability of N₂O in the Tay estuary followed a similar pattern to that reported in other studies (e.g. Dong and Nedwell, 2006) in that the concentrations and saturations usually peaked in the upper estuary suggesting a N₂O source within this area.

Sources of N₂O within estuaries include both external inputs from the upstream river (supersaturated waters from diffuse or point sources in the catchment entering the estuary) and internal inputs from *in-situ* microbial production within benthic and suspended sediments. These microbial processes include denitrification, nitrate ammonification and nitrification, and it is still currently unclear which of these processes acts as the predominant source of N₂O within estuaries (Barnes and Upstill-Goddard,

2011). All of these microbial processes involve or produce NO_3^- and NH_4^+ indicating that dissolved N concentrations and N_2O saturation should be positively correlated. However, unlike Dong et al. (2004) who reported significant positive correlations between N_2O saturation and NO_3^- and between N_2O and NH_4^+ across a number of UK estuaries, N_2O -sat within the Tay estuary appeared negatively correlated in the pooled data, with no significant correlation in individual transects. This negative relationship is likely to be an artefact of the significant positive relationship between N_2O -sat and temperature. Whilst N_2O -sat and temperature are highest in the summer, NO_3^- in the Tay is at its lowest concentrations, conversely in the winter when saturations and temperature are low, NO_3^- in the Tay is at its highest. Barnes and Upstill-Goddard (2011) found similar results in the Humber estuary with highest N_2O saturations occurring when river-borne DIN was at its lowest in the MTZ. Hence, it is likely that in the Tay estuary temperature is the main controlling factor behind N_2O saturations and that this indicates a microbial sources of N_2O . A significant positive relationship between CO_2 -sat and N_2O -sat (Table 2) was observed in the pooled data and in 4 of the 7 transects. This likely results from shared environmental controls, for example microbial respiration leads to a reduction in dissolved oxygen, which in turn can promote denitrification in the presence of NO_3^- . The process of denitrification also produces CO_2 , further strengthening the relationship.

On close inspection of N_2O spatial patterns a clear decrease in N_2O -sat was observed within the MTZ on a number of occasions, but notably in April 2009, July 2009 and June 2010. The MTZ is thought to be an area of possible intense nitrification and microbial activity with the latter already highlighted by our CO_2 data which in general displays clear defined peaks in the turbidity zone. The MTZ has been found to be a source of N_2O in other estuaries (Barnes and Upstill-Goddard, 2011) and it is unclear why the peaks of N_2O are out of phase with the TSS peaks and CO_2 peaks for example in July 2009.

Combined Ordination Analysis: Principal Component and Cluster Analysis

PCA and cluster analysis was performed on the entire dataset to help better understand the biogeochemical functioning of the Tay estuary, with the specific aim in understanding temporal and spatial variability in gas saturations.

The PCA generated a PC1xPC2 ordination plane which explained 55.2 % of the total variation in the dataset. PC3 explained a further 15.4 % of the total variation and PC4

explaining 8.4 %. The main variable coefficients (eigenvectors) generated are displayed in Table (3) and on the whole the variances from all variables are well represented in PC1 and PC2. The eigenvectors are also plotted in Fig.6A which highlights the linear combinations that define the PCs, and since the eigenvectors at large are of comparable length, this indicates that determinand variability is equally represented on the PC1xPC2 plane.

Table 3. Coefficients of variables (eigenvectors) calculated from the principal component analysis of the entire dataset. PC = principal component.

Variable	PC1	PC2	PC3	PC4
N ₂ O-sat	-0.343	0.054	-0.023	0.155
CH ₄ -sat	-0.330	-0.041	0.447	-0.093
CO ₂ -sat	-0.306	0.306	-0.012	0.253
Temperature	-0.374	-0.364	-0.012	0.109
NO ₃ -N	0.142	0.391	0.347	-0.349
NH ₄ -N	0.277	-0.014	0.323	-0.208
pH	0.328	-0.302	-0.136	-0.203
DO	0.379	0.265	-0.104	0.032
DO %	0.211	0.216	-0.407	0.479
Conductivity	0.167	-0.476	-0.196	-0.152
TSS	0.238	-0.033	0.498	0.493
TSS-LOI	0.252	-0.343	0.343	0.435

Cluster analysis was also performed on the dataset to establish if there were groups of samples that shared similar environmental conditions. Four clusters of data points were identified and plotted in the PC1xPC2 plane (Fig. 6B). This combined approach enabled the identification of several biogeochemical states within the estuary which were broadly defined by spatially by salinity and temporally by temperature.

Cluster A was spatially separated within the estuary by salinity (represented by conductivity), with the high salinity sites from each of the 7 sampling transects found within this cluster (Conductivity > 10 mS cm⁻¹). Other characteristic features of cluster A, were low concentrations of NO₃ (< 0.5 mg N l⁻¹), a broad range of temperature (10 –

19 °C) and DO-sat (87 – 100 %), moderate TSS (22 – 50 mg l⁻¹), high pH (7.5 – 9) and moderate to high NH₄ (0.03-0.07 mg N l⁻¹). GHG saturations were generally low especially for CO₂ and CH₄, but with a broader range found in N₂O influenced by possible intense nitrification associated with possible pollution near the city of Dundee. However, overall the conditions in cluster A are highly representative of waters that are strongly influenced by the coastal sea with a strong dilution mechanism.

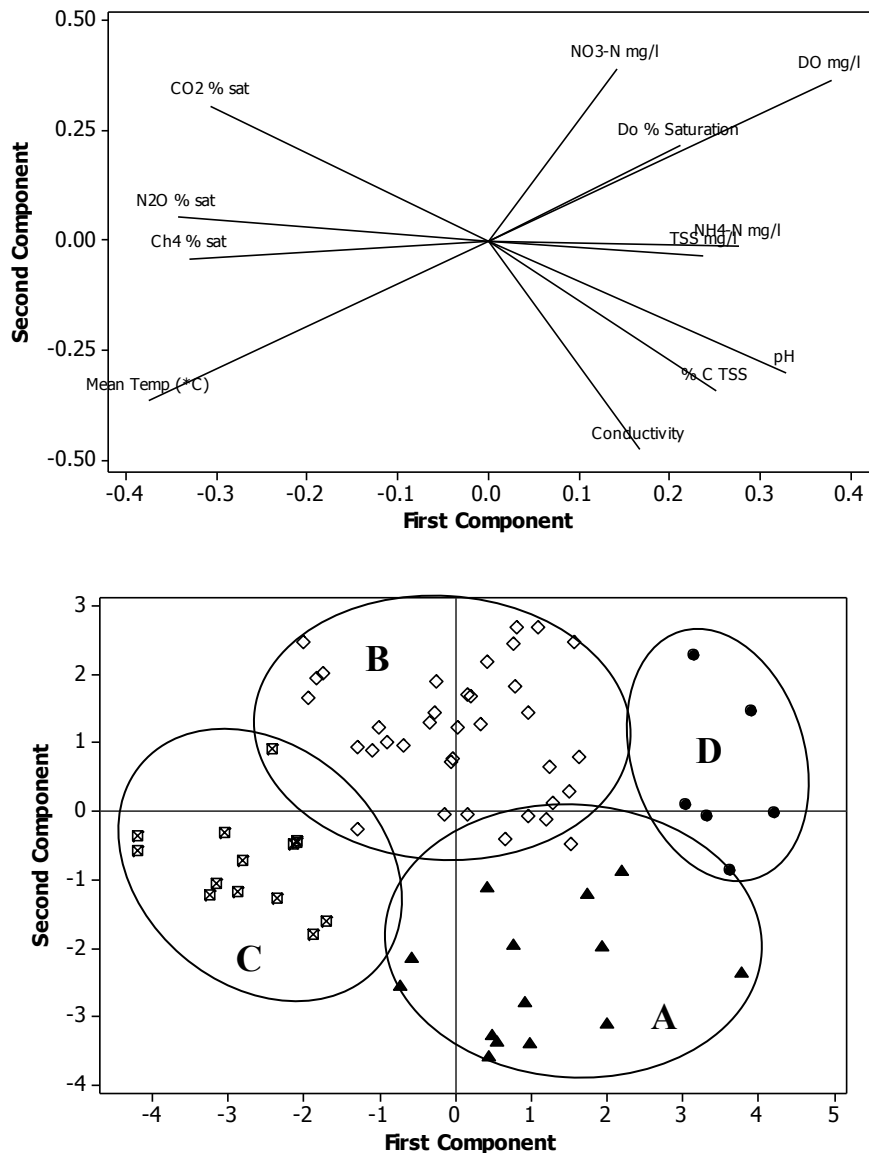


Fig.6. (A) Projections of eigenvectors on the PC1xPC2 ordination plane. (B) Results of k-means cluster analysis plotted on the PC1xPC2 plane.

Clusters B and C are generally spatially defined within the estuary by low to moderate salinity, representing sites from the upper and middle estuary. However they are split

seasonally by temperature. Cluster B displays a broad range in temperature (3 – 15 °C) and DO-sat (92 – 100%) with data points mainly from spring and autumn transects, displaying generally high NO₃ concentration (0.2 – 1.1 mg N l⁻¹), and moderate TSS (0 – 131 mg l⁻¹). A broad range of N₂O-sat is found, with generally moderate to high CO₂ saturations (178 – 628 %), with CH₄ moderate to low. Cluster C is separated from cluster B by temperature, with the highest recorded temperatures (> 15 °C) found within the cluster (July 2009 and June 2010) with other features including low pH (< 7.7) , low DO-sat (83 – 93 %) moderate to low nutrient concentration (NO₃+NH₄). This corresponds to peaks in CH₄ saturation (1700-13100 %), and moderate CO₂-sat (184 – 387 %) and a broad range in N₂O saturation (81 – 188 %).

In summary, cluster C represented conditions present during peak biological processing in the warmest summer months, where freshwater input from the River Tay is at its lowest and water residence time is presumably at its highest. This steady state provides an ideal environmental for microbial processing highlighted by the large reduction in DO saturation typical in the upper/middle estuary, which generally peaks in the MTZ (Figure 2). The reduction in DO combined with high temperatures likely explains the peaks in CH₄ saturation observed within this cluster. Whereas Cluster B typically represents the conditions found in spring and autumn in the upper to middle estuary zone. NO₃ concentrations are higher than in summer in-line with increased river discharge and input. CO₂-sat is also higher than in summer which could highlight the important balance between heterotrophy and autotrophy throughout the seasonal cycle. Heterotrophy has generally been found to dominate during spring and autumn, where as autotrophic processes generally peak in the warmer summer months potentially reducing CO₂-sat.

The final cluster (D) is solely represented by the winter sampling transect which is separated from the other groups by low temperature. Other characteristics that set it apart include low GHG saturations, peaks in nutrient concentration (NO₃ and NH₄), peaks in DO concentration, and a peak in TSS. Generally this cluster represent low productivity, with high river freshwater input combined with greater seasonal wind speeds creating peaks in TSS in the MTZ.

Modelled Gas fluxes

Spatial and seasonal changes in gas flux densities and annual emission were estimated from the four k -wind speed parameterizations, as well as mean surface water temperatures, wind speed and k_{600} for CO₂ (Table 4). Like the dissolved concentrations, the gas flux density and total emissions displayed substantial seasonal and temporal variability within the estuary but were within the range reported in the literature (Table 5).

The largest fluxes for CO₂ occurred in the spring and autumn in the middle estuary. The autumn fluxes corresponded to the highest mean monthly wind speeds and subsequent large k values. The smallest flux occurred in winter, corresponding to the lowest mean temperatures, lowest average monthly wind speed and subsequently mean k values.

The largest fluxes and emission for CH₄ occurred in the middle estuary in summer, and the smallest flux and emission was recorded in the outer estuary in spring. The fluxes and emission of N₂O also followed the seasonal cycle of temperature. The largest fluxes and emissions occurred in the summer and autumn with the smallest flux and emission recorded in the spring, whilst in the winter sampling survey the estuary acted as a sink for atmospheric N₂O.

Substantial spatial variability was also evident in the gas flux calculations. Mean flux density and emission were calculated for the 10 sites in the estuary subdivided into three zones; upper (sites 1-4, permanently freshwater); middle (sites 5-7, mixing zone between fresh and salt water); and lower zone (sites 8-10, permanently saline) (Table 4). Flux densities for all three gases behaved generally in a similar fashion, with highest concentrations observed in the upper and middle estuary with substantially smaller flux densities in the lower estuary. On the whole, annual emissions calculated using method A (mean flux density x estuary area) were substantially higher than those calculated through method B (site flux density multiplied by site/compartiment area).

Estuary emission was also calculated in CO₂ equivalents using the 100 year global warming potentials (IPCC 2007). In the Tay estuary, CO₂ dominated the total emissions budget (~ 98 %), with CH₄ and N₂O each making up approximately 1 % of the total emission budget as CO₂-eq.

The major drivers of seasonal variability in flux density were changes in mean wind

Table 4. Estimated gas flux densities, annual emission and annual emission as CO₂ equivalents using the four gas transfer rate parameterizations: for the whole estuary using methods A and B (see text for explanation); for sub-compartments upper, middle and

	Mean Gas Transfer Rate (<i>k</i>)				Flux Density g C m ⁻² y ⁻¹	CO ₂		CH ₄			N ₂ O		
	C95	RC 01	BO4 S	BO4 T		Annual Emission kg C yr ⁻¹	Annual Emission CO ₂ eq kg yr ⁻¹	Flux Density g C m ⁻² y ⁻¹	Annual Emission kg C yr ⁻¹	Annual Emission CO ₂ eq kg yr ⁻¹	Flux Density g N m ⁻² y ⁻¹	Annual Emission kg N yr ⁻¹	Annual Emission CO ₂ eq kg yr ⁻¹
Whole estuary (a)	-	-	-	-	362.2	41.6x 10 ⁶	152 x 10 ⁶	1.017	116998	3.90 x 10 ⁶	0.059	6785	3.18 x 10 ⁶
Whole estuary (b)	6.8	9.4	15.5	25.8	362.2	9.2x 10 ⁶	33.8 x 10 ⁶	1.017	23920	0.79 x 10 ⁶	0.059	1282	0.60 x 10 ⁶
Upper (b)	-	-	-	-	455.8	0.4 x 10 ⁶	1.56 x10 ⁶	1.249	1412	0.05 x 10 ⁶	0.085	84.66	0.04 x10 ⁶
Middle (b)	-	-	-	-	439.0	5.2 x 10 ⁶	18.9x10 ⁶	1.339	13840	0.46 x 10 ⁶	0.074	887.8	0.42 x 10 ⁶
Outer (b)	-	-	-	-	150.6	3.6 x 10 ⁶	13.4 x10 ⁶	0.354	8667	0.29 x 10 ⁶	0.010	309.2	0.14 x10 ⁶
Spring (b)	6.7	9.0	15.5	25.9	105.5	0.9 x 10 ⁶	3.40 x10 ⁶	0.096	613.3	0.02 x 10 ⁶	0.007	22.79	0.01 x10 ⁶
Summer (b)	6.0	8.0	14.6	24.6	79.92	0.6 x 10 ⁶	2.21 x10 ⁶	0.364	2432	0.08 x 10 ⁶	0.020	210.9	0.10 x 10 ⁶
Autumn (b)	11.3	16.9	20.1	32.4	158.5	1.0 x 10 ⁶	3.49 x10 ⁶	0.305	2056	0.07 x 10 ⁶	0.031	79.80	0.04 x 10 ⁶
Winter (b)	5.0	6.6	13.2	22.6	34.74	0.04x10 ⁶	0.16x10 ⁶	0.150	1903	0.06 x 10 ⁶	-0.002	-85.28	0.04 x 10 ⁶

Spring = April 09 & April 2010; Summer = June 2009 & 2010, July 2009; Autumn = September 2009; Winter = February 2010 a, b = emission calculation method A or B.

speed and the variability of dissolved gas saturation. The main driver of net gas emission in different areas of the estuary was the changes in estuarine area from site to site whilst flux density was driven by the variability in dissolved concentrations. Flux estimates of CH₄ and N₂O were largest in the summer months and lowest in the winter/spring highlighting the importance of the temperature cycle in the fluxes of CH₄ and N₂O. CO₂ fluxes were highest in the spring and early autumn and lowest in winter, corresponding well with the main changes in the growing season and annual temperature cycle. Heterotrophy (and CO₂ production) is dominant in spring after the sudden increase in temperature. Autotrophy gradually becomes more important as the day length increases and conditions become more favourable for GPP. In autumn GPP collapses and heterotrophy becomes dominant again before temperature decreases in winter resulting in a seasonal low in the CO₂ flux.

The gas fluxes reported in this study are subject to a number of uncertainties and possible error. In general errors in emission estimations arise from a number of factors: 1) uncertainties in k wind parameterizations; 2) bias in spatiotemporal coverage of dissolved gas concentrations; and 3) scaling errors.

One of the largest uncertainties in calculating gas fluxes within estuarine environments revolves around the methods used to calculate the gas transfer velocity; k . Currently a suitable and undisputed method does not exist, with considerable debate still ongoing among biogeochemists, ecologists and physicists (Borges and Abril, 2011). Since this study did not measure k directly within the Tay estuary, we adopted an approach used in several other studies (Ferrón et al., 2007; Barnes and Upstill-Goddard, 2011; Bozec et al., 2012) and used parameterizations developed in other estuaries to calculate k . Although the k wind speed approach we utilised in this study allows for a bracketed mean flux, the overall uncertainty remains large. For example the fluxes calculated using the C95 relationship are on average a quarter of the magnitude of those calculated using the BO4T (Thames estuary) parameterization. This variation in fluxes highlights that k wind parameterizations are site specific and could lead to substantial errors in fluxes when utilized in other estuaries with contrasting environmental conditions. However, by using a range of parameterizations instead of focusing on one, it is hoped to bracket the emission estimations into a likely range with the caveat that this still provides an emission estimate with considerable uncertainty.

Table 5. Concentrations, range and mean of saturations and annual emissions of CH₄, CO₂ & N₂O in other UK estuaries and the Tay.

Estuary Site	nmol l ⁻¹	Range of gas saturation % (mean)	Flux Density	Annual Emission	References
	N ₂ O		g m ⁻² yr ⁻¹	g N ₂ O yr ⁻¹	
Tweed	-	96 – 110 (100.4)	0.05	-	Barnes and Owens 1998
Thames	11.2-93	93-681 (321)	1.11	-	De Bie et al 2002
Colne	(197.3)	(992.9)	5.42	-	Dong et al 2004
Stour	(24.9)	(119.9)	0.33	-	Dong et al 2004
Deben	(32.6)	(186.9)	0.60	-	Dong et al 2004
Conwy	(18.6)	(113.6)	0.14	-	Dong et al 2004
Humber	-	157-6506 (395.7)	1.23	4.0 x 10 ⁸	Barnes and Upstill-Goddard 2011
Forth	-	98 – 313 (152.3)	0.25	1.3 x 10 ⁷	Barnes and Upstill-Goddard 2011
Tamar	-	99 – 210 (145)	0.13	5.8 x 10 ⁶	Barnes and Upstill-Goddard 2011
Tyne	-	98 – 280 (123.5)	0.12	5.8 x 10 ⁵	Barnes and Upstill-Goddard 2011
Tees	-	106 – 2118 (383)	1.11	1.1 x 10 ⁷	Barnes and Upstill-Goddard 2011
Tay Estuary	-	100-118 (107)	0.04	4.0 x 10 ⁶	Barnes and Upstill-Goddard 2011
Tay Estuary	-	69 – 188 (118.6)	0.06	6.8 x 10 ⁶	This study (emission calculation method a)
Tay Estuary	-	69 – 188 (118.6)	0.06	1.3 x 10 ⁶	This study (emission calculation method b)
	CH ₄		g m ⁻² yr ⁻¹	kg C yr ⁻¹	
Thames	1.7- 269	150 - 6700	-	-	Middelburg et al. (2002)
Tyne	13.5 -654	4500 - 20000	-	-	Upstill-Goddard et al. (2000)
Humber	13 – 667	370 - 21000	-	-	Upstill-Goddard et al. (2000)
Tay	4.5 - 361	100 - 13134	1.02	23921	This Study (method b)
	CO ₂ pCO ₂		g C m ⁻² d ⁻¹	kg C yr ⁻¹	
Thames	505-5200	-	3483	-	Frankignoulle et al. 1998
Tamar	380-2200	-	1441	-	Frankignoulle et al. 1998
Tay	214 -2186	66 - 644	992	9.2 x 10 ⁶	This study (method b)

Moreover, the variation in flux density and annual emission both spatially and temporally highlight the importance of removing spatial and temporal bias in sample coverage. For example, summer emissions of N₂O were 925 % higher than spring and 264 % higher than autumn, and would have resulted in significant over estimations without the seasonal coverage. However, the seasonal saturation and emission values in this study are still relatively uncertain, mainly due to only having one winter and autumn sampling transect, and it remains unclear if these cover typical autumn and winter estuarine conditions.

Spatial coverage is also important in estuarine studies. Flux densities were highest in the upper estuary and total estuarine mean concentrations would be substantially lower without this spatial coverage. This highlights important difficulties in up-scaling using mean saturation values to calculate total estuarine emission, especially when the highest flux densities are located in the upper estuary with the smallest total area. This is highlighted through the different results from the two emission calculation methods (Table 4 & Table 5), with the estimations from method A (using the estuary mean flux density and total area) being substantially higher than those from method B (using the mean of site compartment emissions). These emission estimations in themselves are both inherent with error as total estuarine area varies significantly over tidal cycles in macrotidal estuaries with large intertidal areas (such as in the Tay). This links with another possible limitation of the study which is a lack of spatial coverage within the estuary. It is possible that the widely spaced samplings points within the estuary transect fail to capture fine scale spatial hotspots in GHG fluxes associated with intertidal areas.

The flux estimates in this study also do not take into account emissions from exposed intertidal areas at low tide or potential emission from CH₄ ebullition. CH₄ ebullition has been found to occur in freshwater estuarine marshes and mudflats, but predominantly only occurs at low tide linked to changes in hydrostatic pressure. Moreover, ebullition is superseded in vegetated sediments by plant-mediated transport ((Borges and Abril 2011). Fluxes from intertidal areas in estuaries are known to be important. Recently Borges and Abril (2011), estimated on the global estuarine scale, that 3% of total flux of CH₄ was sourced from tidal flats, and 36 % from tidal marshes compared to only 4% from well mixed estuarine waters (the remaining emissions associated with mangrove sediments and stratified systems). Taking into consideration the impact of intertidal areas in the Tay

estuary, the flux estimates in this study would likely underestimate total CH₄ emission and therefore only represent emissions from estuary waters only, with no account for ebullition.

Previous studies have found that CH₄ loss through tidal pumping to the main estuary channel is 2 -4 times greater than direct diffusion of CH₄ from exposed intertidal sediments at low tide (Kelley et al., 1995). Intertidal areas and the influence of 'tidal pumping' create further uncertainty in the emission estimates. The temporal study at site 5 highlighted that GHG saturations vary temporally over tidal cycles, increasing 65% with CH₄-sat increasing from 4963 to 8168 % over 8 hours as the tide dropped. N₂O and CO₂ however showed more modest changes with CO₂-sat increasing by 18 % and N₂O increasing by 9 %. However, since this was a spot survey in the warmest time of the year (August) it is not known if the magnitude of these temporal increases would be replicated at other times of the year especially if the controlling factors behind the increase were temperature controlled microbial production. However, previous studies have found that tidal pumping of CH₄ is a major driver in CH₄ temporal and spatial distribution in estuarine waters (Middleburg et al., 2002; Grunwald et al., 2009) and overall at the ecosystem scale this could be an important evasion pathway (Borges and Abril 2011).

Summary

On the whole, these estimations of seasonal and annual CH₄, CO₂ and N₂O emissions are important for future global GHG budgeting. Emission factors for aquatic systems (especially for N₂O) are still highly uncertain (Ivens et al. 2011) despite extensive modelling studies over the last 20 years. Recent work has suggested that the contribution of European estuaries to global N₂O may be substantially overestimated (Barnes and Upstill-Goddard, 2011). This over estimation may be in part because of a bias towards nutrient impacted estuaries in the literature or the continuing uncertainty in what drives GHG production in aquatic environments and how this changes from system to system. This study of the Tay provides much needed estimates from a relatively unpolluted estuary, to redress the reported bias in studies towards nutrient impacted estuaries. We have also provided separate temporal and spatial estimates for fluxes that could help reduce uncertainty associated with global GHG budgets.

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References

Abril, G. and Iversen, N.: Methane dynamics in a shallow non-tidal estuary (Randers Fjord, Denmark), *Mar. Ecol.-Prog. Ser.*, **230**: 171-181, 2002.

Abril, G. and Borges, A. V.: Carbon dioxide and methane emissions from estuaries, in: *Greenhouse Gas Emissions - Fluxes and Processes: Hydroelectric Reservoirs and Natural Environments*, edited by Tremblay, A., Varfalvy, L., Roehm, C. and Garneau, M., Springer-Verlag, 187-207, 2004.

Abril, G., Commarieu, M.-V. and Guérin, F.: Enhanced methane oxidation in an estuarine turbidity maximum, *Limnol. Oceanogr.*, **52**: 470-475, 2007.

Abril, G., Riou, S.A., Etcheber, H., Frankignoulle, M., de Wit, R. and Middelburg, J. J.: Transient, tidal time-scale, nitrogen transformations in an estuarine turbidity maximum–fluid mud system (the Gironde, South-west France), *Estuar. Coast. Shelf S.*, **50**: 703–715, 2000.

Balls, P.: Nutrient behaviour in two contrasting Scottish estuaries, the Forth and Tay, *Oceanol. Acta*, **15**: 261–277, 1992.

Balls, P. W.: Nutrient inputs to estuaries from nine Scottish east coast rivers; influence of estuarine processes on inputs to the North Sea, *Estuar. Coast. Shelf S.*, **39**: 329-352, 1994.

Balls, P., Brockie, N., Dobson, J. and Johnston, W.: Dissolved oxygen and nitrification in the upper Forth estuary during summer (1982–92): patterns and trends, *Estuar. Coast. Shelf S.*, **42**: 117–134, 1996.

Bange, H. W.: Nitrous oxide and methane in European coastal waters, *Estuar. Coast.*

Shelf S., **70**: 361-374, 2006.

Barnes, J. and Owens, N. J. P.: Denitrification and nitrous oxide concentrations in the Humber estuary, UK, and adjacent coastal zones, *Mar. Poll. Bull.*, **37**: 247–260, 1998.

Barnes, J. and Upstill-Goddard, R. C.: N₂O seasonal distributions and air-sea exchange in UK estuaries: Implications for the tropospheric N₂O source from European coastal waters. *J. Geophys. Res.*, **116**, 2011.

Barnes, J., Ramesh, R., Purvaja, R., Nirmal Rajkumar, A., Senthil Kumar, B., Krithika, K., Ravichandran, K., Uher, G. and Upstill-Goddard, R.: Tidal dynamics and rainfall control N₂O and CH₄ emissions from a pristine mangrove creek. *Geophys. Res. Lett.*, **33**: 2006.

Billett, M. F. and Moore, T. R.: Supersaturation and evasion of CO₂ and CH₄ in surface waters at Mer Bleue peatland, Canada. *Hydrol. Process.*, **22**: 2044–2054, 2008.

Billett, M. F., Palmer, S. M., Hope, D., Deacon, C., Storeton-West, R., Hargreaves, K. J., Flechard, C. and Fowler, D.: Linking land–atmosphere–stream carbon fluxes in a lowland peatland system. *Global Biogeochem. Cy.*, **18**: 2004.

Borges, A. V. and Abril, G.: Carbon dioxide and methane dynamics in estuaries. In: Wolanski, E. Melusky D.S.(eds.) *Treatise on Estuarine and Coastal Science*. Waltham: Academic Press., **5**: 119–161, 2011.

Borges, A. V., Delille, B., Schiettecatte, L.-S., Gazeau, F., Abril, G. and Frankignoulle, M.: Gas transfer velocities of CO₂ in three European estuaries (Randers Fjord, Scheldt, and Thames). *Limnol. Oceanogr.*, **49**: 1630-1641, 2004.

Bouillon, S., R. Connolly, and S. Y. Lee (2008), Organic matter exchange and cycling in mangrove ecosystems: Recent insights from stable isotope studies, *J. Sea Res.*, **59**, 44–58

Bozec Y, Cariou T, Mace E, Morin P, Thuillier D, Vernet M. 2012. Season dynamics of air-sea CO₂ fluxes in the inner and outer Loire Estuary (NW Europe). *Estuarine, Coastal and Shelf Science*. 100L 58-71

Cai, W.-J., Pomeroy, L. R., Moran, M. A. and Wang, Y. C.: Oxygen and carbon dioxide mass balance for the estuarine- intertidal marsh complex of five rivers in the southeastern US. *Limnol. Oceanogr.*, **44**: 639-649, 1999.

Clark, J. F., Schlosser, P., Simpson, H. J. Stute, M., Wanninkhof, R. and Ho, D. T.: Relationship between gas transfer velocities and wind speeds in the tidal Hudson River determined by the dual tracer technique, in: *Air-Water Gas Transfer: Selected Papers from the Third International Symposium on Air-Water Gas Transfer*, 785–800, 1995.

Clarke KR, Warwick RN. Changes in marine communities: an approach to statistical analysis and interpretation, 2nd edn. PRIMER-E Ltd, Plymouth 2001

Cloern, J.E., Andrea, E.A., Cole, E.C., Wong, R.L.E., Arthur, J.F., Ball, M.D.: River discharge controls phytoplankton dynamics in the northern San Francisco Bay estuary. *Estuar. Coast. Shelf S.*, **16**: 415–429, 1983

Cole, J. J., Caraco, N. F.: Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. *Mar. Freshwater Res.*, **52**: 101-110, 2001.

Crump, B. C., Baross, J. A. and Simenstad, C. A.: Dominance of particle-attached bacteria in the Columbia River estuary, USA. *Aquat. Microb. Ecol.*, **14**: 7–18, 1998.

Crutzen, P. J.: Methane's sinks and sources, *Nature*, **350**:380–381, 1991.

Crutzen, P. J. and Schmailzl, U.: Chemical budgets of the stratosphere. *Planet. Space Sci.*, **31**: 1009–1032, 1983.

De Angelis, M. A. and Scranton, M. I.: Fate of methane in the Hudson River and estuary. *Global Biogeochem. Cy.*, **7**: 509–523, 1993.

De Bie, M.J.M., Middelburg, J.J., Starink, M., Laanbroek, H.J.. Factors controlling nitrous oxide at the microbial community and estuarine scale. *Marine Ecology Progress Series* **240**: 1 –9. 2002

De Wilde, H. P. J., and de Bie, M. J. M.: Nitrous oxide in the Schelde estuary: production by nitrification and emission to the atmosphere, *Mar. Chem.*, **69**: 203–216, 2000.

Denman, K. L., Brasseur, G., Chidthaisong, A., Ciais, P., Cox, P. M., Dickinson, R. E., Hauglustaine, D., Heinze, C., Holland, E., Jacob, D., Lohmann, U., Ramachandran, S., da Silva Dias, P. L., Wofsy, S. C. and Zhang, X.: Couplings between changes in the climate system and biogeochemistry, in: *Climate Change, 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., Miller, H. L., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 500-587, 2007.

Dong, L. F., Nedwell, D. B., Colbeck, I. and Finch, J.: Nitrous oxide emission from some English and Welsh rivers and estuaries, *Water Air Soil Poll.*, **4**: 127–134, 2004.

Dong, L. F., Nedwell, D. B. and Stott, A.: Sources of nitrogen used for denitrification and nitrous oxide formation in sediments of the hypernutrified Colne, the nitrified Humber, and the oligotrophic Conwy estuaries, United Kingdom. *Limnol. Oceanogr.*, **51**: 545–557, 2006.

Ferrón, S., Ortega, T., Gomez-Parra, A. and Forja, J. M.: Seasonal study of dissolved CH₄, CO₂ and N₂O in a shallow tidal system of the bay of Cadiz (SW Spain). *J. Marine Syst.*, **66**: 244-257, 2007.

Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D. W., Haywood, J., Lean, J., Lowe, D. C., Myhre, G., Nganga, J., Prinn, R., Raga, G., Schulz, M. and Van Dorland, R.: Changes in atmospheric constituents and in radiative forcing, in: *Climate Change, 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., Miller, H. L., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 130-234, 2007.

Frankignoulle, M. and Borges, A. V.: European continental shelf as a significant sink for atmospheric carbon dioxide. *Global Biogeochem. Cy.*, **15**: 569-576, 2001.

Frankignoulle, M. and Middelburg, J. J.: Biogases in tidal European estuaries: the BIOGEST project. *Biogeochemistry*, **59**: 1-4, 2002.

- Frankignoulle, M., Abril, G., Borges, A., Bourge, I., Canon, C., Delille, B., Libert, E. and The'ate, J.-M.: Carbon dioxide emission from European estuaries. *Science*, **282**: 434-436, 1998.
- Hanson, R. S. and Hanson, T. E.: Methanotrophic bacteria, *Microbiol. Rev.*, **60**: 439–471, 1996.
- Hope, D., Palmer, S. M., Billett, M. F. and Dawson, J. J. C.: Variations in dissolved CO₂ and CH₄ in a headwater catchment: an investigation of soil-stream linkages. *Hydrol. Process.*, **18**: 3255–3275, 2004.
- Howarth, R. W.: Coastal nitrogen pollution: A review of sources and trends globally and regionally. *Harmful Algae*, **8**: 14-20, 2008.
- Howarth, R. W., Billen, G., Swaney, D., Townsend, A., Jaworski, N., Lajtha, K., Downing, J. A., Elmgren, R., Caraco, N., Jordan, T., Berendse, F., Freney, J., Kudeyarov, V., Murdoch, P. and Zhao-Liang, Z.: Regional nitrogen budgets and riverine N & P fluxes for the drainages to the North Atlantic Ocean: natural and human influences. *Biogeochemistry*, **35**: 75-139, 1996.
- Garnier, J., Cébron, A., Tallec, G., Billen, G., Sebilo, M. and Martinez, A.: Nitrogen behaviour and nitrous oxide emission in the tidal Seine River Estuary (France) as influenced by human activities in the upstream watershed. *Biogeochemistry*, **77**: 305-326, 2006.
- Garnier, J., Billen, G., Even, S., Etcheber, H. and Servais, P.: Organic matter dynamics and budgets in the maximum turbidity zone of the Seine Estuary (France). *Estuar. Coast. Shelf S.*, **77**: 150-162, 2008.
- Goosen, N. K., Kromkamp, J., Peene, J., van Rijswijk, P. and van Breugel, P.: Bacterial and phytoplankton production in the maximum turbidity zone of three European estuaries: the Elbe, Westerschelde and Gironde. *J. Marine Syst.*, **22**: 151–171, 1999.
- Grunwald, M., Dellwig, O., Liebezeit, G., Schnetger, B., Reuter, R. and Brumsack, H.-J.: A novel time-series station in the Wadden Sea (NW Germany): first results on continuous nutrient and methane measurements. *Mar. Chem.*, **107**: 411-421, 2007.

Grunwald, M., Dellwig, O., Beck, M., Dippner, J. W., Freund, J. A., Kohlmeier, C., Schnetger, B. and Brumsack, H.-J.: Methane in the southern North Sea: sources, spatial distribution and budgets. *Estuar. Coast. Shelf S.*, **81**: 445-456, 2009.

IPCC (2007) Summary for policymakers, in: *Climate Change, 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., Miller, H. L., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2-18, 2007.

Ivens W, Tysmans DJJ, Kroeze C, Lohr AJ, van Wijnen J. 2011. Modeling global N₂O emissions from aquatic systems. *Current Opinion in Environmental Sustainability* **3**:350-358

Jiang, X., W. Ku, R.- L. Shia, Q. Li, J. W. Elkins, R. G. Prinn, and Y. L. Yung (2007), Seasonal cycle of N₂O: Analysis of data, *Global Biogeochem. Cycles*, **21**

Jones Jr., J. B. and Mulholland, P. J.: Carbon dioxide variation in a hardwood forest stream: an integrative measure of whole catchment soil respiration. *Ecosystems*, **1**: 183-196, 1998.

Kelley CA, Martens CS & Ussler III W (1995) Methane dynamics across a tidally flooded riverbank margin. *Limnol. Oceanogr.* **40**: 1112–1129

Kling, G. W., Kipphut, G. W. and Miller, M. C. Arctic lakes and streams as gas conduits to the atmosphere: implications for tundra carbon budgets, *Science*, **251**: 298-301, 1991.

Kremer, J. N., Reischauer, A, and D'Avanzo, C.: Estuary specific variation in the air–water gas exchange coefficient for oxygen, *Estuaries*, **26**: 829–836, 2003.

Kroeze, C., Dumont, E. and Seitzinger, S. P.: New estimates of global emissions of N₂O from rivers and estuaries, *Environm. Sci.*, **2**, 159–165, 2005.

Lilley, M. D., de Angelis, M. A. and Olson, E. J.: Methane concentrations and estimated fluxes from Pacific Northwest rivers. *Mitt. Int. Verein Limnol.*, **25**: 187–196, 1996.

Liss, P. S. and Slater, P. G.: Flux of gases across the air-sea interface. *Nature*, **247**: 181–

184, 1974.

Liss, P. S. and Merlivat, L.: Air-sea exchange rates: introduction and synthesis, in: (The Role of Air-Sea Exchange in Geochemical Cycling, edited by: Buat-Ménard, P., Reidel, Dordrecht, 113-127, 1986.

Megonigal, P. J. and Neubauer, S. C.: Biogeochemistry of tidal freshwater wetlands, in: Coastal Wetlands: An Integrated Ecosystem Approach, edited by: Perillo, G. M. E., Wolanski, E., Cahoon, D. R. and Brinson, M. M., Elsevier, 535-562, 2009.

Middelburg, J. J., Nieuwenhuize, J., Iversen, N., Høgh, N., de Wilde, H., Helder, W., Seifert, R. and Christof, O.: Methane distribution in European tidal estuaries, *Biogeochemistry*, **59**: 95-119, 2002.

Nassehi, V. and Williams, D.J.A. 1987. A mathematical model for salt intrusion in the Tay Estuary. *Proceedings of the Royal Society of Edinburgh* **92**: 285-297.

Neal, C., Harrow, M., Williams, R. J.: Dissolved carbon dioxide and oxygen in the River Thames: spring–summer 1997, *Science of the Total Environment*, **210**: 205–217, 1998.

Neal, C., House, W.A., Jarvie, H.P., Eatherall, A. The significance of dissolved carbon dioxide in major lowland rivers entering the North Sea, *Sci. Total Environ.* **210**: 187–203, 1998.

OSPAR: Eutrophication Assessment Reports: Tay Estuary, OSPAR Eutrophication Committee, 2006.

Ravishankara A. R., Daniel J. S. and Portmann R. W.: Nitrous oxide (N₂O): The dominant ozone-depleting substance emitted in the 21st century, *Science*, **326**: 123–125, 2009.

Raymond, P. A. and Cole, J. J.: Gas exchange in rivers and estuaries: choosing a gas transfer velocity, *Estuaries*, **24**: 312-317, 2001.

Raymond, P. A., Bauer, J. E., Cole, J. J.: Atmospheric CO₂ evasion, dissolved inorganic carbon production, and net heterotrophy in the York River estuary, *Limnol. Oceanogr.*, **45**: 1707-1717, 2000.

Rigby, M., Prinn, R. G., Fraser, P. J., Simmonds, P. G., Langenfelds, R. L., Huang, J., Cunnold, D. M., Steele, L. P., Krummel, P. B., Weiss, R. F., O'Doherty, S., Salameh, P. K., Wang, H. J., Harth, C. M., Mühle, J. and Porter, L. W.: Renewed growth of atmospheric methane, *Geophys. Res. Lett.*, **35**, 2008.

Sansone, F. J., Holmes, M. E. and Popp, B. N.: Methane stable isotopic ratios and concentrations as indicators of methane dynamics in estuaries, *Global Biogeochem. Cy.* **13**, 463–474, 1999.

Seitzinger, S. P., Kroeze, C. and Styles, R. V.: Global distribution of N₂O emissions from aquatic systems: Natural emissions and anthropogenic effects, *Chemosphere Glob. Chang. Sci.*, **2**: 267–279, 2000.

Upstill-Goddard, R. C., Rees, A. P. and Owens, N. J. P.: Simultaneous high-precision measurements of methane and nitrous oxide in water and seawater by single phase equilibration gas chromatography, *Deep-Sea Res.*, **43**: 1669–1682, 1996.

Upstill-Goddard, R. C., Barnes, J., Frost, T., Punshon, S. and Owens, N. J. P.: Methane in the southern North Sea: low-salinity inputs, estuarine removal, and atmospheric flux, *Global Biogeochem. Cy.*, **14**: 1205-1217, 2000.

Upstill-Goddard, R. C.: Air–sea gas exchange in the coastal zone, *Estuar. Coast. Shelf S.*, **70**: 388–404, 2006.

Wanninkhof, R.: Relationship between gas exchange and wind speed over the ocean, *J. Geophys. Res.*, **90**: 7373-7382, 1992.

Weiss, R. F. and Price, B. A.: Nitrous oxide solubility in water and seawater, *Mar. Chem.*, **8**: 347–359, 1980.

Wiesenburg, D. A. and Guinasso, N. L. Jr.: Equilibrium solubilities of methane, carbon monoxide and hydrogen in water and seawater, *J. Chem. Eng. Data*, **24**: 356–360, 1979.

Williams, D.J.A. and West, J.R. 1975. Salinity distribution in the Tay Estuary. *Proceedings of the Royal Society of Edinburgh (B)* **75**: 29-39

World Meteorological Organization (WMO) (2011) The State of Greenhouse Gases in the Atmosphere Based on Global Observations through 2010. World Meteorological

Organization Greenhouse Gases Bulletin No. 7. World Meteorological Organization.
<http://www.wmo.int/gaw>

Zappa, C. J., Raymond, P. A., Terray, E. A. and McGillis, W. R.: Variation in surface turbulence and the gas transfer velocity over a tidal cycle in a macro-tidal estuary, *Estuaries*, **26**: 1401–1415, 2003

4. Paper II

Seasonal and spatial fluxes of the greenhouse gases N₂O, CO₂ and CH₄ in the River Tay, Scotland: Hotspots of GHG emission

J. F. Harley, L. Carvalho, K. V. Heal, B. Rees, U. Skiba

Seasonal and spatial fluxes of the greenhouse gases N₂O, CO₂ and CH₄ in the River Tay, Scotland: Hotspots of GHG emission.

J. F. Harley, L. Carvalho, K. V. Heal, R.M Rees, U. Skiba

Abstract

River networks act as a link between components of the terrestrial landscape, such as soils and groundwater, with the atmosphere and oceans, and are now believed to contribute significantly to global budgets of carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O). However there is still great uncertainty in GHG fluxes from rivers, how they vary spatially and temporally. We investigated the temporal and spatial dynamics of CH₄, CO₂ and N₂O saturation and estimated emission in the River Tay catchment, Scotland, over a two year period. Hotspots of emission were evident across the river catchment, with significant temporal and spatial variability in saturation and emission in GHGs.

Estimated CH₄ emission density, across the freshwater river sites, ranged from 1720 to 15500 $\mu\text{g C m}^{-2} \text{d}^{-1}$ with a freshwater catchment wide mean of 4640 $\mu\text{g C m}^{-2} \text{d}^{-1}$, and in general decreased from upland to lowland sites along the main river stem, with notable peaks of emission in a lowland tributary and at the outflow of a lowland loch. This corresponds well with the main drivers of spatial variability which include allochthonous inputs from gas rich soil waters and in-stream production in fine grained carbon rich sediments. CH₄ production also varied seasonally, with temperature and seasonal variation in sediment quality the predominant driving factor.

Estimated CO₂ emission densities from the surface waters across the catchment ranged from 517 to 2550 $\text{mg C m}^{-2} \text{d}^{-1}$ with a catchment mean flux density of 1500 $\text{mg C m}^{-2} \text{d}^{-1}$. Emission densities on the whole increased along the main river stem from upland sites to lowland sites, with higher emission densities in lowland tributaries. Seasonally, CO₂ emission density was highest in late summer and autumn and lowest in winter at most sites, highlighting the importance in seasonal environmental controls such as temperature, light, and substrate availability.

Estimated N₂O emissions also showed considerable spatial and seasonal variation across the river catchment with emission densities ranging from 176 to 1850 $\mu\text{g N m}^{-2} \text{d}^{-1}$ with a mean emission density of 780 $\mu\text{g N m}^{-2} \text{d}^{-1}$. Mean emission densities were highest in the lowland tributaries and lowest in the upland river. On the whole, N₂O emission across the freshwater catchment was driven by increases in nutrient concentration (NO₃⁻, NH₄⁺)

which in turn was related to increases in the proportion of agricultural area in the catchment..

Introduction

The concentrations of CH₄, CO₂ and N₂O in the atmosphere have been increasing significantly since pre-industrial times (Forster 2007), which indicates a global imbalance between sources and sinks. CH₄ over the last decade has stabilised to a degree (~ 1.8 ppmv from 1999 to 2005) but unexpectedly increased again in 2007 (Rigby et al. 2008) highlighting the sources and sinks are dynamic, evolving and still to be fully understood. To better understand the potential imbalances in greenhouse gases budgets it is necessary to quantify all known sources and sinks in the form of regional and global budgets. The contributions of aquatic environments such as streams and rivers to the budgets of CH₄, CO₂ and N₂O are thought to be significant but highly uncertain (IPCC 2007). This uncertainty is fuelled by the existing evidence that suggests GHG fluxes from these aquatic environments are highly variable both on temporal and spatial scales (Garnier et al 2009). Carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) are important greenhouse gases (GHGs) that contribute to global warming (IPCC 2007).

CH₄:

Wetlands are known to be a major source of global CH₄ emissions contributing 20 – 40% of the estimated global output (Houghton et al., 2001). CH₄ production in lentic wetlands results from the decomposition of organic matter via the microbial process of methanogenesis in anaerobic sediments. However, much less is known about CH₄ production and emission in rivers, streams and lakes, probably because they are thought to be a far smaller source compared to lentic wetlands and have therefore received little study. Recent work, however, suggests that emissions could be strongly related to organic matter accumulation, with macrophytes trapping sediment and providing organic carbon for methanogenesis (Sanders et al., 2007).

CO₂:

Relatively little is known about the dynamics of CO₂ fluxes in aquatic systems, despite being of significant ecological significance within rivers and lakes, and connecting

terrestrial carbon stores (such as peatland) to the atmosphere and ocean (Dawson and Smith, 2007). Moreover, it has been recently shown to be an important and significant component of regional carbon budgets (Billett et al., 2004; Teodoru et al., 2009). On the whole, CO₂ concentrations are controlled by a host of dynamic processes that vary both spatially and seasonally encompassing both allochthonous inputs and autochthonous inputs/outputs. Allochthonous inputs include those from groundwaters and soil water inputs that carry CO₂ derived from geological weathering and soil respiration. Autochthonous inputs include heterotrophically respired CO₂ from in stream mineralization of organic matter and autotrophic respiration. Outputs include photosynthetic uptake by biota in the water column and exchange with the atmosphere (Cole and Caraco, 2001; Finlay, 2003; Jones et al., 2003; Baker et al., 2008; Battin et al., 2008; Dawson et al., 2009).

N₂O:

The most important global source of N₂O is agriculture (Mosier et al., 1998), and emissions have been increasing because of an increase in animal production and nitrogen (N) fertilizer use. The increased availability of reactive N to agricultural catchments has enhanced the biogenic production of N₂O. The direct emissions from agricultural catchments are reasonably well known with a wealth of research in this area. The indirect emissions, those associated with the leaching and runoff of dissolved inorganic nitrogen (DIN) to aquatic systems, are among the most uncertain in the global N₂O budget (De Klein et al., 2007) and the methods used to model global emissions from aquatic systems have been under constant revision over the last 15 years (Ivens et al., 2011). N₂O is produced as a by-product from the microbial transformation of ammonium (NH₄⁺) and nitrate (NO₃⁻) in nitrification and denitrification respectively. Nitrification oxidises NH₄⁺ to nitrites (NO₂⁻) and NO₃⁻ in aerobic conditions in sediments and in the water column. Denitrification is the anaerobic dissimilatory reduction of NO₃⁻ to N₂ and only occurs in anoxic environments, predominantly in sediments.

In this paper, we present the spatial and temporal variations of dissolved CH₄, CO₂ and N₂O across key sites in the River Tay catchment and compare these concentration and estimates of total fluxes of these gases to the atmosphere with other rivers in the UK and Europe. The River Tay is the UK's largest river in terms of mean annual discharge, therefore a potentially important source of GHG to understand. Emissions from the Tay

can also be considered representative of many northern European rivers draining large areas of peatland and agricultural land, but with relatively low levels of point source pollution from urban areas. Understanding the spatial and temporal variation can therefore help us understand the contribution of these types of rivers catchments to global budgets

Study Site

The River Tay catchment has an area of 4587 km² and is located in the southern part of the Scottish Highlands, extending from the mountain of Ben Lui in the west to the mouth of the estuary at Dundee, with a maximum altitude of 1210 mAOD. The following information is sourced from the National River Flow Archive (NRFA 2012). Mean annual flows of 169 m³ s⁻¹, as measured at Balathie gauging station with data extended from 1950 to present. The catchment is mainly steep, comprising mountains, heather moorland and rough grazing in the upper catchment, extending to area of arable farming, grassland and urban in the lowland catchment. The bedrock in the catchment is main impermeable with metamorphic and granite, mixed with old red sandstone in the lower catchment.

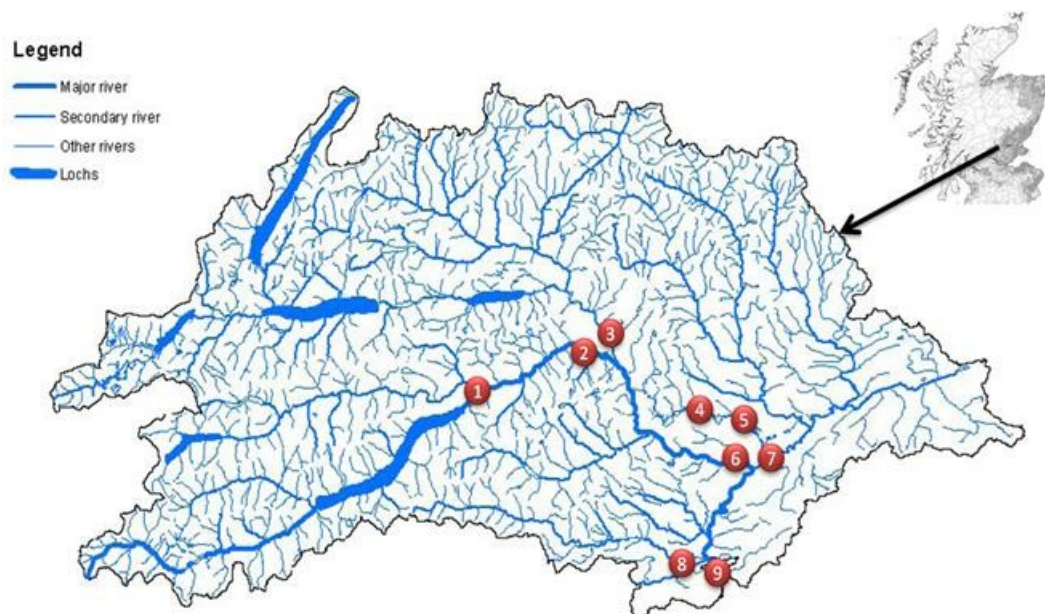


Figure 1: Map of River Tay catchment with river sampling sites.

This is predominantly overlain by superficial deposits such as boulder-clay. The mean annual rainfall for the catchment is 1425 mm, derived from the Standard-period Average Annual Rainfall (SAAR) map with a large gradient in rainfall between the upland and lowland catchment. Various factors affect runoff in the upper catchment including extensive regulation by hydro-electric power, and in the lower catchment through industrial/agricultural abstraction.

Methods

Water Sampling and Analysis

Water from the River Tay and its tributaries was sampled monthly for chemical analysis and determination of GHG concentrations at 9 sites between February 2009 and December 2010. The locations of sampling sites were based on existing flow gauging and water sampling sites of the Scottish Environment Protection Agency (SEPA) and were designed to encompass the entire catchment from the source of the main River Tay at the outflow of Loch Tay at Kenmore (Site 1) to the tidal limit at Perth (Site 9), whilst also covering the major tributaries such as the River Tummel (site 3) in the uplands, and the Rivers Isla (site 7) and Almond (Site 8) in the lowlands. Sites 4 and 5 were chosen to complement the lotic sites, covering the inflows and outflows of a small loch, Loch of Clunie, situated in the lowlands to examine the gas concentration and flux changes associated with a lake along a river continuum. Moreover, lowland lakes are expected to function as an important processing 'hot spot' for GHGs.

Information on landuse and catchment characteristics are displayed in Table 1. Landuse information for each sub-catchment was sourced from the National River Flow Archive website (www.ceh.ac.uk/data/nrfa).

Measurement of Dissolved CH₄, CO₂ and N₂O

The widely used headspace technique (Kling et al. 1991; Billett et al. 2004; Hope et al. 2004; Billett et al. 2008), in which a known volume of water is equilibrated with a known volume of ambient atmosphere, was used to determine the concentrations of dissolved CH₄, CO₂ and N₂O. Gas concentrations were determined in surface waters sampled in triplicate from each site with values reported as the mean. For each sample, three 60 ml syringes (with 3 way valve) were used to extract 40 ml of river water each

from 10 cm below the surface, after which 20 ml of ambient air was added. The syringes were then shaken vigorously underwater for 1 min, equilibrating the headspace and water at water temperature. The headspace gas was then immediately injected into pre-evacuated 12 ml gas tight vials for later analysis using gas chromatography. A further sample of ambient atmosphere was injected into a 12 ml gas tight vial, and water temperature and atmospheric pressure were recorded. N₂O and CH₄ were then analysed on a HP5890 Series II gas chromatograph (Hewlett Packard (AgilentTechnologies) UK Ltd., Stockport, UK) fitted with an electron capture detector (ECD) and flame ionisation detector (FID) with accuracy of 30 ppb for N₂O and 70 ppb for CH₄, respectively. CO₂ was measured simultaneously on another HP5890 Series II gas chromatograph. The concentrations of dissolved CO₂, CH₄ and N₂O were calculated from the concentrations measured from the headspace using the functions for the Bunsen solubility given by Wiesenburg and Guinasso (1979) for CH₄, and Weiss and Price (1980) for CO₂ and N₂O. Saturation values for the gases were calculated as the ratio of the concentration of the dissolved gas and the expected equilibrium water concentration.

Water Chemistry

Temperature, DO/DO-sat and pH were measured *in situ* at 10 cm below the water surface using a Hydrolab Quanta Probe (Hach) at each sample location. One 1 litre bottle (plastic) was filled with water using a custom designed extension pole to sample the main water stream from the river edge. The water sample was filtered on site, through a pre-combusted (500°C for 10 h) Whatman GF/F 0.7 µm, 47 mm diameter filter paper, using a Nalgene filtration unit and an electric vacuum pump, to obtain sub-samples for analysis of Total Dissolved Nitrogen (TdN), NO₃⁻, NH₄⁺, Total Dissolved Carbon (TdC) and dissolved organic carbon (DOC). The filtered sub-samples were transferred to small plastic sample tubes (Sterilin 50 ml high clarity polypropylene) and transferred to an ice box for transportation to the laboratory fridge until analysis. DOC and TdC were analysed on a Pollution and Process Monitoring (PPM) LABTOC analyser using ultraviolet oxidation and infra-red gas analysis. The difference between TdC and DOC was used to calculate DIC. Total dissolved nitrogen (TdN), NO₃⁻ and NH₄⁺ were analysed simultaneously on a Skalar San plus automated flow analyser using standard colorimetric methods. NO₃⁻ was determined using copper/cadmium reduction and sulphanilamide/NEDD reaction to form a red/purple colour whose absorbance was

measured at 540 nm. TdN was determined through oxidation/digestion by peroxodisulfate/UV digestion. NH_4^+ was determined using the hypochlorite reaction, to form chloroamine, reacting with nitroprusside and salicylate to form an idophenol dye whose absorbance was measured at 640 nm.

Estimating CH₄, CO₂ and N₂O gas fluxes

Gas flux density across the air-water interface depend on the concentration gradient and the gas transfer velocity (k) and can be calculated through the following equation:

$$F = k_i(Gas_d - Gas_{eq}) \quad (1)$$

Where F is the flux density of gas ($\text{m}^2 \text{h}^{-1}$), k_i (m h^{-1}) is the gas transfer velocity, Gas_d ($\mu\text{mol m}^{-3}$) is the dissolved gas concentration and Gas_{eq} ($\mu\text{mol m}^{-3}$) is the dissolved concentration at equilibrium with the relevant atmospheric concentration. Gas exchange between surface waters and the atmosphere varies according to saturation of the relevant gas and the gas transfer velocity. For CH_4 , CO_2 and N_2O , which are sparingly soluble gases, the gas transfer velocity is driven by turbulence at the water-air boundary (Liss and Slater, 1974) and a number of different parameterizations have been adopted to model turbulence and gas transfer rate. In this study we followed the method set out by Garnier et al. (2009) used to calculate fluxes in the River Seine, which is adapted from Wanninkhof (1992) and Borges et al. (2004):

$$k_{600} = 1.719 \times v^{0.5} \times d^{-0.5} \quad (2)$$

where k_{600} is the gas transfer velocity (cm h^{-1}), v is the water velocity (cm s^{-1}) and d is the water depth (m).

Water velocity was estimated for each site on each sampling occasion using the following equation (Eq.3):

$$Q = V \times A \quad (3)$$

Where Q is discharge ($\text{m}^3 \text{s}^{-1}$), V ($\text{m}^{-1} \text{s}^{-1}$) is water velocity and A is cross sectional area. Cross sectional area was estimated using channel width at each gauging station (m), and stage height (m). This equation calculates the mean water velocity for all depths at each of the gauging stations. Datasets of flow and stage height at 15 minute intervals for the

two year sampling period for the main gauging stations in the catchment were provided by SEPA. At the sites that had no nearby gauging stations (Site 7, 5 and 4) the mean water velocity calculated across the catchment was used as a substitute.

Gas transfer velocity is influenced by temperature and salinity with the degree of influence being different for each gas. Following the method set out by Ferrón et al. (2007) we used the k_{600} values from each of the parameterizations to calculate k_i for each gas in question at the recorded temperature in the field using the following expressions (Eq. 4):

$$k_i \div k_{600} = (Sc_i \div Sc_{600})^n \quad (4)$$

The Schmidt numbers, Sc_i , is the ratio of the kinematic viscosity of water over the diffusivity of the gas and is calculated through the formulations given by Wanninkhof (1992).

Dissolved fluxes (export) of both GHGs and other water chemistry determinants for the key river sites (where discharge data was present) were calculated using two methods. Firstly, daily fluxes, specific to each sampling day, were estimated based on the mean discharge on day of sampling multiplied by determinant concentration and hereby referred to as daily export. For longer timescales, such as annual fluxes, it was necessary to interpolate between widely spaced concentration measurements. The widely used method of Walling and Webb (1985) was utilised to estimate the annual load based on the monthly concentration measurement and means of discharge data (discharge data at 15 mins intervals between Feb 2009 and Dec 2010). These estimates are accompanied with a calculation of standard error of the flux estimates and this was calculated using the methods set out in Hope et al., (1997b).

Statistical analysis

Data was analysed using a combination of Excel and Minitab (v 16) statistical software. Pearson's correlation coefficients were calculated to examine associations between the measured gas saturations and other measured determinants after data was checked for normality and transformed if required. To determine the statistical difference between sites, a one-way ANOVA with a Tukey test was performed on the data. Where data were

non-normal, and resistant to transformation, a non-parametric Mood's median test was utilised. The significance level used was $p < 0.05$.

The complete dataset was also analysed using a combined ordination approach, through a combination of principal component analysis (PCA) and cluster analysis. PCA is frequently used to analyse large, multivariate environmental datasets (Clarke and Warwick, 2001), in the aid of simplifying data and allowing for the recognition of patterns and sample similarity. Combined with k-means cluster analysis, it was used here to define the controls on gas saturation over temporal and spatial scales, indentifying distinct environmental conditions. The data included in the PCA was not normalised, standardized or transformed before analysis, therefore correlation instead of covariance analysis was used.

Results and Discussion

Spatial and Temporal Patterns of CO₂ saturations in relation to Catchment Characteristics and Water Chemistry

CO₂-sat ranged between 117 %, on the upper-most main stem site (S1-KEN), and 720 % on the lowland tributary (S7-ISL) with the saturations never falling below atmospheric equilibrium. Mean CO₂-sat ranged between 184 % at site S1-KEN to 383 % at S7-ISL. The mean and range of saturations are similar to those reported in previous studies from a range of aquatic environments in northern temperate regions, from small streams (Billett et al., 2007) to large rivers (Dawson et al., 2009; Neal et al., 1998; Raymond et al., 1997).

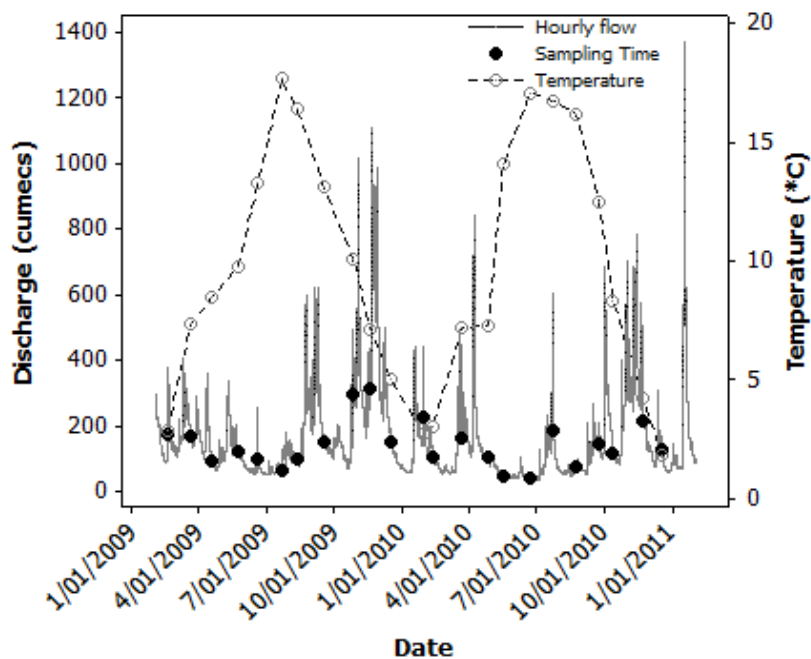


Figure 2. Discharge (15 minute intervals) between 2009-2010 at Balathie gauging station representing site 9 (PER) and water temperature (this study) and sampling points (this study).

Mean CO₂ saturations in the Tay fall into the lower range of those reported in the literature, similar to other oligotrophic waters such as the River Dee (282 -689 % saturation range of annual means; Dawson et al., 2009) and the River Tweed (10 – 420 % saturation annual range; Neal et al., 1998) and much less than some nutrient impacted rivers in eastern United Kingdom such as the River Trent (970 % sat mean; Neal et al., 1998), chalk streams in SE England such as the River Pang (catchment mean 1980 %

sat) and Lambourn (1680 % sat) (Griffiths et al., 2007).

Table 1 .) For each sampling site, a) Catchment area and land cover characteristics and b) Mean saturations of CH₄, CO₂ & N₂O, and mean water chemistry (n=207). Letters indicate statistical difference between sites for GHGs using one-way ANOVA with Tukey Family Error rate (p<0.05).

Site and Name	Catchment Area km ²	Percentages of Main Land Use										
		Forest	Grass-land	Arable Land	Mountain, Heath & Bog	% Built-up Areas	% Peat Drift					
a)	km ²											
S1 KEN	601	12.0	35.4	0.1	47.4	0.1	0.0					
S2 PIT	1149	13.9	31.0	0.7	50.9	0.1	0.2					
S3 TUM	1670	13.2	15.8	0.2	67.1	0.1	12.1					
S4 IN	94	23.8	34.1	18.1	20.2	0.7	-					
S5 OUT	94	23.8	34.1	18.1	20.2	0.7	-					
S6 KIN	3210	16.3	22.4	0.7	57.3	0.1	6.3					
S7 ISL	1377	15.0	30.1	22.4	30.7	1.1	0.0					
S8 ALM	175	22.1	32.0	3.8	41.9	0.2	0.0					
S9 PER	4587	16.7	25.9	7.3	50.9	0.4	5.0					
b)												
	CH ₄ -sat %	CO ₂ -sat %	N ₂ O-sat %	pH	DO-sat %	TdC mg l ⁻¹	DOC mg l ⁻¹	DIC mg l ⁻¹	TdN mg N l ⁻¹	NO ₃ mg N l ⁻¹	NH ₄ mg N l ⁻¹	DON mg N l ⁻¹
S1 KEN	1643 ^{bc}	184 ^D	119 ^C	6.7	97	6.60	3.33	3.27	0.34	0.12	0.02	0.11
S2 PIT	1939 ^{bc}	209 ^{CD}	122 ^C	6.8	97	7.07	3.45	3.62	0.47	0.13	0.02	0.23
S3 TUM	2185 ^{bc}	221 ^{CD}	120 ^C	6.8	96	7.12	4.85	2.27	0.66	0.14	0.03	0.37
S4 IN	722 ^d	273 ^{BC}	130 ^C	7.1	95	16.0	4.60	11.4	0.52	0.36	0.03	0.15
S5 OUT	1651 ^{bc}	346 ^A	189 ^B	7.3	85	17.4	4.25	13.1	1.09	0.75	0.03	0.30
S6 KIN	963 ^d	227 ^{CD}	136 ^C	6.9	95	7.68	4.02	3.66	0.47	0.25	0.03	0.21
S7 ISL	6649 ^a	383 ^A	232 ^A	7.2	90	15.4	3.47	12.2	2.78	2.17	0.06	0.85
S8 ALM	761 ^d	312 ^{AB}	185 ^B	7.1	91	10.1	3.11	6.99	1.35	1.17	0.04	0.15
S9 PER	915 ^d	253 ^{BCD}	144 ^C	6.9	95	8.90	3.85	5.05	0.82	0.66	0.04	0.13

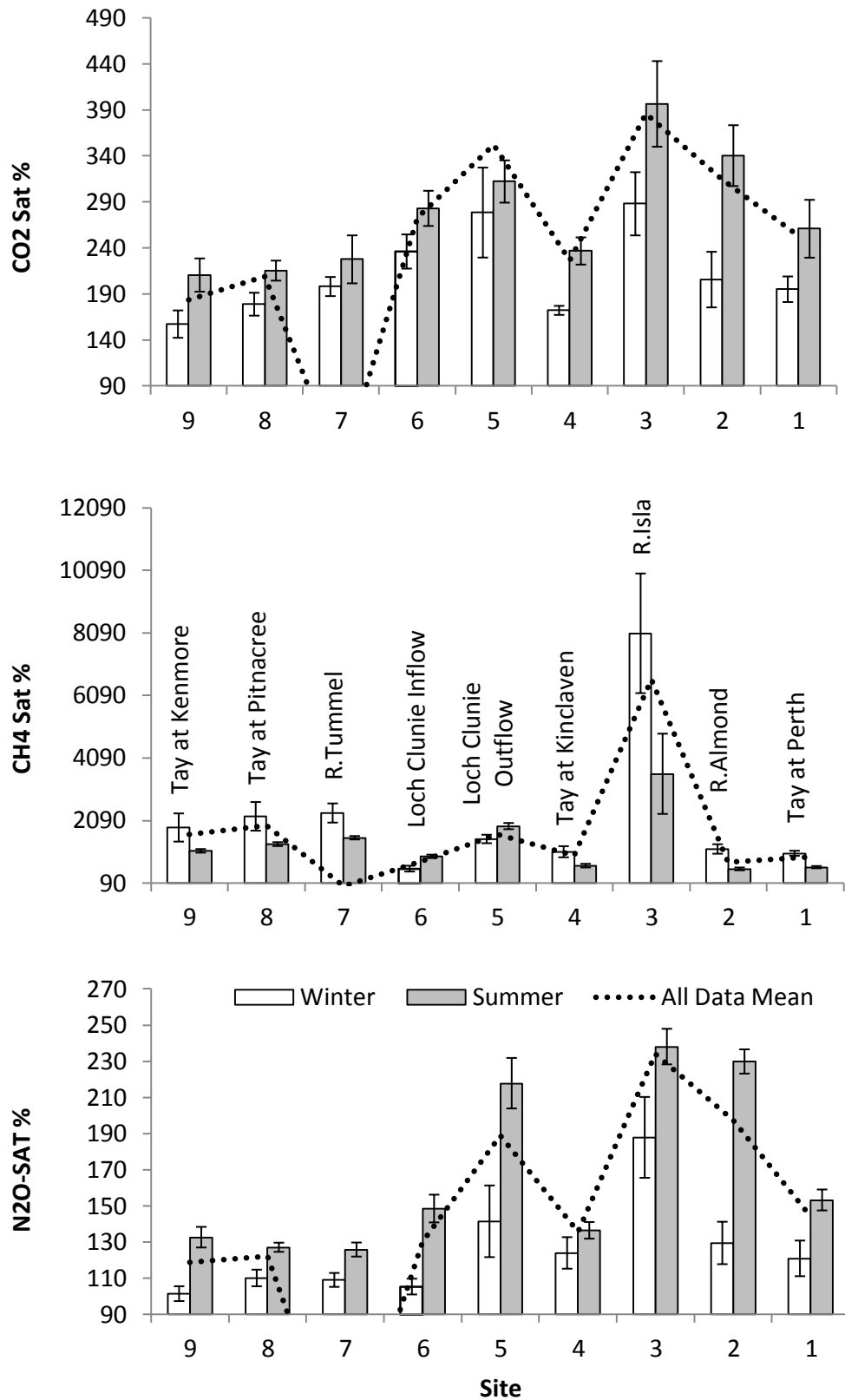


Figure (3) Mean winter, summer and all data values (n=207) for CO₂, CH₄ and N₂O. Winter (December, January, February) Summer (June, July, August). Error bars equal the standard error of the mean

The spatial and temporal distribution of CO₂-sat is dependent on the allochthonous (ie groundwater, soil pore-water) and autochthonous (in-stream production/consumption) inputs/outputs to the river system. The allochthonous CO₂ inputs are dependent on the dynamics of the terrestrial-stream connectivity, something which is known to vary substantially between upland headwaters and lowland rivers. For example, many upland streams and rivers are known to be highly connected to their surrounding terrestrial environment particularly in catchments dominated by hydrologically responsive organic soils (Billett et al., 2007; Billett and Moore, 2008). Lowland rivers in general are less dependent on terrestrial inputs with the exception of some chalk rivers/streams that are dominated by groundwater flow (Griffiths et al., 2009). Instead, CO₂-sat is controlled by the delicate balance between heterotrophy and autotrophy which becomes progressively more important in a downstream direction in lowland rivers, as conditions for the establishment of biotic communities improve. Allied to this, is the continual loss/gain of CO₂ to and from the atmosphere, as stream water concentrations equilibrate with the atmosphere. This is controlled by the gas transfer velocity which itself is dependent on the water temperature, depth, velocity and wind speed, all of which can vary across catchments (Dawson et al., 2009).

CO₂ saturations varied substantially across the 9 sites in the catchment (Figure 3). Although sites 1, 2 and 3 represent the upland catchment, the river flow is already substantial, with mean annual values of 47, 58 and 74 m³ s⁻¹, respectively (NRFA 2012). In terms of CO₂ concentrations and water chemistry the three upland sites are reasonably comparable (Table 1). There is a small increase in mean CO₂-sat from site 1 to site 2 (2 sample T-Test: $p > 0.02$). This could be either linked to increased autotrophy in Loch Tay over the annual cycle or a net input of CO₂ from the un-sampled River Lyon.

Mean CO₂-sat, combined with DOC is also higher at site 3 compared to site 1 and 2 which may reflect the higher proportion of peatland in the upper Tummel catchment (Table 1) and the river being more hydrologically connected to surrounding soils. However on the whole, the high discharge of the river results in the dilution of soil derived determinants so that CO₂-sat and DOC are substantially lower than found in other upland aquatic environments such as small streams (Billett et al., 2004) with concentrations of gas often being observed to decrease with distance from original source as water equilibrates with atmosphere.

Table 2: Pearson's correlation coefficients. Spatial relationships between GHGs and water chemistry and land use using the 2 year mean site values.

	CH₄- sat†	CO₂- sat	N₂O- sat	Flow- Q	pH	T°C	DO mg l⁻¹	Do-sat %
CH ₄ -sat†	-	0.41	0.53	-0.38	0.31	0.28	-0.24	-0.24
CO ₂ -Sat	0.34	-	0.95 ^{***}	-0.33	0.94 ^{***}	-0.07	-0.42	-0.88 ^{***}
N ₂ O-Sat		0.95 ^{***}	-	-0.39	0.93 ^{***}	0.05	-0.34	-0.83 ^{***}

	NO₃ mg N l⁻¹	NH₄ mg N l⁻¹	TdN mg N l⁻¹	DON mg N l⁻¹	DOC mg l⁻¹	TdC mg l⁻¹	DIC mg l⁻¹
CH ₄ -sat†	-0.25	0.72 ^{**}	0.71 ^{**}	0.92 ^{***}	-0.13	0.23	0.25
CO ₂ -Sat	0.89 ^{***}	0.81 ^{***}	0.86 ^{***}	0.64 [*]	-0.09	0.84 ^{***}	0.87 ^{***}
N ₂ O-Sat	0.96 ^{***}	0.89 ^{***}	0.94 ^{***}	0.70 ^{**}	-0.33	0.67 ^{**}	0.73 ^{**}

	Wood- land	Arable & horticultu re	Grassland	Mountain, heath, bog	Built-up areas
CH ₄ -sat†	-0.46	0.38	-0.06	-0.08	0.51
CO ₂ -Sat	0.55	0.84 ^{***}	0.28	-0.69 ^{**}	0.85 ^{***}
N ₂ O-Sat	0.33	0.70 ^{**}	0.26	-0.54	0.75 ^{**}

† Transformed ($\sqrt[2]{\text{Ch4-sat}}$) p value = * <0.1 ** <0.05 *** <0.01

On the whole, mean saturations of CO₂ across the River Tay catchment increased in a downstream direction along the main river stem with saturations significantly higher in the lowland tributaries (Sites 7 & 8) compared to all other river sites ($p < 0.00$). This is mirrored by a marked change in land use through the river continuum with areas of agriculture and built-up land increasing substantially. Highest saturations of CO₂ were found at site 7 (maximum of 720 %) which also contained the largest percentage of arable land and built-up areas (with 89.6 % of the total arable land and 80 % of the total built-up areas) and also the highest concentrations of both TdC and TdN of the river sites.

The spatial distribution of CO₂ appears to be defined by the large scale environmental changes occurring in the catchment with CO₂ concentrations increasing as the land use

switches from mountainous moorland/heath/bog to agricultural land. This is supported by correlation analysis with mean CO₂-sat found to correlate with a number of measured water chemistry determinants, spatially across the catchment (Table 2). Significantly and positively correlated with pH, TdN, NO₃⁻, NH₄⁺, TdC and DIC; as well as significantly and negatively with DO-sat. Also, significantly correlated with percentage of arable land and built-up areas in each catchment, whilst negatively correlated with the percentage mountain, heath and bog. This indicates that as the agricultural component of land use increases, there is a concurrent rise in CO₂ and nutrient concentrations (TdN and TdC).

In many rivers, the dominance of net heterotrophy decreases with increasing river order as autotrophic processes take over (Webster 2007). This change in trophic dominance is fuelled by increasing light, temperature and nutrients combined with a general habitat improvement (gradient decrease, channel width increase, decreased riparian shading) leading to an increase in algal and biofilm establishment (Webster 2007; Battin et al., 2008). The change in trophic dominance along a river continuum should in theory lead to an overall decrease in CO₂ concentrations/saturations, especially in the summer months (maximum time of GPP/photosynthesis). This has been found to be the case in some low nutrient rivers such as the River Dee where saturations have been driven below atmospheric equilibrium in the summer/autumn periods and annual means decrease along the main stem continuum (Dawson et al., 2009; Neal et al., 1998). However in the Tay, the mean spatial CO₂-sat increases along the river continuum, despite its relatively low nutrient status. There are a number of possible reasons for this spatial pattern in the river continuum. Firstly, autotrophic establishment in the Tay could be limited to a few key areas, such as backwater sites, in the main channel as discharge and flow velocity remain high throughout the entire river main-stem (Figure 5). The high river velocity is likely to reduce the establishment of biota and rooted macrophytes compared to other lowland rivers with substantial biotic development (Saunders, 2007). In conjunction, inputs from the main lowland tributaries, that have a net heterotrophic signal, may increase the CO₂-sat at the lowland Tay sites thus offsetting any autotrophic influence.

The River Isla (Site 7) remains net heterotrophic throughout the year (CO₂ sat > 100 %) despite it having all the characteristics of a river suited to autotrophic dominance. Reduced flow velocity, combined with increased nutrient load and substantial sediment

availability would indicate plant, algal and biofilm establishment should increase. The effects of autotrophic establishment at this site may be offset by high CO₂ production in organic rich sediments in the river and high mineralization in the surrounding soils. This pattern of net heterotrophy is common in other studied rivers. Jones et al. (2003) found that CO₂ remained typically supersaturated across 417 streams and rivers in the United States, attributed to organic matter decomposition exceeding photosynthetic uptake.

The River Almond (Site 8) also remains heterotrophic throughout the year. This could be due to the tree lined riparian zone with over-hanging forest canopy reducing incident light reaching the water surface which lowers water temperatures, thus reducing the potential for autotrophic activity (Malcolm et al., 2008) and can significantly change the dynamics of CO₂ (Finlay, 2003).

Another reason for the CO₂-sat spatial patterns found in this study could lie in the limited spatial coverage in the upland catchment. The River Tay and Tummel (Sites 1, 2 and 3) at the upland sampling points are already at these points large established rivers, downstream of large deep lochs. It is more than likely additional sampling points, higher up in the catchment where peaty soils dominate, would capture larger fluxes and saturations of CO₂ as found in other studies (Dawson et al., 2009). CO₂ sourced from soil waters, such as in peatland catchments, is known to de-gas quickly in the stream continuum (Billett et al., 2004), and could easily account for the lower saturations found at site 1, 2 and 3.

Seasonality and Temporal Distribution:

Figure 4a shows the temporal variability of CO₂-sat and CO₂- daily export at key sites, both along the main river stem and on the major tributaries, representing the major aquatic landscapes of the catchment. Sites 2 and 3 represent the two main sections of river in the upland catchment, the Western Tay catchment and River Tummel catchment respectively. Sites 7 and 9 represent the major lowland sites, site 7 being the major lowland tributary and site 9 is the catchment end-member. Overall a similar temporal distributional pattern of CO₂-sat occurs across the catchment sites with the major difference being in the magnitude of fluxes rather than temporal differences. Saturations increased from winter to spring both in the main stem sites and in the major tributaries, with several peaks (particularly April and June) and troughs (May) in concentration.

CO₂-sat decreased during the height of the summer (July/August) before maximum saturations occurred in autumn and early winter (September/October).

Seasonal differences in temperature are a major control on biotic activity, which includes both community respiration and photosynthetic processes (Jarvie et al., 2001). Autotrophic processes are further controlled by the availability of light reaching the water surface, and the differences between winter and summer are substantial in Scotland (Dawson et al., 2009). Saturations of CO₂ are lowest in late winter when both community respiration and GPP are suppressed by the low temperatures (c.2 – 3 °C Dec/Jan) and low light availability. With recovering temperatures in early spring CO₂-sat increases as community respiration increases. In mid spring to early summer it appears that autotrophic processes begin to take hold as temperatures and light availability increase. This creates a pattern of peaks and troughs in CO₂-sat as the balance between heterotrophy/autotrophy changes through April to June. This is mirrored by changes in DO-sat and pH in 2009 with peaks of DO and pH coinciding with troughs of CO₂-sat (Figure 6) By midsummer, autotrophic processes become more dominant as GPP peaks with light availability and river flows decrease allowing for increased biotic establishment and CO₂-sat drops close to the annual low. This was especially prevalent in the summer of 2009 with a clear saturation minimum in July and August when maximum study temperatures occurred (river discharge and water temperature shown in figure (2)). In the following year however, although saturations were again lower in July, the saturation in August was close to the annual maximum. This could be for a number of reasons. The overall balance between heterotrophy/autotrophy can be influenced by discharge events causing disturbance to autotrophic community group habitats such as anchorage sites in the stream bed/sediments (Battin et al., 2008). The effects of discharge on autotrophy are particularly strong when the process is at its peak in the summer, for example discharge events in the summer months were linked to increases in CO₂-sat in the River Dee (Dawson et al., 2009). It is possible that the large saturation of CO₂ in August 2010 was linked with the discharge event at the end of July 2010. This discharge event might not only have disturbed autotrophic communities but in doing so it would have delivered a fresh wave of organic material to benthic sediments thus increasing benthic respiration and creating a peak in CO₂-sat across the whole catchment.

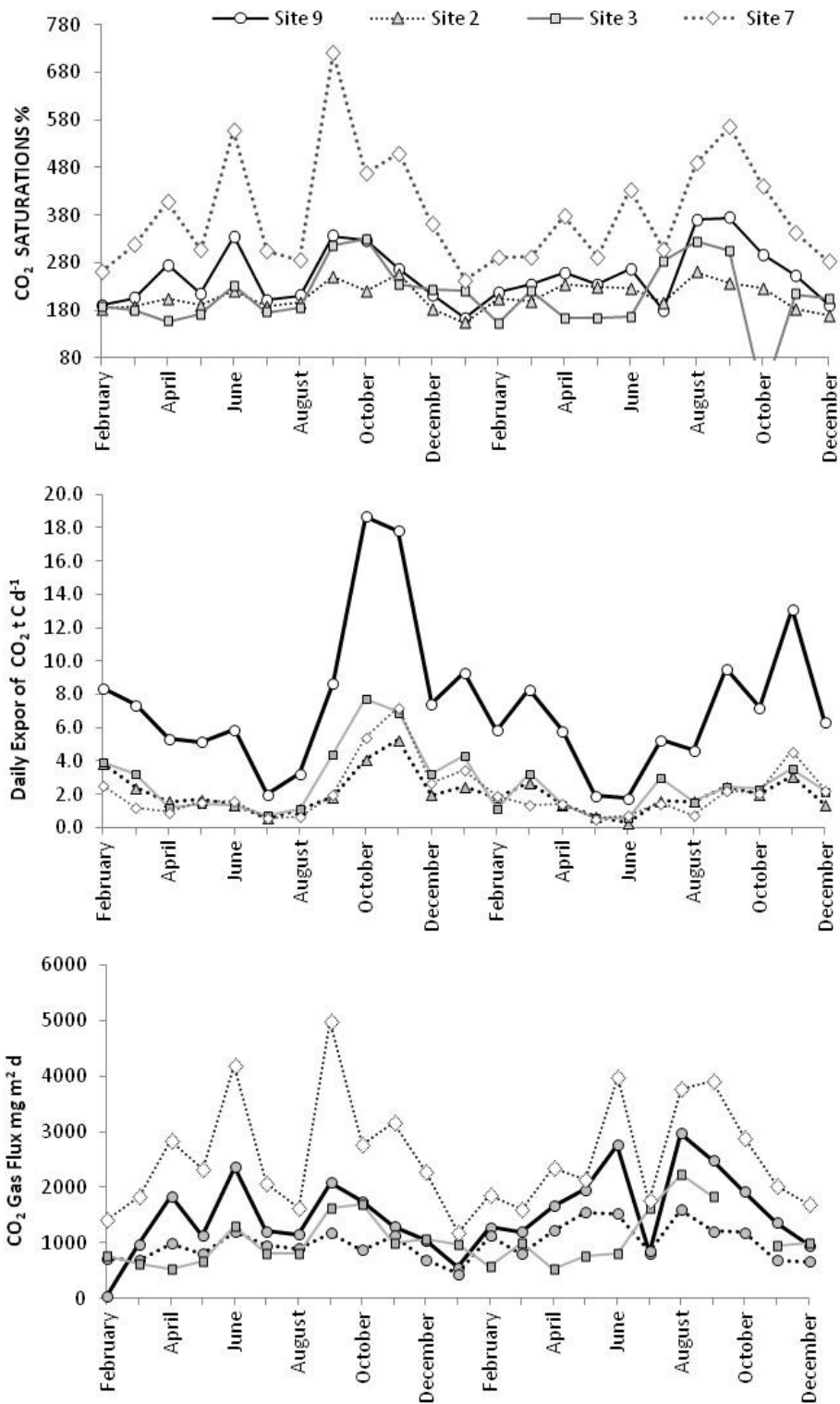


Figure 4a: Temporal dynamics of CO₂ saturations, CO₂ daily dissolved export and CO₂ emission density at key sites.

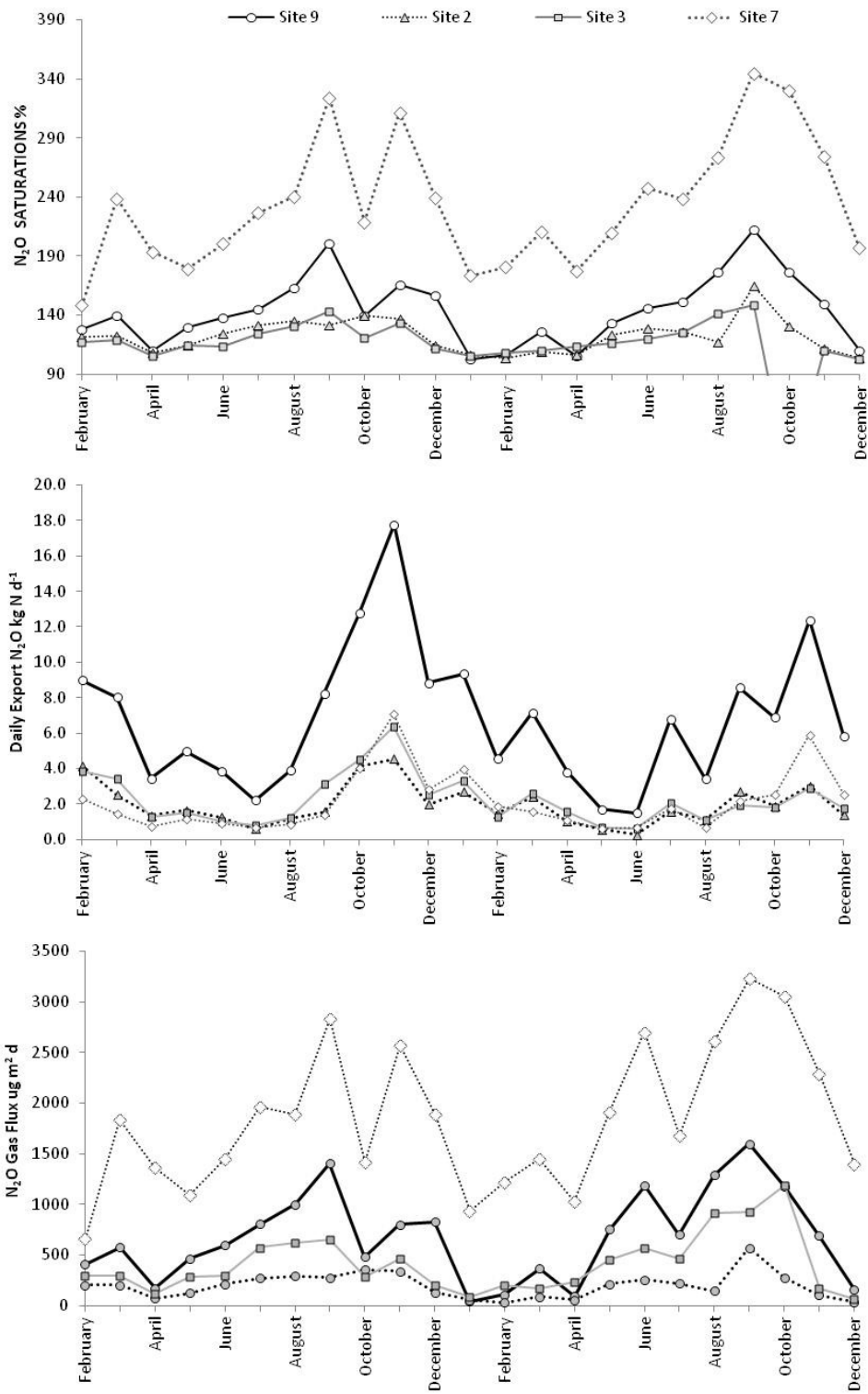


Figure 4b: Temporal dynamics of N₂O saturations, N₂O daily dissolved export and N₂O emission density at key sites.

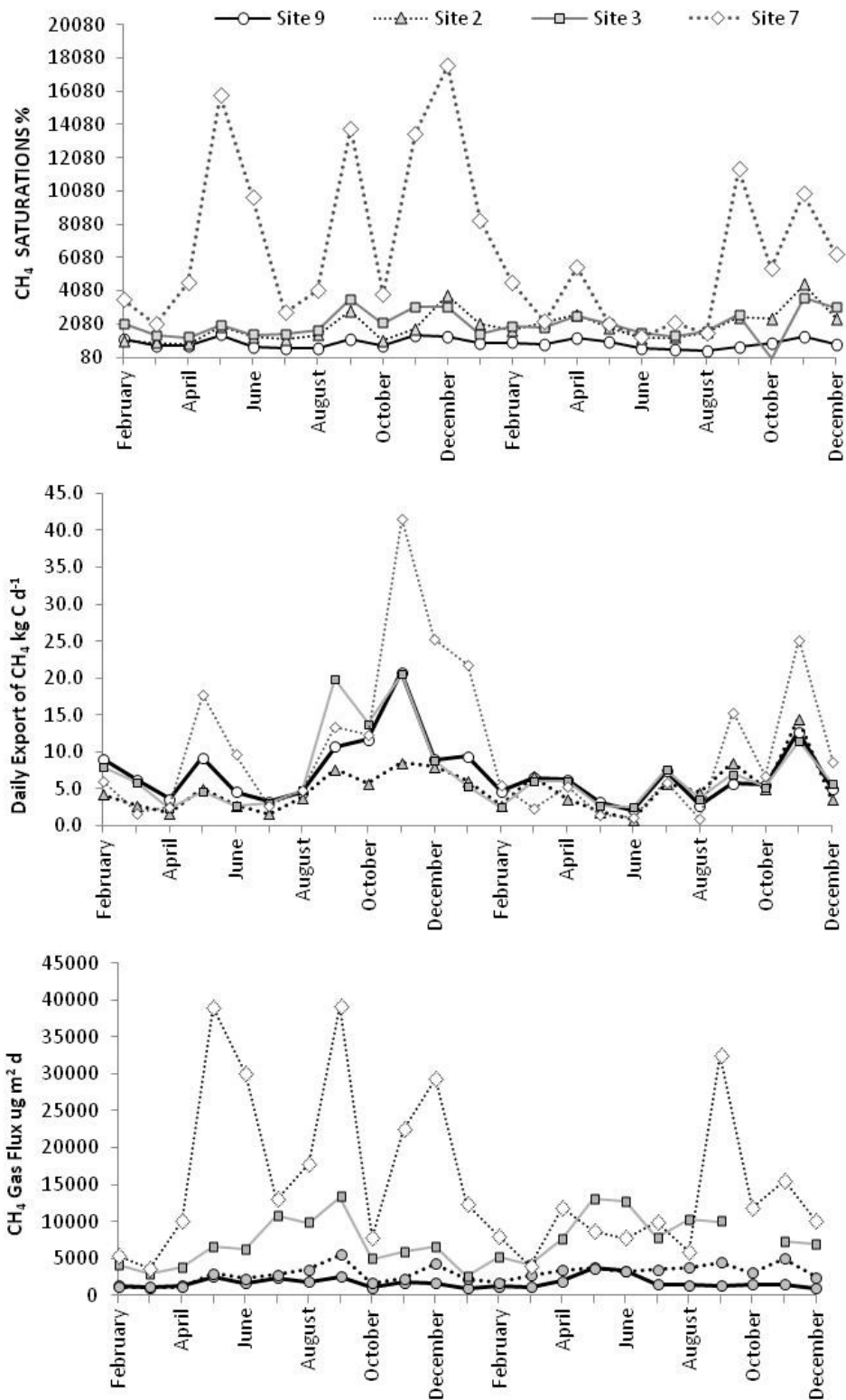


Figure 4c: Temporal dynamics of CH₄ saturations, CH₄ daily dissolved export and CH₄ emission density at key sites

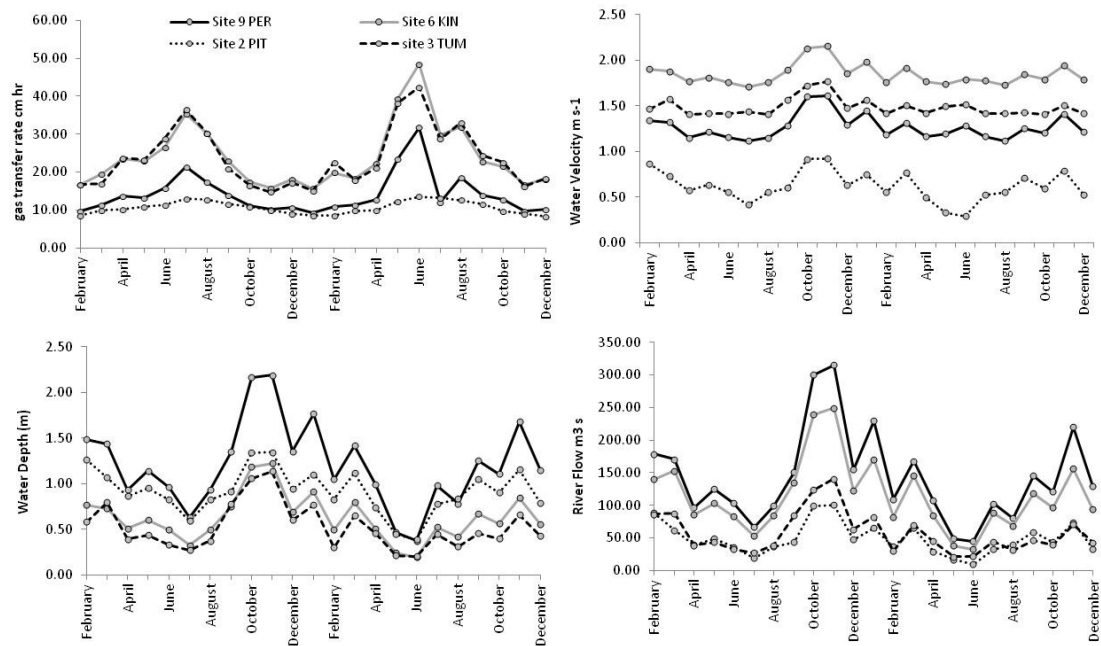


Figure (5) Gas Transfer Rate, Water Velocity, Water Depth and River Flow at 4 key sites, the two main-stem lowland sites (Site 9 PER, Site 6 KIN) and two upland sites (S3 TUM and Site 2 Pit).

Hydrological flow paths could play another important role in regulated CO₂-sat across the river catchment. Streams and rivers receive groundwater discharge from a number of differing flow paths; both from shallow sources such as riparian soils, and deeper groundwater. On the whole, whilst upland streams are known to be heavily influenced by shallow flowpaths, particularly in upland peaty catchments (Dawson et al., 2009), in general lowland rivers receive water primarily from groundwaters, particularly during summer baseflow conditions, with flowpaths becoming increasingly shallow in autumn/winter linked with increasing rainfall and river discharge. Groundwater either deep or shallow transports dissolved gases into the river channel as it is generally super-saturated with CO₂ (Kling et al., 1991; Jones and Mulholland 1998; Cole and Caraco 2001). Changes in flow path could control temporal CO₂ changes within the catchment as observed in other river and streams (Jones and Mulholland 1998). However in the autumn and winter, the increases in monthly rainfall likely trigger changes in flow-path towards shallow and surface flow and saturations of CO₂ increase in late summer into autumn coinciding with the changes in river flows. The increase in CO₂-sat is likely to be controlled by a number of factors. Changes in flow path could be flushing CO₂ rich soil waters into the river, or the same soil waters rich in nutrients (C, N and P) could be stimulating increased in-stream production with increases

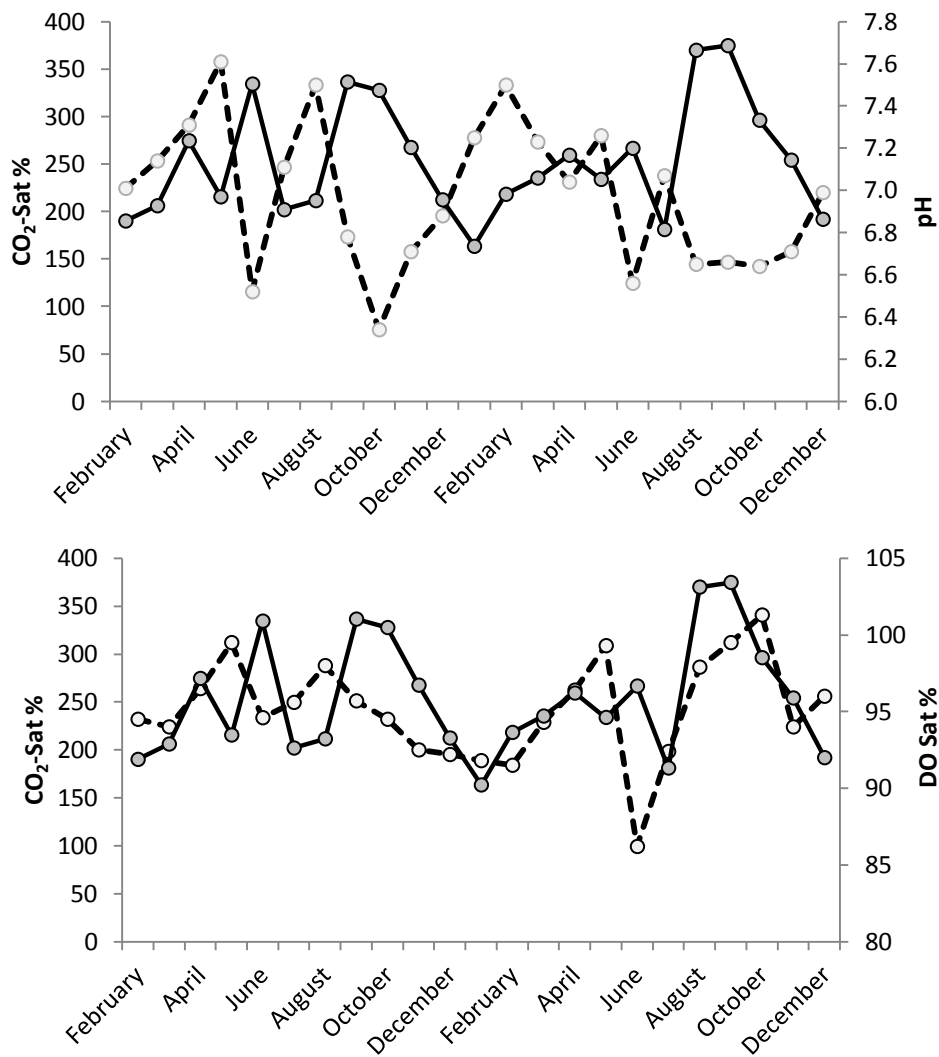


Figure (6) CO₂-sat (black line) plotted with pH and DO-sat at the S9 River Tay at Perth.

in the availability of organic material as gross primary productivity ebbs in autumn. In late autumn and winter saturations and export of CO₂ drop due to seasonal lows in temperature possible reducing catchment wide CO₂ production both in the river and within catchment soils.

Spatial and Temporal Patterns of N₂O saturations in relation to Catchment Characteristics and Water Chemistry

N₂O-sat ranged from 96 %, at site 1, to a high of 345 %, at site 7, with saturations being equal to or above atmospheric equilibrium throughout the year. The mean and range of

saturations are similar to those reported in previous studies from a range of aquatic environments, including those from other UK rivers (mean values from 117 to 389 %) (Dong et al., 2004). Mean N₂O saturation at Site 9 Perth (144 %) falls into the lower range of those reported in the literature for other UK rivers, similar to other oligotrophic rivers in the UK such as the Conwy (mean 137 %), Mawddach (mean 117 %) and Dovey (mean 127 %) (Dong et al., 2004). Mean saturation in the River Isla Site 7 (232 % sat) is similar to other rivers that have a high nutrient loads in the south east of England, such as the River Trent (mean 228 %), and River Ouse (mean 218%) but lower than some highly nutrified rivers such as the river Orwell (mean 389 %) and river Stour (mean 297 %) (Dong et al., 2004).

In general, mean spatial saturations of N₂O followed a similar pattern to the saturations of CO₂, increasing in a downstream direction along the main river stem, with saturations significantly higher in the lowland tributaries (Sites 7 & 8) and outflow of Loch of Clunie compared to all other site (Figure 3). This high spatial variability in N₂O across catchments/regions has been reported in a number of other studies, from small streams (Beaulieu et al., 2008; Baulch et al., 2011), and rivers (Cole and Caraco, 2001).

Like CO₂, the spatial distribution of N₂O appears to be defined by the large scale environmental changes occurring in the catchment with N₂O concentrations increasing as the dominant land uses switch and nutrient load increases. This is supported by correlation analysis (Table 2). Spatially, N₂O-sat was found to correlate with a number water chemistry variables. Significantly and positively correlated with CO₂-sat, pH, TdN, NO₃⁻, NH₄⁺, TdC and DIC; as well as significantly and negatively with DO-sat. Also significantly positively correlated with percentage of arable land and built-up areas whilst negatively correlated with percentage mountain, heath and bog. This indicates that as the agricultural component of land use increases, there is a concurrent rise in N₂O and nutrient concentrations (TdN and TdC), with concentrations of TdN and TdC also strongly correlated with agriculture.

The spatial and temporal distribution of N₂O-sat is dependent on the allochthonous (ie groundwater, soil pore-water) and autochthonous (in-stream production/consumption) inputs/outputs to the river system combined with the continual loss/gain from atmospheric evasion. N₂O is produced both in terrestrial environments and in aquatic environments by the processes of nitrification and denitrification. N₂O is produced in

denitrification, an anaerobic process, in an intermediary step of the denitrification pathway ($\text{NO}_3^- > \text{NO}_2^- > \text{NO} > \text{N}_2\text{O} > \text{N}_2$) where N_2O can be both produced and consumed in the process. N_2O is also produced in nitrification, an aerobic process, which oxidises NH_4^+ to NO_3^- , with N_2O being produced as a consequence of the oxidation of the intermediate hydroxylamine. It can also be produced in the nitrifier-denitrification pathway, where organisms play a dual role both oxidising NH_4^+ and reducing NO_2^- to N_2O (Stein and Yung, 2003; Wrage et al., 2001). Other than aeration status, the two processes are also controlled by the amount and quality of substrate (e.g. surface area, grain size, water content), temperature, NH_4^+ for nitrification, and NO_3^- and carbon for denitrification (Baker & Vervier 2004; Inwood et al., 2005; Strauss et al., 2002, 2004). Moreover, particularly for denitrification where many of the denitrifiers are also heterotrophs, the amount and quality of carbon is important not only for N_2O production (Mounier, 2008) but also CO_2 production during denitrification. Therefore it is not surprising that there is such a strong correlation between N_2O -sat and dissolved nutrients such as TdN (NO_3^- and NH_4^+), TdC, with the spatial relationship driven by the proportion of agriculture in the catchment and/or human population. N_2O -sat and CO_2 -sat are also highly spatially correlated and although CO_2 is produced in conjunction with N_2O during denitrification, it is unclear if this is a direct relationship or correlated through the spatial environmental conditions found across the sampling sites. For example, this relationship is unlikely to hold true if the spatial coverage of sampling sites was increased to include additional upland sites dominated by peatland soils, which in line with other work are likely to have high saturations of CO_2 (Dawson et al., 2009) but low saturations of N_2O due to low nutrient status.

Previous studies have reported similar relationships between N_2O (emission) and NO_3^- with Stow et al., (2005) reporting a relationship of $r^2 = 0.52$ using data from their own study and that of Cole and Caraco (2001).

Temporal Distribution of N_2O across the catchment

Figure 4b shows the temporal variability of N_2O -sat, daily dissolved N_2O export and N_2O emission flux density at key sites, both along the main river stem and on the major tributaries, representing the major aquatic landscapes of the catchment. Overall a similar temporal distributional pattern of N_2O -sat occurs across the catchment sites, with the range and magnitude of saturations increasing from the upland sites to the lowland sites.

In general, there was a small spike in N₂O-sat in early spring before falling again towards the middle to end of spring. Saturations then steadily climbed through the summer as temperatures increased, peaking in September in both years. In 2009 a large decrease in October was observed before saturations increased again in November. From November onwards, in both years, saturations fell to close to a seasonal low in winter.

Although strong correlations between N₂O-sat and other water chemistry variables were found using the 2 year mean values for each sampling site, the relationships breaks down using the monthly temporal data in individual sites. As mentioned previously, there is a wealth of research on spatial and temporal controls of nitrification and denitrification in a range of aquatic environments, there is far less knowledge on the importance of these processes in the production and emission of N₂O. Allied to this is the vast complexity of the various controls of denitrification/nitrification, that alter on spatial and temporal scales. For example, organic matter dynamics in water, sediment and soils are extremely complex, leading to hotspots of N₂O production. Add to this the seasonal changes in temperature and nutrient load and it leaves a complicated scenario that means it is still not possible to estimate N₂O production from river water chemistry (Garnier et al., 2009). Moreover, it is unlikely that there is only one driving factor, but a host of different factors that combine to drive temporal changes in N₂O.

The temporal patterns in N₂O observed across the Tay catchment are likely to result from a combination of hydrology related C and N delivery and temperature. Like CO₂, seasonal switches in flow path could partly control the N₂O-sat distributions observed across the catchment. Riparian soils are likely to provide ideal conditions for both denitrification and N₂O production with high C availability and anoxic conditions (Groffman et al., 2002) and shallow groundwater sourced from these areas is likely to contain high concentrations of N₂O. The change to a shallow and surface flow paths in the autumn/winter also delivers higher concentrations of NO₃⁻ to the river, with concentrations peaking in late winter at the time of lowest temperatures and lowest productivity. However the increased NO₃⁻ concentrations through autumn and early winter could stimulate denitrification within benthic river sediments and thus increase N₂O-sat within the river, which would be temporal and spatially dependant on the amount and quality of organic carbon within the river and ambient temperature.

Spatial and Temporal Patterns of CH₄ saturations in relation to Catchment Characteristic and Water Chemistry

Spatial CH₄

CH₄-sat ranged from 722 % at site 5, to a high of 6650 %, at site 7, with saturations being equal to or above atmospheric equilibrium throughout the year. The mean and range of saturations are similar to those reported in previous studies from a range of aquatic environments in northern temperate regions, from small streams to large rivers and lakes (de Angelis and Scranton 1993; Pulliam 1993; Sanders et al., 2007).

In general, CH₄-sat decreased down the main stem sites. Between the two main upper sites, saturations were highest in the Tummel catchment (2185% at site 3 compared to 1939 % at site 2) that also had the catchment highest DOC concentrations and the largest amount of peat coverage (12%). By site 4, the gas saturation has been reduced by half (963 %) but any gas losses between site 4 and 1 are offset by the inputs from the highly supersaturated River Isla, and subsequently there is only a small reduction in overall gas concentrations (915%).

Like CO₂, the spatial distribution of CH₄ appears to be defined by the large scale environmental changes occurring in the catchment with concentrations increasing as the dominant land uses change. Mean site CH₄-sat was significantly correlated with a number water chemistry variables. Significantly and positively correlated with TdN, NH₄, and DON. Also weakly (p=0.1 to p=0.3) positively correlated with percentage of arable land and built-up areas whilst negatively correlated with percentage woodland. It is unclear from this what the dominant controls are, with complex spatial relationships between catchment nutrients, land use and CH₄.

Methane concentrations in river water, like CO₂ and N₂O, are derived from the combination of external inputs, internal production/consumption and the equilibrium with atmospheric concentrations. The production of CH₄ in streams is generated by obligate anaerobic bacteria and therefore limited to pockets of anoxic sediment. Streams and rivers are generally well oxygenated environments, but a steep oxycline has been observed in river sediments over a few cm depth (including in sediments in the River Tay; Unpublished data) meaning CH₄ production is possible in deeper sediments with high organic matter content. Until recently, in aquatic environments, CH₄ production was

thought to be limited to anoxic environments. Grossart et al., 2011 found that microbial methane production occurred in the well-oxygenated water column of Lake Stechlin, Germany. More commonly however, aerobic environments are sites of CH₄ oxidation, which has been found to be a large sink in some rivers (Sanders et al., 2007; de Angelis and Scranton, 1993). For example, in the Hudson River, the turnover of CH₄ due to methanotrophic bacteria ranged from 0.005 to 0.33 d⁻¹ in spring to 0.11-0.70 d⁻¹ in summer. Pulliam (1993) found high methane oxidation in the Ogeechee River that correlated strongly with temperature increasing 0.92 d⁻¹ °C⁻¹. Moreover, the coupling between methanogenesis in sediments at depth and oxidation at or near the sediment water interface has been observed in a number of studies from a range of aquatic environments (Sanders et al., 2007; Trimmer et al., 2010).

Outside the river environment, riparian zones are known sources of CH₄ production and flux, with high organic matter availability and anoxic conditions, and combined with other terrestrial soil zones have been shown to be important and dominant sources of stream water CH₄ in small streams in upland catchments (Jones and Mulholland, 1998). Moreover, a downstream increase of CH₄ has been observed previously with increasing river size linked to increasing zone of anoxia. De Angelis and Lillet (1987) suggest that in rivers, lateral seeps, with the prevalence of seeps increasing down river, are a major source of CH₄. Allied to this is the general change in river morphology down-stream, with areas of sediment increasing, particle size decreasing enhancing anaerobiosis and organic matter decomposition. These classic morphological changes are limited in the main stem River Tay, with water velocity remaining high throughout (Figure 4). This could explain the reduction in CH₄-sat down river, as gas is lost to the atmosphere and oxidation could possible occur in the gravels in the oxic river bed. The River Isla however, fits with the classic view, with the lower river having a reduced flow velocity and increased areas of fine sediment. Production in bed sediments at the site is likely to be enhanced by a riparian zone dominated with shrubs and small trees that delivers and traps organic material within the fine sediment in the river at this site.

Temporal CH₄

Figure 4c shows the temporal variability of CH₄-sat, daily dissolved CH₄ export and CH₄ emission flux density at key sites, both along the main river stem and on the major tributaries, representing the major aquatic landscapes of the catchment.

Overall a similar temporal distributional pattern of CH₄-sat occurs across the catchment sites, with the range and magnitude of saturations generally decreasing from the main stem upland sites to the lowland sites, with the exception of the River Isla (Site 7). In general, there was a large spike in CH₄-sat in spring in both sampling years before falling again towards and into summer. Saturations then substantially increased in late summer into autumn before declining in winter. In 2009 a large decrease in October was observed before saturations increased again in November.

The temporal patterns in CH₄-sat observed across the Tay catchment are likely to result from a combination of seasonal switches in flow paths and temperature and organic matter controlled production. Riparian soils are likely to provide ideal conditions for methanogenesis and CH₄ production with high C availability and anoxic conditions and shallow groundwater sourced from these areas is likely to contain high concentrations of CH₄. Temporal changes in seasonal CH₄-sat is likely controlled by seasonal changes in rainfall and river discharge, especially in the upper catchment where soil waters sourced from organic soils are likely to be important. In the lower catchment, especially in Loch of Clunie (S6-S5) and River Isla (s7), *in-situ* production is likely to be highly significant, with temperature and availability of organic carbon likely strong controlling factors.

Controls on GHG saturations

A series of further analysis was conducted to better understand the controlling factors in GHG saturations throughout the river. Through a combination of best subset, stepwise regression and combined ordination analysis, a number of controlling factors were highlighted. Using best subset and stepwise regression (Table 3) on the full dataset of river sites highlighted that temperature, NO₃ concentration and discharge best predicted nitrous oxide saturations in the river catchment ($r^2=0.61$). CO₂ saturations were best predicted using temperature, NO₃, DIC and pH variables ($r^2=0.50$) and CH₄-sat best predicted with temperature, NO₃, DON, DO and pH ($r^2=0.56$).

Table (3) Results from the best subset and stepwise regression analysis using all data from the main river sites (Site 1,2,3,6,7,8,9) with data from the inflow and outflow of Loch of Clunie (4 and 5) excluded. (N=161)

	T	P		T	P		T	P
a) N ₂ O Sat ($r^2= 0.61$)			b) CO ₂ Sat ($r^2= 0.50$)			c) CH ₄ Sat ($r^2=0.56$)		
T °C	9.83	0.000	T °C	5.72	0.000	T °C	-4.28	0.000
NO ₃ -N	13.7	0.000	NO ₃ -N	7.58	0.000	NO ₃ -N	-2.95	0.000
Flow	3.03	0.003	DIC	8.75	0.000	DON-N	10.96	0.004
			pH	-4.17	0.000	DO	-2.83	0.000
						pH	-2.83	0.005

To further the analysis of the dataset, principal component analysis was combined with k-means cluster analysis. The PCA generated a PC1xPC2 plane that explained 63% of the total variation in the data. A further 9% was explained by PC3 and 7% by PC4. Examination of the variable coefficients (eigenvectors) suggested that the variance of all variables on the PC1xPC2 plane were well represented (Table 4). The patterns of coefficients in PC1 suggest that all three gases are correlated to NO₃, NH₄, DON, TdN, DIN, TdC. In PC2, the coefficients of the GHGs are reduced, with temperature and DO and DOC explaining the majority of variation.

Table (4) Coefficients of variables (eigenvectors) calculated from the PCA analysis using the main river sites (Sites 1,2,3,6,7,8,9). PC= principal component.

Variable	PC1	PC2	PC3	PC4
N ₂ O-sat	0.291	-0.160	-0.021	0.403
CH ₄ -sat	0.248	0.156	0.326	-0.015
CO ₂ -sat	0.290	-0.167	0.140	0.463
Temperature	0.052	0.616	0.093	0.091
NO ₃ -N	0.359	0.164	-0.025	0.012
NH ₄ -N	0.311	0.032	-0.049	0.125
DON-N	0.306	0.119	0.233	-0.343
TdN-N	0.363	0.166	-0.120	-0.120
DOC	-0.027	-0.240	0.623	-0.454
DIC	0.362	-0.048	-0.114	0.026
TdC	0.356	-0.096	0.047	-0.092
pH	0.184	0.127	-0.426	-0.422
DO	-0.058	0.612	-0.070	0.090
Flow	-0.139	0.105	0.464	0.256

Cluster analysis was also performed and plotted on the PC1xPC2 plane, finding 4 main clusters that had individual environmental conditions (Figure 7). Cluster A was represented by the generally nutrient poor river sites, separated from cluster B by temperature, with temperature above and below 10 °C in clusters A and B respectively. Overall sites represented in cluster A/B were generally the main-stem river sites both from the upland (Sites 1,2,3) and lowland (Sites 6 and 9). Cluster A generally exhibited the lowest nutrient concentration (TdN < 1.0 mg N L⁻¹; NH₄ <0.05) linking with the high temperature indicating that this cluster represents a typical summertime biogeochemical state, where nutrient load is reduced. Cluster B nutrient load displays an overall larger range (TdN 0.1 – 2 mg N l⁻¹) than cluster A, with temperatures below 10 °C representing late autumn, winter and early spring. This corresponds to greater flows, and greater leaching of nitrogen in the catchment which elevates river water nutrient concentrations.

These clusters are separated from cluster C and D mainly by a combination of nutrient load and GHG saturation. Cluster C is mainly represented by the lowland tributary S8

ALM and cluster D by S7 ISL. Nutrient load is highest in cluster D (TdN 1 – 3 mg N l⁻¹) which also exhibits highest saturations of N₂O and CH₄. CO₂-sat is higher in both cluster C and D compared to cluster A and B. The elevated GHG and TdN likely reflects the greater proportion of agriculture within the smaller tributary catchments in Cluster C and D.

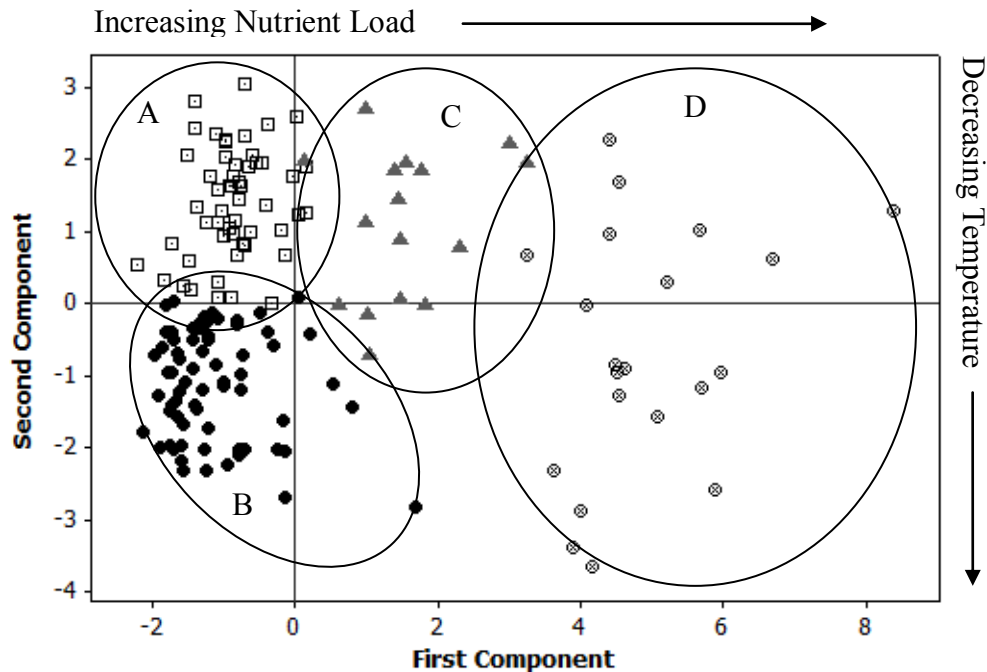


Figure (7) PC1xPC2 ordination plane and the results of k-means cluster analysis plotted on the PC1xPC2 plane.

Temporal and spatial controls on GHG fluxes and hot spots of GHG emission in the River Tay Catchment:

Fluxes of GHGs in aquatic environments are comprised of two main components: the dissolved flux (export of dissolved gases both above and below atmospheric equilibrium) and the emission flux (to the atmosphere). Figure 4 (a,b,c) highlights the spatial and temporal importance of the dynamic between the dissolved daily export flux (total dissolved export including concentrations above and below atmospheric equilibrium) and the estimated atmospheric flux (gas emission density) of all three GHGs at key river sites.

The balance between export and emission is controlled by both the temporal changes in

gas transfer rate, as well as the temporal changes in gas saturation and river discharge. Gas emission rates are directly controlled by the amount of dissolved gas above equilibration (the saturation) and the rate of gas transfer across the aqueous boundary.

The gas transfer rate is controlled by temperature and the processes that drive turbulence at the water surface which in rivers include, water depth and velocity (Demars and Manson 2012). Since gas transfer rates was not directly measured in this study, it was estimated based on the frequently referenced conceptual relationship of O'Connor and Dobbins (1958) for oxygen reaeration, which is adapted by Borges et al., (2004) for CO₂, and using the Schmidt numbers of Wanninkhof (1992) this can also be used to express the gas transfer velocity of N₂O and CH₄. The emission densities of CO₂, CH₄ and N₂O in this study are therefore estimates.

Both depth and velocity are directly related to river flow, with higher flows leading to increased depth and velocity but a lower gas transfer rate. Gas transfer rates are generally at the lowest during autumn and winter when flows are greatest and are at the maximum during the summer when flows and depth are at the lowest increasing the gas transfer velocity through increased turbulence. The dissolved flux export is therefore controlled both by the dissolved concentration of gas and discharge but also gas emission rate. Increases in discharge can therefore mask large fluxes of GHG, which is exported in the dissolved flux rather than being emitted to the atmosphere. Therefore to fully understand fluxes of GHG within the Tay it is important both to understand the emission fluxes but also how much is being exported downstream.

CO₂ fluxes showed considerable spatial, temporal and seasonal variation across the catchment with temporal dynamics of emission density and dissolved export shown in Figure 4(a) with mean and seasonal means shown in Table 5.

Mean CO₂ emission densities, across the 9 river sites, ranged from 517 to 2553 mg C m⁻² d⁻¹ with a catchment mean flux density of 1498 mg C m⁻² d⁻¹. Mean emission densities were highest in the lowland tributaries (Site 7 and 8) and the outflow of Loch of Clunie (Site 5). Emission densities on the whole, increased along the main river stem from upland sites to lowland sites, with lowland sites strongly influenced by tributaries. Likewise, the dissolved export flux increases along the river continuum with highest export rates at site 9 Perth, in line with the increased inputs from the major tributaries.

Comparisons of mass fluxes from site to site indicated that in general sinks were greater than sources of CO₂ along the lowland river continuum, with the majority of discrepancy most likely due to gas evasion to the atmosphere. However above site 6, there was an additional source of CO₂ in the main-stem river. This is likely to be sourced from the unsampled River Braan and possible further additions through in-stream production.

Emission density of CO₂ was comparable, but at the lower end of the scale, to other rivers in the UK and around the world as would be expected for a relatively nutrient poor, high flow river. For example, Hellings et al., (2001) report a CO₂ efflux in the range of 1210 to 5404 mg C m⁻² d⁻¹ (4200-18750 μmol CO₂ m⁻² h⁻¹) from a lowland section of the River Scheldt, which on the whole is highly polluted compared to the River Tay. A similar range of values were reported by Trimmer et al., 2009 in a lowland chalk river in the South of England.

CO₂ flux showed pronounced temporal variability throughout the two year monitoring period. CO₂ emission density generally increased from winter to summer at all four of the key sites shown in Figure 4a reaching a peak in June before substantial reduction in July/August. During the same period the dissolved export flux was generally low, reaching an annual low in July/August. This was followed by substantial increases both in emission and dissolved export through late summer and autumn before reducing again in winter. CO₂ emission density peaked in September in both years, however peaks in export flux of CO₂ occurred in October/November highlighting a sustained source of CO₂ throughout the autumn period. Overall these temporal patterns in CO₂ flux highlight the importance of seasonal environmental controls such as temperature, light, and substrate availability in controlling magnitude of fluxes.

CH₄ fluxes showed considerable spatial, temporal and seasonal variation across the catchment with temporal dynamics of emission density and export shown in Figure 4(c) with mean and seasonal means shown in Table (5). CH₄ emission densities, across the main river sites, ranged from 1719 to 15487 μg C m⁻² d⁻¹ with a catchment wide mean of 4635 μg C m⁻² d⁻¹. In general, emission densities decreased down river along the main river stem. The highest emission densities was observed in the River Isla (Site 7) which were substantially higher than any other site (15488 μg C m⁻² d⁻¹) but displayed significant temporal variability (SD. 11013 μg C m⁻² d⁻¹).

Dissolved export flux varied throughout the catchment, with largest fluxes observed at site 7 corresponding to the high CH₄-sat and emission densities. Overall highest emissions and export fluxes were seen in summer/autumn. Comparisons of mass fluxes indicated that in general sinks were greater than sources of CH₄ along the river continuum. This observation was most pronounced in the lower catchment, with substantial reduction in export at Site 9 compared to inputs. This is likely to be through a combination of gas evasion and possible CH₄ oxidation in the oxygen rich gravels in the main river stem.

Emission density of CH₄ was comparable, to other rivers in the UK. For example, Sanders et al., 2007 found CH₄ flux rates from 5.7 to 12970 $\mu\text{g C m}^{-2} \text{d}^{-1}$ with a mean of 4035 $\mu\text{g C m}^{-2} \text{d}^{-1}$ in a chalk stream in the South of England impacted by agriculture.

N₂O emissions also showed considerable spatial and seasonal variation across the catchment. Emission densities, across the main river sites, ranged from 176 to 1846 $\mu\text{g N m}^{-2} \text{d}^{-1}$. Mean emission densities were highest in the lowland tributaries (Site 8 and 7) and lowest in the upland river. In general, emission increased down river along the main river stem with fluxes substantially higher in the lowland tributaries. Seasonally emission densities followed similar patterns to CO₂, being higher in summer and autumn and lowest in winter suggestion temperature is an important control in production in the catchment, as well as seasonally changes in the gas transfer rate which also peaks in summer and reaches a minimum in winter.

Comparisons of mass fluxes indicate that on the whole sinks are greater than sources in the lowland river stem. This is likely due to atmospheric evasion being greater than in-stream production of gases. In September 2009 and 2010, mass fluxes indicate that additional sources of N₂O were found in the lowland river stem between site 9 and site 6. This could either be linked to increases in in-stream production, or additional inputs from unmonitored small tributary streams. Additionally, there is further discrepancy in the main stem river above site 6 with an unaccounted for source of N₂O. Again this is likely to be sourced from the unsampled River Braan and/or other sources (in-stream production/additional inputs).

The mean catchment emission density of 780 $\mu\text{g N}_2\text{O-N m}^{-2} \text{d}^{-1}$ was comparable, but at the lower end of the scale, to other rivers in the UK. For example, Dong et al., (2004)

Table 5. Overall mean and mean seasonal emission densities and emission density as CO₂eq * at key sites within the River Tay

	S9 PER		S8 ALM		S7 ISL		S6 KIN		S5 OUT	
	Emission	CO₂eq	Emission	CO₂eq	Emission	CO₂eq	Emission	CO₂eq	Emission	CO₂eq
CO₂ mg C m⁻² d⁻¹										
Mean	1514	2025	1925	2575	2553	3414	1278	1709	2114	2828
Winter	937.1	1253	979.1	1309	1684	2253	690	923	1601	2142
Spring	1462	1956	1613	2157	2183	2919	1373	1836	1465	1959
Summer	1877	2510	2189	2928	2899	3878	1593	2130	2024	2708
Autumn	1816	2430	2764	3697	3299	4413	1359	1817	3514	4700
N₂O µg N m⁻² d⁻¹										
Mean	682.8	116.6	1366	233.2	1846	315.2	681.1	116.3	1221	208.5
Winter	308.9	52.74	422.1	71.99	1221	208.4	427.1	72.92	566.4	96.70
Spring	404.7	69.08	1038	177.2	1446	246.8	531.9	90.81	903.8	154.3
Summer	929.5	158.7	1820	310.7	2048	349.6	800.5	136.7	1689	288.4
Autumn	1026	175.1	2028	346.2	2566	438.1	922.4	157.5	1616	275.9
CH₄ µg C m⁻² d⁻¹										
Mean	1719	20.95	1605	19.56	15488	188.7	2996	36.52	4811	58.63
Winter	1216	14.82	1814	22.11	13042	158.9	2216	27.00	2279	27.77
Spring	1952	23.79	1394	16.99	12903	157.3	3039	37.03	4081	49.74
Summer	2024	24.66	1858	22.64	14060	171.3	3705	45.15	8491	103.5
Autumn	1601	19.51	1389	16.92	21538	262.5	2896	35.29	3803	46.34

* CO₂eq mg l⁻¹ m⁻² hr⁻¹

Table 6. Annual Fluxes (dissolved export and emission) of GHGs and dissolved CO₂, CH₄, TdC, DOC, N₂O, TdN, NO₃ and NH₄.

Water Area	Emission Density mg C m ² d ⁻¹		Dissolved Flux kg C yr ⁻¹ x 10 ³				Emission kg C yr ⁻¹ x 10 ³	
km²	CO₂	CH₄	CO₂	CH₄	TdC	DOC	CO₂	CH₄
110.4	1498	4.634	2968	2.879	44067	19793	36944	109

Water Area	Emission Density μg N m ² d ⁻¹	Dissolved Flux kg N yr ⁻¹ x 10 ³				Emission kg N yr ⁻¹ x 10 ³
km²	N₂O	N₂O	TdN	NO₃	NH₄	N₂O
110.4	781	2.72	4330	3553	131.4	10.61

reported mean emission densities in the range of 769 to 2651 μg m⁻² d⁻¹ (Rivers Colne, Stour, Orwell, Deben, Trent, Ouse and Conwy). However the hotspot sites, those that had substantial levels of agriculture, were in the middle to high end of the range reported by Dong et al., (2004).

Emission densities were also used to calculate total freshwater emission and this was compared to annual dissolved fluxes at S9 PER (freshwater catchment outlet) (Table 6). Since approximately 93 % of the freshwater in the Tay catchment lies within the lower range of N₂O emission densities modelled in the study, the catchment data was further split into two sections (low emission density i.e. the upland catchment and high emission density i.e. the lowland catchment). A large portion of this freshwater in the upland catchment is contained in deep oligotrophic lakes (approximately 50 km²; Loch Tay, Loch Tummel and Loch Rannoch). Lakes generally have gas transfer rates far smaller than those found in rivers, therefore the mean estimated emission densities for the river sites in this study would likely overestimate the total emission in the upland zone. Instead, the emission from the upland lakes was estimated using mean values found in the literature for other nutrient poor upland lakes. N₂O lake emission was calculated from Whitfield, Aerne and Baulch (2011), who reported a mean N₂O emission rate of 36.4 μg m⁻² d⁻¹ from a 162 nutrient poor headwater lakes in Ireland. Alin and Johnson

(2007), through a synthesis of literature CO₂ emissions from a number of large lakes, reported a mean emission flux of 78 g C m⁻² yr⁻¹ from 62 lakes at a latitude of 50-60 deg. CH₄ was calculated using a mean of 690.9 µg C m⁻² d⁻¹ taken from 177 Swedish Lakes (Juutinen et al., 2009).

Using the literature values, and the mean upland and lowland river emission densities for CO₂, CH₄ and N₂O a total emission rate was calculated for freshwater in the Tay catchment (Table 6) based on a total freshwater area of 110.4 km² (NRFA 2012). Since the total emission flux for freshwater is calculated by multiplying by the mean flux density for catchment compartments (i.e. upland/lowland river and lake), it therefore makes the assumption that the mean emission density would hold true if the spatial coverage within the Tay catchment was expanded. Many upland rivers and streams (not fully represented by the sampling sites in this research) have been found to have high CO₂ and CH₄ emission densities, particularly in peatland-dominated catchments such as the upper River Tummel. Moreover, the dissolved concentrations and therefore saturation and emission have been found to vary significantly over a number of temporal scales, for example Dawson et al., (2009) found substantial variation in week sampling of the River Dee. The monthly sampling conducted in this study could likely have missed key temporal hotspots of GHG sources and sinks and consequently the catchment mean flux density may be an under or overestimate.

Of the total carbon flux, 46 % was estimated to being directly emitted to the atmosphere as CO₂, with the rest made up of dissolved fluxes exported to the estuary in river water (TdC = 54 %; DOC = 24 %). Estimated CH₄ emission only contributed 0.17 % of the total flux of C from freshwaters.

Fluxes of nitrogen were also compared, with the majority of the flux exported as a dissolved fraction. For example, 79 % of the total N flux was exported as NO₃ to the estuary with only 0.24 % of total N flux emitted to the atmosphere from freshwater in the catchment.

Estimated emission densities (Table 5) and total emissions were also compared as CO₂ equivalents to calculate the significance of the global warming potential of each gas. Flux densities as CO₂ equivalents across the sites was dominated by CO₂, for example at S9 PER, CO₂ made up 93.6 % of the total CO₂ equivalents, N₂O a further 5.4 % and CH₄

only 0.97 %.

Summary

The dynamic balance between external input, internal production/consumption, and export both downstream and to the atmosphere for all three GHGs is evident across the Tay catchment with considerable spatial and temporal variability. It is evident, that even in a relatively low pollution river like the Tay that spatial hotspots of gas emission and export still exist highlighting the importance of spatial coverage in monitoring greenhouse gas flux from catchments. In the Tay catchment, flux hotspots included lowland tributaries and a lowland lake, both of which were heavily impacted by agricultural landuse. Moreover, landuse was the predominant driver of GHG emission across the catchment, with increases in the flux of N₂O linked to increases in agricultural derived nutrients. Whilst CH₄ was generally higher in the upland catchment, linked to soil waters derived from peaty soils, hotspots of emission occurred in the lowland River Isla and Loch of Clunie sites, with these hotspots likely fuelled by *in-situ* production linked to increased availability of substrate.

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References

- Alin S.R, Johnson T.C. 2007. Carbon cycling in large lakes of the world: A synthesis of production, burial, and lake-atmosphere exchange estimates. **21**: issue 3
- Baker MA, and Vervier P. 2004. Hydrological variability, organic matter supply and denitrification in the Garonne River ecosystem. *Freshwater Biol.* **49**:181–190.
- Baker MA, Cumberland S, Hudson N. 2008. Dissolved and total organic and inorganic carbon in some British rivers. *Area* **40**: 117-127
- Battin, TJ, Kaplan LA, Findlay S, Hopkinson CS, Marti E, Packman AI, Newbold JD, and Sabater F. 2008. Biophysical controls on organic carbon fluxes in fluvial networks, *Nat. Geosci*, **1**, 95– 100
- Baulch HM, Dillon PJ, Maranger R, Schiff SL. 2011. Diffusive and ebullitive transport of methane and nitrous oxide from streams: Are bubble-mediated fluxes important?

Beaulieu, J.J., C.P. Arango, S.K. Hamilton, and J.L. Tank. 2008. The production and emission of nitrous oxide from headwater streams in the Midwestern USA. *Glob. Change Biol.* **14**:878–894.

Billett MF, Palmer SM, Hope D, Deacon C, Storeton-West R, Hargreaves KJ, Flechard C, Fowler D. 2004. Linking land-atmosphere-stream carbon fluxes in a lowland peatland system. *Global Biogeochemical Cycles* **18**: GB1024

Billett MF, Garnett MH, Harvey F. 2007. UK peatland streams release old carbon dioxide to the atmosphere and young dissolved organic carbon to rivers. *Geophysical Research Letters* **34**: L23401

Billett MF, Moore TR. 2008. Supersaturation and evasion of CO₂ and CH₄ in surface waters at Mer Bleue peatland, Canada. *Hydrological Processes* **22**: 2044–2054.

Borges, A. V., Delille, B., Schiettecatte, L.-S., Gazeau, F., Abril, G. and Frankignoulle, M. 2004. Gas transfer velocities of CO₂ in three European estuaries (Randers Fjord, Scheldt, and Thames). *Limnol. Oceanogr.*, **49**: 1630-1641

Clarke KR, Warwick RN. 2001 Changes in marine communities: an approach to statistical analysis and interpretation, 2nd edn. PRIMER-E Ltd, Plymouth

Cole JJ, Caraco NF. 2001. Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. *Marine and Freshwater Research* **52**: 101–110.

Dawson JJC, Smith P. 2007. Carbon losses from soil and its consequences for land-use management. *Science of the Total Environment* **382**: 165–190.

Dawson J. Soulsby C. Hrachowitz M. Speed M. Tetzlaff D. 2009. Seasonality of epCO₂ at different scales along an integrated river continuum within the Dee Basin, NE Scotland. *Hydrological Processes* **23**, 2929 – 2942

De Angelis, M.A., Lilley, M.D., 1987. Methane in surface waters of Oregon estuaries and rivers. *Limnology and Oceanography* **32**: 716–722.

De Angelis, M. A. and Scranton, M. I. 1993 Fate of methane in the Hudson River and estuary. *Global Biogeochem. Cy.*, **7**, 509–523

De Klein C, Novoa RSA, Ogle S, Smith KA, Rochette P, Wirth TC, McConkey BG, Mosier A, Rypdal K, Walsh M et al.: Chapter 11: N₂O emissions from managed soils, and CO₂ emissions from lime and urea application. 2006 *IPCC Guidelines for National Greenhouse Gas Inventories*. 2007:1 54.

Dong, L. F., Nedwell, D. B., Colbeck, I. and Finch, J. 2004. Nitrous oxide emission from

- some English and Welsh rivers and estuaries, *Water Air Soil Poll.*, **4**, 127–134
- Ferrón, S., Ortega, T., Gomez-Parra, A. and Forja, J. M. 2007. Seasonal study of dissolved CH₄, CO₂ and N₂O in a shallow tidal system of the bay of Cadiz (SW Spain). *J. Marine Syst.*, **66**, 244-257
- Finlay JC. 2003. Controls of streamwater dissolved inorganic carbon dynamics in a forested watershed. *Biogeochemistry* **62**: 231–252
- Forster, P., V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D.W. Fahey, J. Haywood, J. Lean, D.C. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, M. Schulz, and R.V. Dorland. 2007. Changes in atmospheric constituents and in radiative forcing. p. 129–234. In S. Solomon et al. (ed.) *Climate Change 2007: The physical science basis. Contributions of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge Univ. Press, Cambridge, United Kingdom and New York
- Garnier, J., Billen, G., Vilain, G., Martinez, A., Silvestre, M., Mounier, E., and Toche, F. 2009. Nitrous oxide (N₂O) in the Seine river and basin: observations and budgets, *Agr. Ecosyst. Environ.*, **133**, 223–233
- Griffiths J, Nutter J, Binley A, Crook N, Young A, Pates J. 2007. Variability of dissolved CO₂ in the Pang and Lambourn chalk rivers. *Hydrology and Earth System Sciences* **11**: 328–339.
- Groffman, P.M., A.J. Gold, D.Q. Kellogg, and K. Addy. 2002. Mechanisms, rates, and assessment of N₂O in groundwater, riparian zones, and rivers. p. 159–166. In J.V. Ham et al. (ed.) *Non-CO₂ greenhouse gases: Scientific understanding, control and policy aspects*. Millpress, Rotterdam, the Netherlands.
- Grossart HP, Frindte K. Dziallas C. Eckert W. Tang KW. 2011. Microbial methane production in oxygenated water column of an oligotrophic lake.
- Hellings L. Dehairs F. Van Damme S. Baeyens W. 2001 Dissolved inorganic carbon in a highly polluted estuary (the Scheldt) *Limnol Oceanogr.*, **45** (6) 1406-1414
- Hope, D., Billett, M.F., Milne, R., Brown, T.A.W., 1997b. Exports of organic carbon in British rivers. *Hydrological Processes* **11**, 325-344
- Hope D, Palmer S, Billett MF, Dawson JJC. 2004. Variations in dissolved CO₂ and CH₄ in a first-order stream and catchment: an investigation of soil–stream linkages. *Hydrological Processes* **18**: 3255–3275.
- Houghton, R. A. and Hackler, J. L. 2001. Carbon flux to the atmosphere from land-use changes: 1850 to 1990. ORNL/CDIAC 131, NDP-050/R1
- Inwood, S.E., J.L. Tank, and M.J. Bernot. 2005. Patterns of denitrification associated with land use in nine midwestern headwater streams. *J. N. Am. Benthol. Soc.* **24**:227–245.

IPCC (2007) Summary for policymakers, in: *Climate Change, 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., Miller, H. L., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2-18, 2007.

Ivens WPMF, Tysmans DJJ, Kroeze C, Lohr AJ, Van Wijnen J. 2011. Modelling global N₂O emissions from aquatic systems. *Current Opinion in Environmental Sustainability*. **3**: 350-358

Jarvie HP, Neal C, Smart R, Owen R, Fraser D, Forbes I, Wade A. 2001. Use of continuous water quality records for hydrograph separation and to assess short-term variability and extremes in acidity and dissolved carbon dioxide for the River Dee, Scotland. *Science of the Total Environment* **265**: 85–98.

Jones, J. B., and P. J. Mulholland (1998a), Influence of drainage basin topography and elevation on carbon dioxide supersaturation of stream water, *Biogeochemistry*, **40**, 57–72.

Jones JB Jr., Stanley EH, Mulholland PJ. 2003. Long-term decline in carbon dioxide supersaturation in rivers across the contiguous United States. *Geophysical Research Letters* **30**: 1495

Juutinen S, Rantakari M, Kortelainen P, Huttunen J.T, Larmola T, Alm J, Silvola J, and Martikainen P.J. 2009. Methane dynamics in different boreal lake types. *Biogeosciences* **6**: 209-223

Kling GW, Kipphut GW, Miller MC. 1991. Arctic lakes and streams as gas conduits to the atmosphere: implications for tundra carbon budgets. *Science* **25**: 298–301.

Liss, P. S. and Slater, P. G.: Flux of gases across the air-sea interface. *Nature*, **247**, 181–184, 1974.

Malcolm IA, Soulsby C, Hannah DM, Bacon PJ, Youngson AF, Tetzlaff D. 2008. The influence of riparian woodland on stream temperatures: implications for the performance of juvenile salmonids. *Hydrological Processes* **22**: 968–979.

Mosier, A., C. Kroeze, C. Nevison, O. Oenema, S. Seitzinger, and O. van Cleemput. 1998. Closing the global N₂O budget: Nitrous oxide emissions through the agricultural nitrogen cycle- OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. *Nutr. Cycling Agroecosyst.* **52**:225–248.

Mounier, E., 2008. Emissions d'oxyde nitreux, de nitrification et bactéries de nitrifiantes. Ph.D. Univ. Paris 6 (UPMC), ED Géosciences et Ressources Naturelles, 216 pp.

Neal C, House WA, Down K. 1998. An assessment of excess carbon dioxide partial pressures in natural waters based on pH and alkalinity measurements. *Science of the Total Environment* **210/211**: 173–185. Neal C, House WA, Jarvie HP, Eatherall A. 1998b. The significance of dissolved carbon dioxide in major lowland rivers entering the North Sea. *Science of the Total Environment* **210/211**: 187–203.

Neal C, Watts C, Williams RJ, Neal M, Hill L, Wickham H. 2002. Diurnal and longer term patterns in carbon dioxide and calcite saturation for the River Kennet, south-eastern England. *Science of the Total Environment* **282/283**: 205–231.

NRFA 2012; The national river flow archive. www.ceh.ac.uk/data/nrfa/

Pulliam WM. 1993. Carbon dioxide and methane exports from a southeastern floodplain swamp. *Ecological Monographs* **63(1)**: 29-53

Raymond PA, Caraco NF, Cole JJ. 1997. Carbon dioxide concentration and atmospheric flux in the Hudson River. *Estuaries* **20**: 381–390.

Rigby, M., Prinn, R. G., Fraser, P. J., Simmonds, P. G., Langenfelds, R. L., Huang, J., Cunnold, D. M., Steele, L. P., Krummel, P. B., Weiss, R. F., O’Doherty, S., Salameh, P. K., Wang, H. J., Harth, C. M., Mühle, J. and Porter, L. W. 2008 Renewed growth of atmospheric methane, *Geophys. Res. Lett.*, **35**, L22805.

Sanders IA, Heppell CM, Cotton JA, Wharton G, Hildrew AG, Trimmer M. 2007. Emission of methane from chalk streams has potential implications for agricultural practices. *Freshw Biol* **52**:1176–86.

Stein, L. Y., and Y. L. Yung. 2003. Production, isotopic composition, and atmospheric fate of biologically produced nitrous oxide, *Annu. Rev. Earth Plant Sci.*, **31**, 329– 356.

Stow, C.A., Walker, J.T., Cardoch, L., Spence, P., Geron, C., 2005. N₂O emissions from streams in the Neuse River watershed, North Carolina. *Environmental Science and Technology* **39**, 6999-7004.

Strauss, E.A., N.L. Mitchell, and G.A. Lamberti. 2002. Factors regulating nitrification in aquatic sediments: Effects of organic carbon, nitrogen availability, and pH. *Can. J. Fish. Aquat. Sci.* **59**:554–563

Strauss E.A, Richardson WB, Bartsch LA, Cavanaugh JC, Bruesewitz DA, Imker H, Heinz JA, Soballe DM. 2004. Nitrification in the Upper Mississippi River: patterns, controls, and contribution to the NO₃ budget. *J.N. Am, Benth.Soc.* **23**: 1-14

Teodoru CR, del Giorgio PA, Prairie YT, Camire M. 2009. Patterns in pCO₂ in boreal streams and rivers of northern Quebec, Canada. *Global Biogeochemical Cycles* **23**: GB2012

Trimmer M, Hildrew AG, Jackson MC, Pretty JL, Grey J. 2009. Evidence for the role of methane-derived carbon in a free-flowing, lowland river food web. *Limnol Oceanogr* **54**:1541–7

Trimmer M, Maanoja S, Hildrew AG, Pretty JL, Grey J.2010. Potential carbon fixation via methane oxidation in well-oxygenated riverbed gravels. *Limnol Oceanogr.* **55**:560–8

Wanninkhof, R.N., 1992. Relationship between wind speed and gas exchange over the ocean. *J. Geophys. Res.* **97**, 7373–7382

- Webster JR. 2007. Spiralling down the river continuum: stream ecology and the U-shaped curve. *Journal of the North American Benthological Society* **26**: 375–389.
- Weiss, R.F., Price, B.A., 1980. Nitrous oxide solubility in water and seawater. *Mar. Chem.* **8**, 347–359.
- Whitfield C.J, Aherne J, Baulch H.M. 2011. Controls on greenhouse gas concentrations in polymictic headwater lakes in Ireland. *Science of the Total Environment*. **410-411**: 217-225
- Wiesenburg, D. A. and Guinasso, N. L. Jr.1979. Equilibrium solubility's of methane, carbon monoxide and hydrogen in water and seawater, *J. Chem. Eng. Data*, **24**, 356–360
- Wrage, N., Velthof, G.L., Beusichem, van, M.L., Oenema, O., 2001. Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol. Biochem.* **33**: 1723– 1732.

Chapter 5. Paper III

Seasonal temperature and nutrient controls on the production of the greenhouse gases N₂O, CO₂ and CH₄ in lakes in the River Tay Catchment, Scotland

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Seasonal temperature and nutrient controls on the production of the greenhouse gases N₂O, CO₂ and CH₄ in lakes in the River Tay Catchment, Scotland

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Abstract

The production of the greenhouse gases CH₄, CO₂ and N₂O in a temperate, lowland lake show strong seasonal changes with the production, of N₂O in particular, also impacted by both nitrate and carbon concentrations in the sediments. We studied lake sediment slurries collected during spring, summer and late autumn from a small Scottish loch in the River Tay catchment (Loch of Clunie). Gas production varied seasonally, with greatest production of CH₄ in the summer and generally highly correlated to temperature. CO₂ production was greatest in summer and in autumn, with lowest production rates in spring. N₂O had highest production rates in spring and summer with no detectable production in the autumn. Semi-potential denitrification rate experiments, and semi-potential N₂O production rate experiments showed that sediments supported higher production rates when NO₃⁻ and temperature were non-limiting.

Nitrate additions to the spring sediment had a strong impact on N₂O production, but no effect on either CH₄ or CO₂. Carbon additions, as glucose, to the summer sediments had a strong impact on CH₄ and CO₂ production but little impact on N₂O production. However, additions of both glucose and NO₃⁻ lead to a substantial increase in N₂O production, but little additive effect on CH₄ and CO₂ production compared to glucose only addition. We also incubated sediments collected from a chain of lochs (including Loch of Clunie) along a known nutrient gradient. CO₂ production was observed to increase along with water column nutrient concentrations. However there was no statistically significant production of either N₂O or CH₄, which may have been due to the low temperatures limiting production. Potential denitrification experiments showed N₂O production increased along the loch chain highlighting the importance of nutrient loading linked to the proportion of catchment agriculture within the catchment.

Introduction

Freshwaters are important sources of greenhouse gases, methane (CH₄), carbon dioxide (CO₂) and nitrous oxide (N₂O). This is through the microbial processing of nutrients (carbon and nitrogen), predominantly occurring within lake sediments. Excess nutrient loading to lakes can stimulate primary production which can have knock-on effects on greenhouse gas production by microbial communities, and this clearly has both regional and global significance (IPCC, 2007).

Increases in primary production increase the overall organic matter supplied to lake sediments which in turn increases mineralisation of carbon. This can result in depletion of dissolved oxygen, and through lake stratification can lead to anoxic conditions in bottom waters (Smith et al., 1999) which can increase the rate of anaerobic microbial processing. Increases in nutrient concentrations can also affect microbial degradation pathways of carbon with the specific microbial process dependent on the maximum energy yield available. This thermodynamic energy yield, which results from oxidation reactions with various electron acceptors decreases in the order of O₂, NO₃⁻, Mn⁴⁺, Fe³⁺, SO₄²⁻ and CO₂ (Capone and Kiene, 1988). Therefore if anoxic conditions are present, as is common in many lake sediments, nitrate (NO₃⁻) becomes the preferred electron acceptor with organic carbon (C) reduced through denitrification. The denitrification rate can, therefore, potentially be dependent on the supply of available C and/or NO₃⁻. Under low nitrate conditions, carbon in anoxic conditions is generally degraded through methanogenesis in freshwater sediments (Capone and Kiene, 1998), producing CH₄.

Although, on the whole, CH₄ is found only in small concentrations in the atmosphere (<2 ppmv), it accounts for up to 20 % of the total radiative forcing (IPCC 2007). Lakes are now recognised as important sources of atmospheric CH₄ both regionally and globally. An estimated 24 % of all wetland emissions of CH₄ are contributed from large lakes in Europe (Saarnio et al., 2008). Small lakes however, have been shown to have a proportionally large significance in terms of element fluxes in the landscape (Cole et al., 2007). Moreover, small lakes have been shown to have relatively large emissions, in terms of unit area, of both CH₄ and CO₂ in arctic, boreal and temperate lakes (Bastviken et al., 2004; Kortelainen et al., 2004; 2006; Walter et al., 2007). Methane is further processed in oxygenated conditions, with aerobic oxidation of CH₄ to CO₂ in both sediments and the water column. Thus O₂ gradients in lakes can lead to both the consumption and production of CH₄. Eutrophication induced depletions in O₂ stimulate

CH₄ production, particularly during lake stratifications, and also reduce CH₄ oxidation possibly leading to increased emissions of CH₄ to the atmosphere (Kortelainen et al., 2000). Increased nutrient loading can also inhibit both the production and consumption of CH₄. Ammonium (NH₄) can interfere with the oxidation of CH₄ through competition for the key enzyme in CH₄ oxidation (Bédard and Knowles, 1989). However evidence for the importance of this within soils and sediments remains unclear, with the effects of NH₄ on CH₄ oxidation appearing contradictory in a number of reports from wetland soils and sediments (Conrad and Rothfuss 1991; Cai and Mosier, 2000). Moreover, in a direct incubation of lake sediment cores, Liikanen and Martikainen (2003) found no direct influence of NH₄ on the flux of CH₄.

CO₂ is produced in freshwater lakes through mineralization of organic carbon both in the water column and in the sediments, and the relative importance of respiration is largely dependent on water column depth (Algesten et al., 2005). For example, in shallow lakes bed sediments remain in contact with the upper warm mixed water column, compared with the cold hypolimnetic water in deep thermally-stratified lakes. This favours mineralization (CO₂ production) in shallow lakes, compared with relatively greater amounts of C burial in deep lakes. The main sink for CO₂ produced in lakes is typically atmospheric evasion, however, uptake by plants and algae during photosynthesis, which on the whole is controlled by both light and nutrient availability, is another important process, which can result in highly variable spatial (vertical water column) and temporal uptake. Fuelled by both allochthonous and autochthonous carbon inputs, CO₂ production (through respiration) often dominates over the consumption of CO₂ by primary producers (Duarte and Prairie 2005), which often results in super saturation of CO₂ in lake waters and sediments.

Nitrous oxide is produced through the microbial processing of nitrogen, either directly from aerobic nitrification, with the oxidation of NH₄ to nitrite (NO₂), or from an intermediate step in anaerobic denitrification, where NO₃⁻ is ultimately reduced to molecular nitrogen (N₂). Key elements of the production of N₂O in freshwater sediments are therefore the quantity of NH₄⁺, NO₃⁻ and O₂, but also the quantity and quality of organic carbon. In relatively unpolluted freshwaters, NH₄⁺ concentrations in sediments can be very important as they fuel coupled nitrification-denitrification processes (where nitrification in oxygenated upper sediment layers supply the NO₃⁻ for denitrification in deoxygenated deeper sediments). In high nutrient loading scenarios, coupled

nitrification-denitrification becomes less important as a free supply of water column NO_3^- , and eutrophication-induced anoxia, limits nitrification activity. However the amount of N_2O produced relative to N_2 in denitrification can still be important in freshwater sediments, but with the vast majority of NO_3^- reduced to N_2 .

Previously in chapter 4 we monitored the temporal variability of GHG from numerous sites in the River Tay catchment including the inflow and outflow of a small lake. This work highlighted that the lake, Loch of Clunie, was a hotspot for GHG emission in the catchment. The aims of the study are to firstly examine in more detail the temporal variability in GHG from Loch of Clunie and then examine the importance and controls of GHG production from lake sediments using laboratory experiments of cores taken from the loch. We firstly investigate the influence of NO_3^- , carbon and season on GHG production in sediments from Loch of Clunie. We hypothesise that increases in NO_3^- concentration will increase production of N_2O and CO_2 , whilst suppressing CH_4 production. We also hypothesise that additions of labile carbon will stimulate CO_2 and CH_4 production and in combination with additions of NO_3^- will stimulate an increased rate of N_2O production.

To further analyse the importance of nutrient concentrations on GHG production in lakes we conducted further laboratory studies on cores from 3 lochs that form a chain (including Loch of Clunie) along a gradient in nutrients and landuse.

Methods

Site description

Experiments were carried out in a chain of lakes in the River Tay Catchment, Scotland (Figure 1). The three lakes (referred to as lochs in Scotland) are among a chain of five lochs connected along the Lunan Burn, a small tributary of the River Isla and from there flow into the River Tay. The three lochs are Loch of Butterstone, Loch of Clunie and Loch of Marlee (also known as Loch of Drumellie). The majority of the work in this study focuses on Loch of Clunie, which sits approximately in the centre of the catchment. This loch was chosen to tie in with the temporal monitoring of GHG concentrations described in Chapter 4 and originally, because of its position in the landscape, its moderate nutrient status and general ease of access. The chain of lakes on the whole was selected because they span a nutrient gradient, with agricultural landuse becoming increasingly dominant through the catchment, which results in a gradient in

nutrient load. On the whole the catchment is undulating and hilly, with peaks of 400 m. The geology is mainly of mixed permeability bedrock that is two-thirds overlain by superficial deposits. There is a significant amount of mixed arable land use in the catchment, rough grazing and natural woodlands.

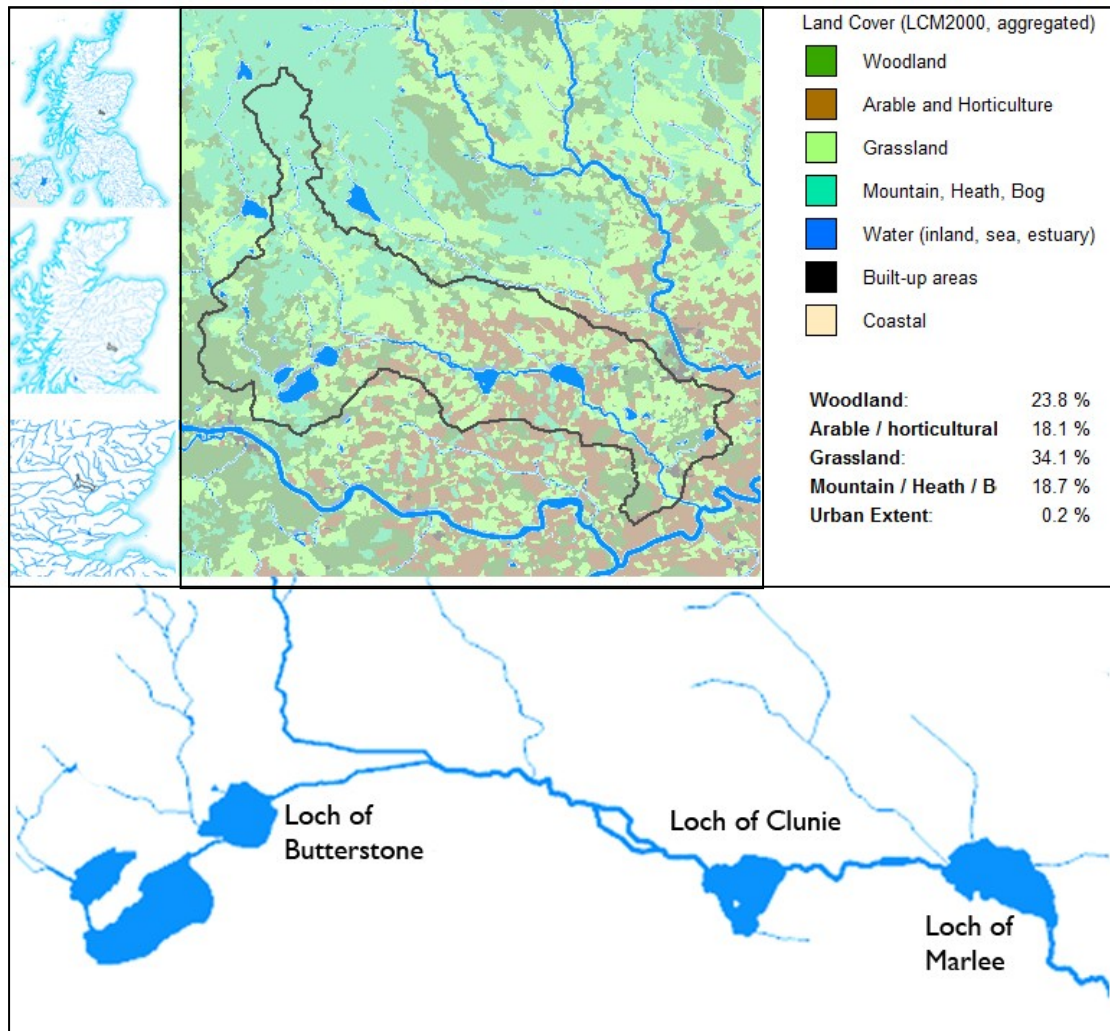


Figure (1) Lunan catchment with associated land use. Images and data sourced from NRFA (2012).

Sediment Collection and Basic Slurry Assay

The experimental work in this paper examines the GHG processing within loch sediments and is broadly split into two groups. Group A is a series of experiments examining the production of GHGs from Loch of Clunie, and Group B examines production of GHGs from two additional lochs that together with Loch of Clunie form a chain of lochs on the Lunan Burn (Figure 1).

Sediment samples (0-10 cm depth) were collected using a coring device (a Plexiglas cylinder, diameter 7 cm) from a small inflatable boat. Samples were collected from 2 metres water depth at the same location at all sampling times, with the position recorded using a handheld GPS device. The top 10 cm of sediment were collected from multiple cores, placed in an air tight sealable bucket and mixed on site to convert to a slurry. Water column temperature, pH and dissolved oxygen (DO) was recorded using a hand held device (Hach Quanta Probe). A four litre sample of surface water was taken and stored in a large plastic flexible container. Both sediment and water were transported back to the laboratory in an ice-box and sediment was stored overnight at *in-situ* temperature in a temperature controlled room. The water samples were stored at 4 °C.

The day after collection, sediments were further mixed in each bucket for 3 minutes using a stirring rod. Subsamples were extracted quickly ensuring slurry remained well mixed. 50 ml of slurry was transferred into 300 ml glass jars in triplicate for each treatment in all the experiments. Two sets of triplicate samples were also extracted for the analysis of sediment properties. Samples for sediment properties were dried for 48 h at 105 °C in an oven to determine the dry weight (DW) and the % water content (% WC). One set of triplicate samples were then put in a muffle furnace for 4 hours at 475 °C to determine the loss on ignition (% LOI) which is a measure of the % of organic material in the sediment.

Basic Slurry Procedure

In each jar containing 50 ml sediment slurry, a further 100 ml of *in-situ* site water was added. The water was allowed to reach experimental temperature prior to addition to minimise temperature disturbance. Gas tight rubber septa with grease applied were then placed over the bottle openings to seal the bottles and allow for gas sampling. Two syringe needles with 3-way valves were placed through each septum to minimise potential gas loss through repeated piercings. A length of rubber tubing extending to the base of the bottle was attached to one needle, which allowed for purging of the sediment using N₂ (10 minute purge) and the other needle vented the displaced gas in the experimental bottle. This ensured that the sediments were kept anaerobic throughout the experiment as carried out in other studies examining the production of CO₂, CH₄ and N₂O from sediments (Stadmark and Leonardson, 2005). Anaerobic conditions are generally found in lake sediments, although the top few millimetres may be aerobic depending on the oxygen state of the water column (Maerki et al., 2009).

Experiment Group A: Loch of Clunie

Sediments and water were collected from one site within Loch of Clunie on three occasions, April, June and November 2010. These sediments were used to perform a series of experiments.

In vitro temperature assays were used to determine the production rates of N_2O , CO_2 and CH_4 with no additional amendments at the *in-situ* temperature for each sampling month. The basic sediment procedure above was followed with sediments incubated at 9.6 °C in April, 15.5 °C in June and 6.5 °C in November with samples of gas taken using a gas tight syringe (Hamilton) at 0, 1 and 2 hours of incubation and analysed by gas chromatography within 6 hours.

Additionally, semi-potential denitrification rates were determined where acetylene addition was used to inhibit N_2O reductase allowing for a linear increase in N_2O , which can be used to calculate the rate of denitrification production. The denitrification rate under non-limiting NO_3^- concentrations, at laboratory temperature (20°C) is described as semi-potential as organic carbon can be a limiting factor (Garnier et al., 2010). Two sets of triplicate slurries were used for the denitrification assay. The first set (SPDR) was used to monitor the semi-potential denitrification rate where acetylene gas was introduced after purging to a final concentration of 10 % by volume. KNO_3 was added immediately after to both the SPDR set and the second set of slurries (which is the control set which we calculate the N_2O semi-potential production rate ($N_2O_{S,P}$)) at 8 mg N l^{-1} concentration. All slurry bottles were then shaken for one minute to ensure full equilibration of sediment, water and air. A gas sample was immediately extracted from each bottle using a gas tight syringe with further gas samples extracted after one hour and two hours to calculate the linear increase in N_2O and produce potential denitrification rates. Prior to each gas extraction slurry bottles were shaken vigorously for 1 minute to equilibrate gas between water and sediment slurry.

In April 2010, sediments were also used to examine the influence of NO_3^- on GHG production. After the basic slurry procedure, varying concentrations of NO_3^- as KNO_3 were added to triplicate sediment samples. Concentrations of KNO_3 were added at 0 (control sediment), 2, 4, and 8 mg N l^{-1} KNO_3 with three replicate sediment slurries each. NO_3^- concentrations were chosen to cover both the natural range of *in-situ* concentrations (0-4 mg N L^{-1}) as well as an additional high concentration. The slurries

were incubated at room temperature (20°C) and samples of gas were extracted after 0, 2.5, 5, 24 and 48 hours.

In June 2010, sediments were used to examine the influence of a combination of NO_3^- and carbon (as glucose) on GHG production. After the basic slurry procedure was followed, glucose was added at 10 mg C l^{-1} to one set of triplicate slurries, and glucose (10 mg C l^{-1}) and KNO_3 (8 mg N l^{-1}) added to a further set of slurries, and the final control set had no amendments. Slurries were incubated at 20°C and gas samples taken at 0, 2.5, 5, 24, 48 hours.

Experiment Group B: Chain of Lochs

The final set of experiments tests the hypothesis that concentrations of GHG increase along the chain of lakes as nutrient load increases. Two experiments were conducted, with semi-potential denitrification rate and in the *in-situ* condition production rate (no C or N additions and at *in-situ* temperature) measured.

Firstly one set of replicate sediment samples were incubated *ex-situ* at (6.5°C) for 2 hours using the standard slurry technique to obtain estimates of production rates at *in-situ* temperature.

Secondly, two further replicate sets were incubated at 20°C for the semi-potential denitrification experiments. After the basic slurry procedure was followed, acetylene was added to 10 % by volume in one set of slurries. After which, both sets received 8 mg N l^{-1} KNO_3 . A gas sample was immediately taken, and thereafter at 1 hour and 2 hours.

Analytical Procedure

N_2O and CH_4 were analysed on a HP5890 Series II gas chromatograph (Hewlett Packard (Agilent Technologies) UK Ltd., Stockport, UK) with an electron capture detector (ECD) and flame ionisation detector (FID) with accuracy of 30 ppb for N_2O and 70 ppb for CH_4 , respectively. CO_2 was measured simultaneously on another HP5890 Series II gas chromatograph fitted with a methaniser, facilitating the dual analysis of CH_4 and CO_2 by FID.

The gas concentration C_{gas} in ($\text{N}_2\text{O } \mu\text{g N l}^{-1}$, $\text{CH}_4 \mu\text{g C l}^{-1}$ and $\text{CO}_2 \text{ mg C l}^{-1}$) the samples extracted from the slurry experiments was calculated using the following equation adapted from Garnier et al. (2010):

$$C_{gas} = \frac{1}{V_l} \left[\left(kRT S_{gas} \frac{V_l}{1000} \right) + \left(S_{gas} \frac{V_g}{1000} \right) \right] MW \times 10^6$$

Where k equals the solubility coefficient of the specific gas at experimental temperature ($\text{mol L}^{-1} \text{ atm}^{-1}$), R is the perfect gas constant ($0.082057 \text{ atm L K}^{-1}$), S_{gas} is the concentration of gas derived from GC analysis (expressed in mol L^{-1}), V_l is the liquid volume of the experimental flask, V_g is the gas volume of the experimental flask (mL), and MW is the molecular weight of either C or N in CH_4 (12.01 g mol^{-1}), CO_2 (44.01 g mol^{-1}) and N_2O (44 g mol^{-1}). Gas production for each experiment is estimated as the amount of gas in gas and water phases divided by experimental time and the sediment surface area of the experimental flask (0.0028 m^2) and expressed as $\mu\text{g or mg m}^{-2} \text{ h}^{-1} / \mu\text{g or mg m}^{-2} \text{ d}^{-1}$.

Temperature, DO/DO-sat and pH were measured *in-situ* using a Hydrolab Quanta Probe (Hach) at each sample location. A subsample of water was filtered in the laboratory through a pre-combusted (500°C for 10 h) Whatman GF/F 47 mm diameter filter paper, using a Nalgene filtration unit and an electric vacuum pump, to obtain sub-samples for analysis of NO_3^- , NH_4^+ . The filtered sub-samples were transferred to small plastic sample tubes (Sterilin 50 ml high clarity polypropylene) and frozen until analysis. NO_3^- and NH_4^+ were analysed simultaneously on a Skalar San plus automated flow analyser using standard colorimetric methods. NO_3^- was determined using copper/cadmium reduction and sulphanilamide/NEDD reaction to form a red/purple colour whose absorbance was measured at 540 nm. NH_4^+ was determined using the hypochlorite reaction, to form chloroamine, reacting with nitroprusside and salicylate to form an idophenol dye whose absorbance was measured at 640 nm.

Statistics

All statistical analysis was carried out using Minitab (V16). Data was first tested for normality and transformations were applied if applicable. Impact of seasonal and added NO_3^- and carbon concentrations on GHG production was analysed with a one-way ANOVA. The potential change in gas production between the 3 study lochs was also analysed using one-way ANOVA with Tukey's family error rate test. Treatment effects were significant if $p \leq 0.05$.

Results and Discussion

Temporal variation in GHG at Loch Clunie

The monthly temporal and spatial variation in saturation of CH₄, CO₂ and N₂O are displayed in Figure (2) and relationships between dissolved gases and water chemistry are displayed in Table 1. In general all three gases displayed substantial temporal variability and increased between the inflow and outflow of Loch of Clunie suggesting that the loch is both a source of gas accumulation and likely gas production. Moreover, concentrations of GHG at the outflow of Loch of Clunie were always substantially above atmospheric equilibrium meaning the loch acts as a source of GHGs to the atmosphere.

Saturations of greenhouse gases in aquatic environmental are controlled by the dynamics of their sources and sinks which vary both spatially and temporally. These include: the magnitude of the allochthonous inputs (from upstream, inputs of groundwaters and soil waters), the magnitude of in-situ (sediments and water column) production and consumption of gas, physical mixing processes within the loch and loss to the atmosphere and external transport via the outlet stream. All these processes are influenced by a range of environmental factors to create a dynamic spatial and temporal GHG distributions.

Saturations of CH₄ in general displayed seasonal peaks in spring and summer with yearly maxima reached in September 2009 and August 2010 and minima in the Autumn of both 2009 and 2010 (Fig. 2b). CH₄ was positively correlated with temperature but no other water chemistry properties (Table 1). Maxima in CH₄ concentrations in summer have been observed in other lakes, with Huttunen et al., (2003) finding increased CH₄ flux linked to the increases in temperature and oxygen deficiency near bed sediments in Lake Kevätön, Finland. This observation is backed up by Liikanen, (2002) who found temperature and oxygen availability as important controlling factors in CH₄ production from Lake Kevätön sediment experiments. Other studies (Sanders et al., 2007) have observed that the quantity and quality of organic carbon may also combine to control the dynamics of CH₄ production and therefore water column saturation.

November 2009 and October 2010 (Fig. 2a). Seasonal lows were recorded in early spring, summer and in winter. Saturations of CO₂ were positively correlated with N₂O, and DIC, whilst negatively correlated with pH (Table 1). Dynamics of CO₂ in

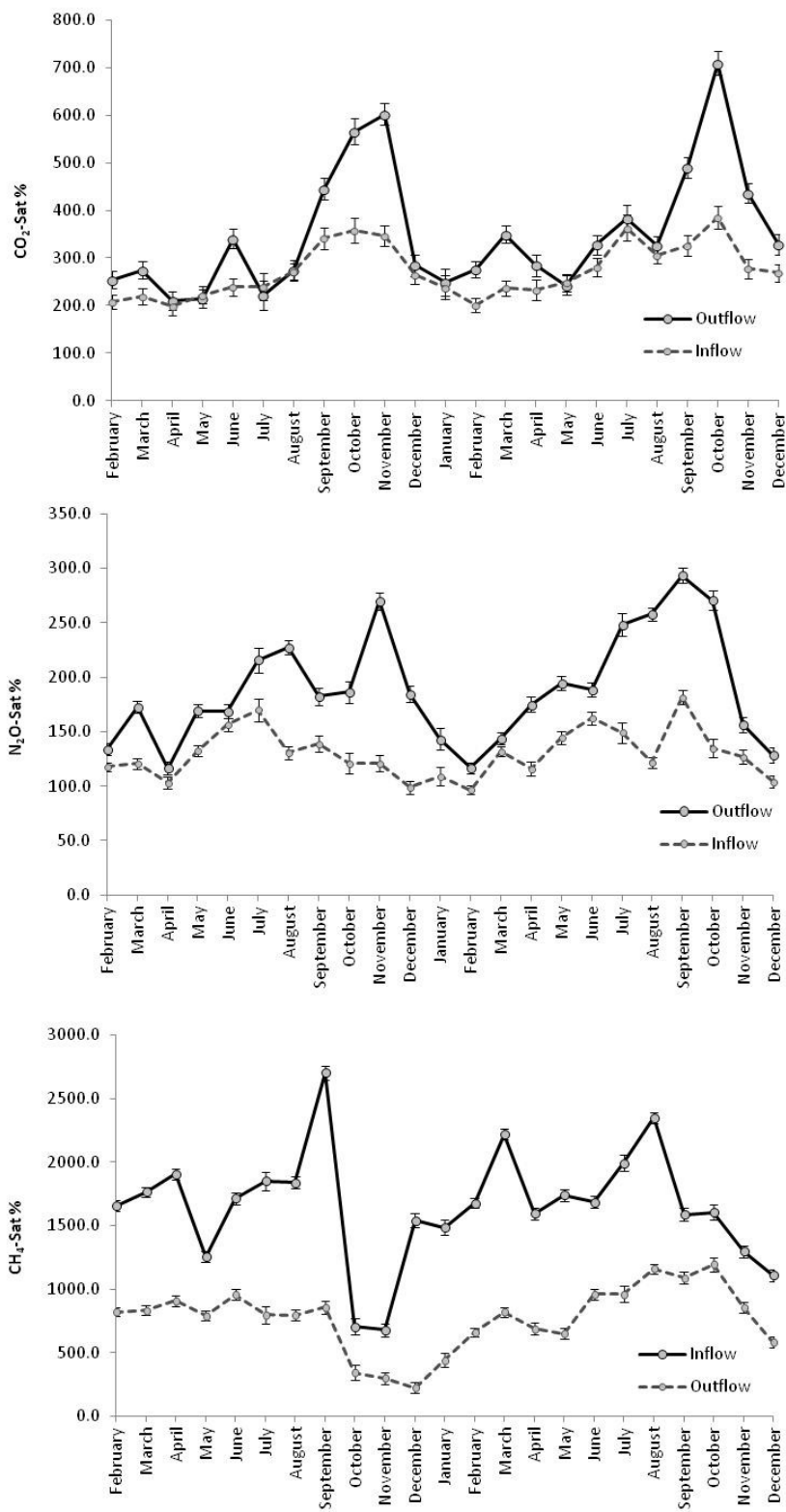


Figure 2: Temporal variability of CO₂, CH₄ and N₂O saturations between February 2009-December 2010 at the inflow and outflow of Loch of Clunie 2010 (Error bars equal standard error of mean, n=3)

freshwaters are known to be complex with a host of processes both producing and consuming CO₂ in the water column and in sediments. However, net heterotrophy (CO₂ saturation > 100 %), has been well documented in lakes (Cole, 1999, Huttunen et al., 2003) both in those that receive increased allochthonous C inputs and those that receive increased C inputs from autochthonous sources driven by increased primary production brought on by eutrophication and increased nutrient loading (Cole et al., 2000). In the latter case, primary production is known to both lead to consumption and production of CO₂ with the dominance of each process varying through the seasons.

Peaks in the saturation of CO₂ were found during late summer and autumn, peaking in Moreover, increases in primary production, fuelled by temperature and light increases, can lead to a decrease in CO₂ in the water column during the spring and summer (often below atmospheric equilibrium). This is followed by the collapse of primary production in autumn, which can lead to intense microbial processing of the organic material in the autumn/winter. The CO₂-sat signal in Loch of Clunie, showing seasonal troughs in spring and summer and peaks in autumn suggest that this seasonal balance between heterotrophy/autotrophy is the primary controlling factor of saturation levels of CO₂.

N₂O saturations showed peaks in the summer and into autumn, with lows through the winter and spring (Fig. 2b). Saturations of N₂O were positively correlated with CO₂, temperature, DO, NO₃⁻ and DIC (Table 1) suggesting biological processes were key in controlling temporal variability.

Table 1: Pearson's correlation relationships between GHG and water chemistry at Loch of Clunie between 2009 and 2010

	N ₂ O- sat %	CO ₂ - sat %	T. °C	pH	DO mg l ⁻¹	NO ₃ mg N l ⁻¹	NH ₄ mg N l ⁻¹	DOC mg l ⁻¹	DIC mg l ⁻¹
CH ₄ -sat	-0.01	-0.34	0.44*	0.40	-0.01	-0.19	0.07	-0.13	-0.25
CO ₂ -sat	0.56**	-	0.01	-0.41*	-0.23	-0.33	-0.20	-0.12	0.51*
N ₂ O-sat	-	0.56**	0.54**	-0.08	-0.67***	-0.47*	-0.20	0.072	0.52*

*p=0.05, ** p= 0.01 ***p = 0.001

Gas production from sediments: Impact of season, and nitrate and carbon Concentrations

It has been widely reported that both nutrient dynamics and temperature in the water column and sediments of lakes varies substantially through the year and therefore are likely to be a major controlling factor in the flux of GHG from sediments to water in lakes (Stadmark and Leonardson 2007; Liikanen et al., 2003). The results from the two year monitoring of Loch of Clunie suggest that there are important seasonal changes in the source of GHG in the loch. The main aim of this study was to examine the importance of sediment sources to the *in-situ* production of GHGs in Loch of Clunie and whether they varied seasonally. This was combined with nutrient addition experiments to attempt to understand some of the controlling factors in GHG production.

The results from the seasonal *in vitro* production assays and semi-potential denitrification assays from experiments conducted from Loch of Clunie sediments are outlined in Table 2 along with properties of the collected sediments and surface water. The *in vitro* production rates for all three gases displayed substantial seasonal variation indicating the season is an important controlling factor in GHG production from sediments. Previous work on lake sediment incubations have found that CO₂, CH₄ and N₂O all increase with temperature, with Liikanen et al. (2002) using a through-flow system finding increases over a temperature range of 6 – 23 °C. Stadmark and Leonardson (2007) found similar results with both CO₂ and N₂O increasing between 13 and 20 °C using sediment slurry incubations.

In our study, *in vitro* CH₄ production rates were highest in June and April with no production or consumption in November, resulting in a positive relationship with temperature. However both CO₂ and N₂O production showed no clear correlation with temperature, indicating that there may be additional important controlling factors in production. *In vitro* CO₂ production rates increased from April to June (possibly associated with increasing temperatures) but production rates did not decline much by November, being reasonably similar to those found in June, despite much cooler temperatures. Conversely, *in vitro* N₂O production rates were lowest in November with the coolest temperatures, where a net consumption of N₂O was observed, but highest in April, not June. This highlights that it is a complex of factors affecting GHG production,

not one dominant rate limiting factor.

Table 2: Experimental Output: *in-vitro* production rates at *in-situ* temperature, semi-potential N₂O and denitrification rates, and properties of sediment and water.

Production at <i>in-situ</i> temperature			N ₂ O Production		Sediment		& Water Properties					
N ₂ O	CH ₄ µg	CO ₂ mg	N ₂ O _{S,P}	SPDR*	LOI	W	DO-	pH	NO ₃	NH ₄	T	
µg N	C m ⁻²	C m ⁻²	mg N	mg N m ⁻²	%	%	% sat		mg N l ⁻¹	mg N l ⁻¹	°C	
m ⁻² hr ⁻¹	hr ⁻¹	hr ⁻¹	m ⁻² hr ⁻¹	hr ⁻¹								
Apr	9.06	241	82.3	0.22	4.23	9.2	54	98.7	7.5	1.1	0.01	9.5
Jun	8.59	338	116	0.19	3.47	8.7	59	86.5	7.6	0.6	0.02	15.6
Nov	-2.91	-	129	0.28	5.57	9.4	57	85.4	7.4	0.9	0.01	6.5

*: Semi-Potential Denitrification Rate LOI %: Loss on ignition W%: percentage water

This conclusion is supported by a range of studies which have observed that sediments collected from different times of the year and incubated at the same temperatures produce different amounts of CH₄ (Boon and Mitchell, 1995; Liikanen et al., 2003; Stadmark and Leonardson 2007) and N₂O (Stadmark and Leonardson 2007).

The results from the SPDR and N₂O_{S,P} assays indicate that this is true for sediments from Loch of Clunie, with higher semi-potential N₂O production and denitrification rates in November and April, compared to June. Since temperature and NO₃⁻ are high in the experimental setup, and identical between the seasonal incubations in the SPDR and N₂O_{sp} assays, we can only assume the seasonal changes in rates observed in the experiments are most likely affected by changes in the quality of the sediment as a substrate for microbial activity. Since there was no statistical relationship between SPDR or N₂O_{S,P} and organic matter content (LOI %; Table 2), we suggest that either the quality of the carbon within the sediment changed, such as more labile organic carbon associated with sedimentation of spring and autumn blooms of algae, or a significant change occurred in the microbial community itself.

The influence of nutrients on the production rates of GHGs were further explored with amendments of NO₃⁻ and carbon as glucose. Figure (3a) displays the results from the nitrate addition experiments conducted on the April sediments, where a series of NO₃⁻ additions were made to replicate sediment samples at room temperature (20°C) and incubated over 48 hours. There was a significant relationship between concentration of

nitrate addition and N₂O concentration after 48 hours (p=0.001). N₂O increased in all treatments with an increase of N₂O production at each step increase of KNO₃, with highest production recorded in the sediment incubated with 8 mg N l⁻¹ KNO₃ and lowest in the control sample with no KNO₃ addition. No significant relationship was found between NO₃⁻ addition and either CH₄ or CO₂. The two highest production rates for both gases were recorded both in the control and the 8 mg N l⁻¹ KNO₃ treatments. For CH₄, production over the course of the experiment did not increase linearly with a dip in concentration after 24 hours, then an increase again after 48 hours. CO₂ continually increased over the 24 hour incubation (technical error ruling out 48 hour sample collection) with a linear increase over the first 5 hours, but showing signs of reaching a plateau after 24 hours.

It is commonly known that denitrification rates are stimulated by increases in NO₃⁻ availability, and therefore the production of N₂O is increased. However much less is known about the effects of NO₃⁻ on CO₂ and CH₄ production under anaerobic conditions. As stated previously, denitrification is a heterotrophic process that utilises both NO₃⁻ and carbon as energy sources, therefore can produce both N₂O and CO₂. Therefore it would be expected that under increasing NO₃⁻ concentration not only would N₂O increase but this would be matched by increases in CO₂. However we did not find any changes in CO₂ production that directly linked to increased inputs of NO₃⁻. The two highest CO₂ production rates were found at both the lowest NO₃⁻ and highest NO₃⁻ concentrations.

Similarly, Stadmark and Leonardson (2007) found that a direct relationship between NO₃⁻ and CO₂ did not occur at low NO₃⁻ concentrations, instead they hypothesised that the production of CO₂ was linked to reductions in Fe and SO₄ and that these processes varied inversely with NO₃⁻ reduction.

Other studies have observed that elevated NO₃⁻ concentrations can reduce the production of CH₄ (Acht nich et al., 1995; Boon and Mitchell 1995; Kluber and Conrad 1998; Roy and Conrad, 1999) and recently Stadmark and Leonardson (2007) found occasional CH₄ production inhibition. However this study finds no link between reduction in CH₄ production with increased NO₃⁻ concentrations. However, in most cases the concentrations of NO₃⁻ in the previous studies have been significantly greater than those used in this study that were chosen to reflect more closely the range of *in-situ* NO₃⁻ concentrations found in freshwaters (but are still substantially greater than what is likely to be found in this lake site).

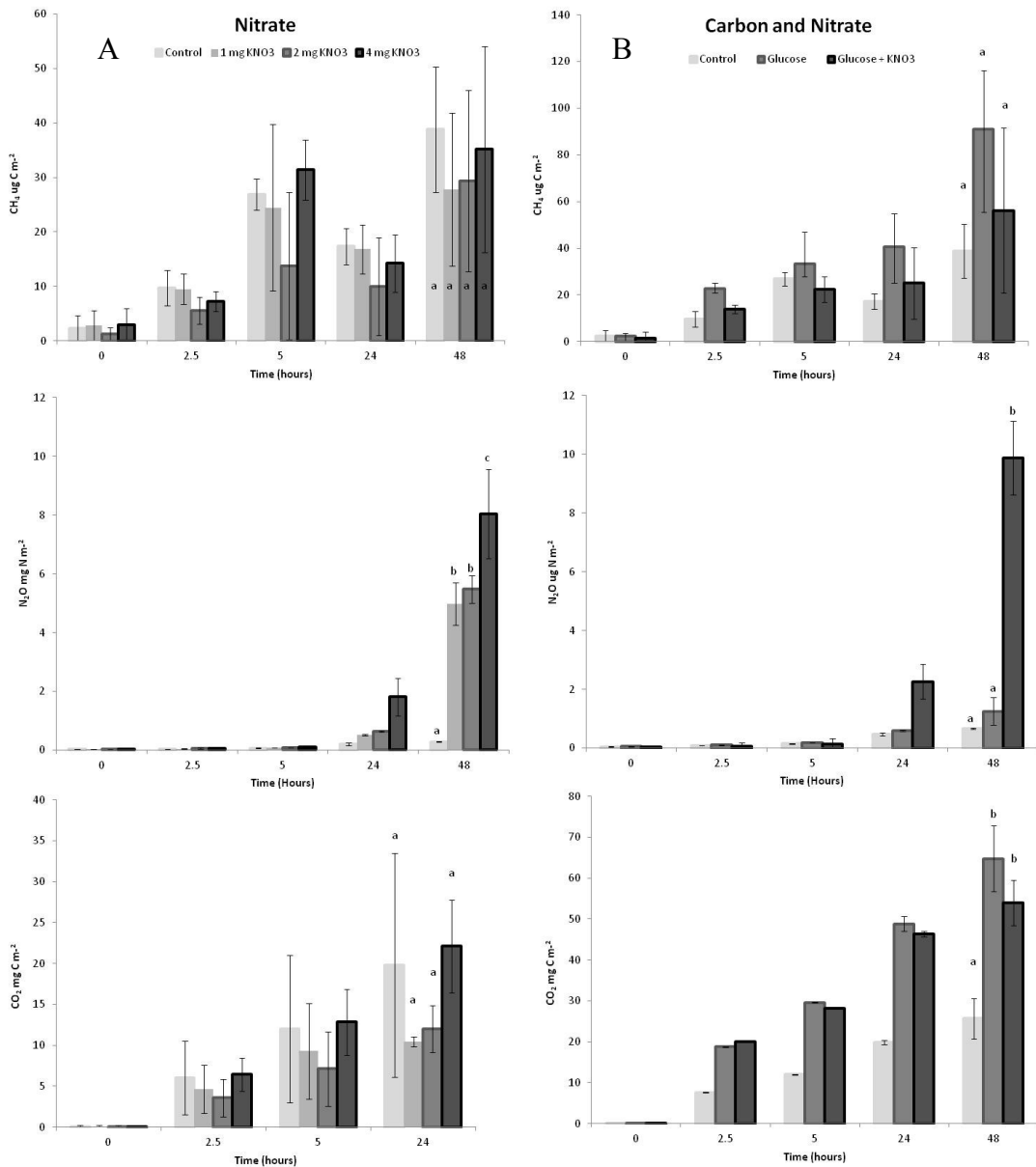


Figure (3) Nutrient addition experiments a) influence of KNO₃ on GHG production and b) influence of carbon and KNO₃ on GHG production. Letters indicate treatment statistical significance ($p=0.05$) using one-way ANOVA with Turkey family error rate test at 48 hrs. Bars equal mean ($n=3$) and error bars equal standard deviation.

More important to the production of CO₂ and CH₄ was the addition of carbon, as glucose, which substantially increased the rate of production of both gases. Figure (3b) displays the results from the carbon and nitrate addition experiments conducted in June on sediments kept at room temperature (20°C). With the simultaneous addition of NO₃⁻ and glucose, production rates of either CH₄ and CO₂ did not differ statistically between those with only glucose added. N₂O however substantially increased after addition of

combined NO_3^- and glucose additions, but with only a small increase with glucose by itself (Figure 3b).

Gas production along a continuum of lochs from nutrient poor to nutrient rich

This study has shown that the nutrients carbon and nitrogen, both in the water column and in sediments, are important controlling factors in greenhouse gas production within a lake environment. It has been clearly shown that N loading in lakes is significantly related to catchment agriculture (Bruesewitz et al., 2011; Schindler 2006). This raises the question of how important are changes in catchment landuse to the increases in nutrient loading and the consequences of this on the potential emission of greenhouse gases from lakes, both in the Tay catchment and elsewhere. This was investigated further through an additional experiment examining *in-situ* production of GHG and denitrification rates through a chain of lochs, Loch of Clunie, Loch of Butterstone (upstream of Clunie) and Loch of Marlee (downstream of Clunie), with the three lochs connected by the Lunan Burn.

Water chemistry was found to change substantially between the three loch sites with NO_3^- and NH_4^+ concentrations increasing downstream as the dominance of agricultural landuse increased (Table 3). Nutrient loading from agriculture is known to alter many dynamics in lakes that can enhance conditions for microbial processing through increase in both allochthonous and autochthonous carbon inputs. This results from the possible increase in soil erosion and organic carbon transport from surrounding terrestrial soils (Chen and Driscoll 2009; Wilson and Xenopoulos 2009). Furthermore, increased in-lake productivity due to nutrient loading increases the rate of organic matter deposition to sediments, fuelling increased microbial productivity, which enhances DO consumption both in the water column and in sediments (Spooner and Maher, 2009) resulting in not only increased denitrification but also release of phosphate and ammonium from sediments.

Table (3) Properties of sediment, *in-situ* gas concentrations and water chemistry along a continuum of loch sites from nutrient poor (site 3) to nutrient rich (site 1).

	Properties of Sediment		<i>In-situ</i> Gas Concentrations and Water Chemistry						
	% LOI	% W	N ₂ O-sat %	CO ₂ -sat %	CH ₄ -sat %	T. °C	pH	DO - Sat	NO ₃ mg N l ⁻¹
Site 1	9.8	59.9	225	345	870	6.9	7.0	81.5	1.45
Site 2	9.4	55.4	195	300	900	6.5	7.4	85.4	0.94
Site 3	8.7	57.5	150	280	895	6.8	7.4	87.2	0.44

The changes in general sediment properties between the 3 lochs were small with LOI % varying from 8.7 to 9.8 % (Table 3). Water content varied from 55.4 to 59.9 %. Since sampling was conducted in November water temperatures at all three loch sites were seasonally low ranging from 6.5 to 6.9 °C. Other water chemistry variables varied linearly between upstream Butterstone Loch (loch 3) and downstream Loch Marlee (loch 1). Both NO₃⁻ and NH₄⁺ increased, with NO₃⁻ concentrations increasing from (0.44 mg N L⁻¹) Butterstone Loch to (1.45 mg N L⁻¹) at downstream in Loch of Marlee (Fig.4) . DO-sat decreased from 87.2 % upstream to 81.5 % downstream. Dissolved concentrations of N₂O in the water column increased through the 3 loch sites with N₂O-sat increasing from 150 % to 225 %. This was matched by increases in CO₂-sat, with an increase from 280 % to 345 %. CH₄-sat varied spatially with highest concentrations at site 2 (300 %) and lowest at site 3 (280 %) (Fig. 4).

Semi-potential denitrification rates experiments were conducted to ascertain if there was a change in microbial processing between the three lakes, when NO₃⁻ concentration was raised to stimulate N₂O production in the experimental setup. SPDR increased substantially through the loch chain, suggesting that there are important changes within the sediments. SPDRs across the 3 lochs was predicted by *in-situ* NO₃ concentrations ($r^2=0.72$ $p=0.031$); sediment organic matter content ($r^2=0.64$ $p=0.05$); *in-situ* DO-sat ($r^2=0.78$ $p=0.020$) and *in-situ* saturations of GHGs such as N₂O ($r^2=0.67$ $p=0.047$) and CO₂-sat ($r^2=0.78$ $p=0.02$).

Factors controlling this increase are likely to be a combination of the increase in the quantity of organic material found in the sediments, and perhaps an increase in the

lability of the carbon within this organic material. There may also be differences in the species and gene expression of the microbial communities between the three lochs. As stated previously, nutrient loading link to agriculture can increase the quantity and quality of carbon supplied to sediments. Potential denitrification rates have previously been shown to alter with catchment agriculture. Bruesewitz et al., (2011) found increases in potential denitrification rates linked to increases in agriculture landuse across 10 lakes in New Zealand. *In-situ* condition rates of denitrification rates have also been shown to increase with N loading with McCrackin and Elser (2010) finding denitrification rates between $0.07 \text{ mg N m}^{-2} \text{ d}^{-1} \pm 0.13$ and $14.0 \text{ mg N m}^{-2} \text{ d}^{-1} \pm 2.5$ across a gradient of atmospheric N deposition with strongest rates at the high N deposition lakes.

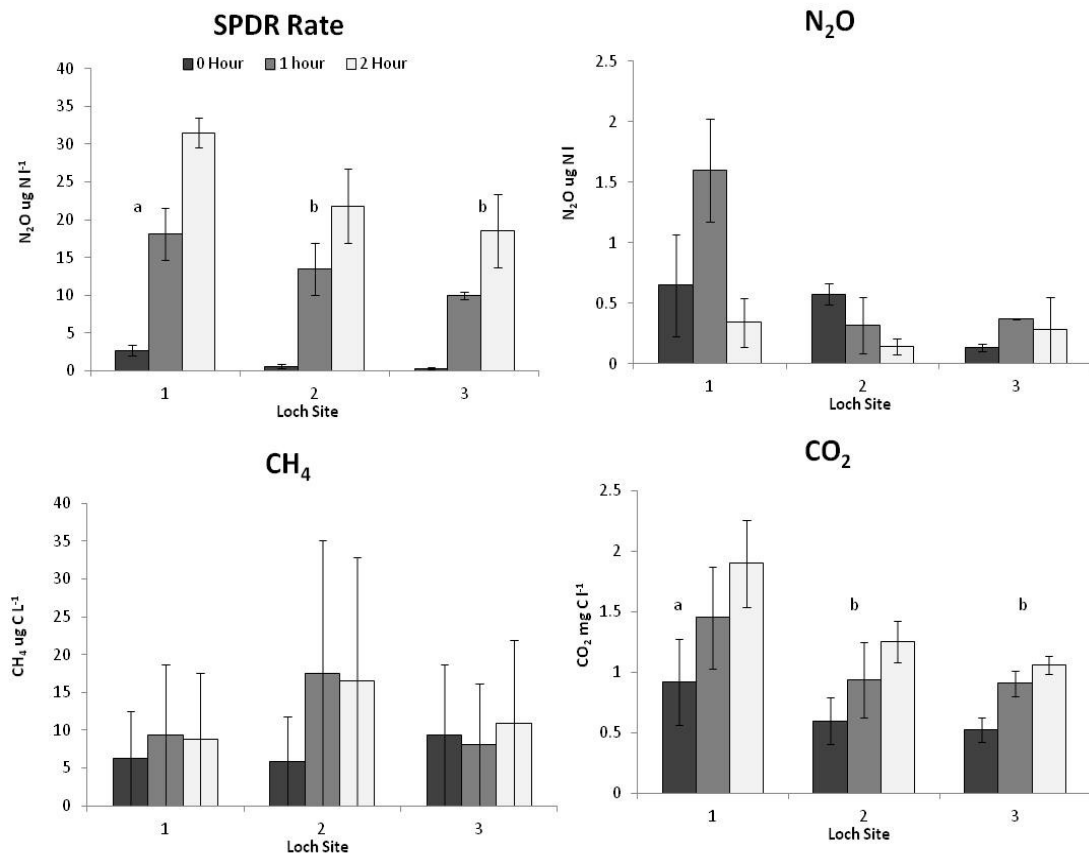


Figure (4) Denitrification potential and the production of N₂O, CH₄ and CO₂ along a continuum of loch sites from nutrient poor to nutrient rich. The Tukeys Family Error Rate result, testing for significant difference between sites is displayed in letters (a, b, c) with different letters indicating a significant difference ($p \leq 0.05$). Error bars equal standard deviation.

We also measured non-amended *in-vitro* production of GHGs at each loch site at the ambient loch temperature with varying results. CO₂ significantly increased across the 3 loch sites ($p \leq 0.01$) with clear hourly production of CO₂ at each loch. There was no significant methane production at any of the lochs with substantial variability between replicates. There also was no sustained production of N₂O at any of the loch sites. This could have been partly due to the low temperatures limiting production with mean temperatures near seasonal lows (6.5 – 6.9 °C). Alternatively NO₃⁻ concentrations were depleted to a point where the first stage of denitrification (NO₃⁻ to N₂O) could be outcompeted by the second stage (N₂O to N₂) resulting in the reduction of overall N₂O concentration through experimental time. Results from the experiments back this view up, with an initial burst of N₂O production (within Loch of Marlee sediments) occurring within the first hour of incubation, followed by substantial reductions in the second hour as NO₃⁻ concentrations potentially were too low to support further N₂O production at the experimental temperature. Both the low NO₃⁻ concentrations and the overall experimental conditions (anaerobic) probably limited the production of N₂O through suppression of coupled nitrification-denitrification. Whilst conditions in the bottom waters of lakes can sometimes mean that sediments remain fully anaerobic, often there is a steep oxygen gradient within sediments with the uppermost layers (often only a few mm) remaining aerobic. This is important in controlling the production of N₂O through the process of nitrification which is often coupled with denitrification in nutrient poor sediments, where the aerobic nitrification of NH₄⁺ to NO₃⁻ fuels further N₂O production both in the nitrification stage and the denitrification stage. In our experiment this nitrification stage is either inhibited fully or greatly reduced by the low O₂ levels resulting from the purging by N₂. This results in the overall reduction in N₂O concentrations during the experiment as the denitrification process consumes N₂O. This highlights that nitrification, rather than denitrification, is most likely very important in regulating N₂O production within lake environments with only moderate to low nutrient loading.

Despite a lack of N₂O production, there was however, sustained microbial processing at all three sites highlighted by the linear increase in CO₂ production. This production increased through the loch chain with highest production rates at the nutrient rich Loch of Marlee. CO₂ production rates were predicted by NO₃ concentrations ($r^2=0.67$, $p=0.014$); in-situ DO-sat ($r^2=0.78$, $p=0.004$); % organic matter ($r^2=0.54$, $p=0.037$) and in-

situ pH ($r^2=0.81$, $p=0.002$). This indicates that it is not only SPDR that is linked to increases in agriculture but also CO₂ production. The production increase ties in with the increase in denitrification potential and it is potentially fuelled by the increase in quality of carbon found within the sediments fuelled by changes in primary production between the 3 lakes.

Conclusions

On the whole, this study shows that GHG production varies seasonally and that both NO₃⁻ and carbon are important controlling nutrients in GHG production in sediment slurries incubated *in-vitro*. Moreover, GHG gas production and potential denitrification was shown to increase along a chain of lochs from nutrient poor to nutrient rich highlighting the importance of catchment land use and nutrient loading to the production of GHGs from lochs.

References

- Achnich C, Bak F, Conrad R. 1995. Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil. *Biol Fertil Soils*. **19**:65–72.
- Algesten G., Sobek S., Bergström A.-K., Jonsson A., Tranvik L.J. & Jansson M. 2005. Contribution of sediment respiration to summer CO₂ emission from low productive boreal and subarctic lakes. *Microbial Ecology*, **50**, 529–535
- Algesten G, Sobek S, Bergstrom AK, Agren A, Tranvik LJ, Jansson M. 2003. Role of lakes for organic carbon cycling in the boreal zone. *Global Change Biol*. **10**:141–7.
- Bastviken D., Cole J., Pace M. & Tranvik L. 2004. Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochem. Cycles* **18**: GB4009. doi:10.1029/2004GB002238.
- Bedard, C., Knowles, R., 1989. Physiology, biochemistry, and specific inhibitors of C₄, NH₄⁺, and CO oxidation by methanotrophs and nitrifiers. *Microbiological Reviews* **53** (1), 68–84.
- Boon PI, Mitchell A. 1995. Methanogenesis in the sediments of an Australian freshwater wetland: comparison with aerobic decay, and factors controlling methanogenesis. *FEMS Microbiol Ecol* **18**:175–90
- Bruesewitz DA, Hamilton DP, Schipper LA. 2011. Denitrification Potential in Lake Sediment Increases Across a Gradient of Catchment Agriculture. *Ecosystems* **14**: 341-352
- Cai, Z.C., Mosier, A.R., 2000. Effect of NH₄Cl addition on methane oxidation by paddy soils. *Soil Biology and Biochemistry* **32**, 1537–1545.

- Capone, D.G., Kiene, R.P., 1988. Comparison of microbial dynamics in marine and freshwater sediments: Contrasts in anaerobic carbon metabolism. *Limnology and Oceanography* **33** (4), 725–749
- Chen, X. Driscoll, C.T. 2009 Watershed Land Use Controls on Chemical Inputs to Lake Ontario Embayments. *Journal of Environmental Quality*. 38 (5): 2084-2095
- Cole, J.J., Pace, S. R. Carpenter, AND J. F. Kitchel. 2000. Persistence of net heterotrophy in lakes during nutrient addition and food web manipulations. *Limnol. Oceanogr.* **45**: 1718 – 1730
- Cole JJ, Caraco NF. 1998. Atmospheric exchange of carbon dioxide in a low wind oligotrophic lake measured by the addition of SF₆. *Limnol Oceanogr.* **43**:647–56.
- Cole JJ, Prairie YT, Caraco NF, McDowell WH, Tranvik LJ, Striegl RG, et al. 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems*. **10**:171–84
- Cole, J.J., 1999. Aquatic microbiology for ecosystem scientists: new and recycled paradigms in ecological microbiology. *Ecosystems* **2**: 215–225.
- Conrad, R., Rothfuss, F., 1991. Methane oxidation in the soil surface layer of a flooded rice field and the effect of ammonium. *Biology and Fertility of Soils* **12**: 28–32.
- Duarte, C.M., Prairie, Y.T., 2005. Prevalence of heterotrophy and atmospheric CO₂ emissions from aquatic ecosystems. *Ecosystems* **8**: 862-870
- Forster, P., V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D.W. Fahey, J. Haywood, J. Lean, D.C. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, M. Schulz, and R.V. Dorland. 2007. Changes in atmospheric constituents and in radiative forcing. p. 129–234. In S. Solomon et al. (ed.) *Climate Change 2007: The physical science basis. Contributions of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge Univ. Press, Cambridge, United Kingdom and New York
- Garnier, J. A., Mounier, E. M., Laverman, A. M., and Billen, G. 2010. Potential denitrification and nitrous oxide production in the sediments of the Seine river drainage network (France), *J. Environ. Qual.*, **39**: 449–459
- Huttunen J.T., Nykänen H., Turunen J. & Martikainen P.J. 2003. Methane emissions from natural peatlands in the northern boreal zone in Finland, Fennoscandia. *Atmos. Environ.* **37**: 147-151
- IPCC (2007) Summary for policymakers, in: *Climate Change, 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., Miller, H. L., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2-18, 2007.
- Klüber HD, Conrad R. 1998. Inhibitory effects of nitrate, nitrite, NO and N₂O on methanogenesis by *Methanosarcina barkeri* and *Methanobacterium bryantii*. *FEMS Microbiol Ecol.* **25**:331–339
- Kortelainen P., Pajunen H., Rantakari M. & Saarnisto M. 2004. A large carbon pool and

- small sink in boreal Holocene lake sediments. *Glob. Change Biol.* **10**: 1648- 1653.
- Kortelainen P., Rantakari M., Huttunen J.T., Mattsson T., Alm J., Juutinen S., Larmola T., Silvola J. & Martikainen P.J. 2006. Sediment respiration and lake trophic state are important predictors of large CO₂ evasion from small boreal lakes. *Glob. Change Biol.* **12**: 1554- 1567.
- Kortelainen P, Huttunen JT, Vaisanen T, Mattsson T, Karjalainen P, Martikainen PJ.2000. CH₄, CO₂ and N₂O supersaturation in 12 Finnish lakes before and after ice-melt. *Verh Internat Verein Limnol.* **27**:1410–4.
- Liikanen A, Martikainen P.J. 2003. Effect of ammonium and oxygen on methane and nitrous oxide fluxes across sediment-water interface in a eutrophic lake. *Chemosphere* **52**: 1287-1293
- Liikanen A., Murtoniemi T., Tanskanen H., Väisänen T. & Martikainen P.J. 2002. Effects of temperature and oxygen availability on greenhouse gas and nutrient dynamics in sediment of a eutrophic boreal lake. *Biogeochemistry* **59**: 269-286.
- Liikanen A., Ratilainen E., Saarnio S., Alm J., Martikainen P.J. & Silvola J. 2003a. Greenhouse gas dynamics in boreal, littoral sediments under raised CO₂ and nitrogen supply. *Freshw. Biol.* **48**: 500-511.
- Liikanen A., Huttunen J.T., Murtoniemi T., Tanskanen H., Väisänen T., Silvola J., Alm J. & Martikainen, P.J. 2003a. Spatial and seasonal variation in greenhouse gas and nutrient dynamics and their interactions in sediments of a boreal eutrophic lake. *Biogeochemistry* **65**: 83-103.
- Maerki, M., Müller, B., Dinkel, C., Wehrli, B., 2009. Mineralization pathways in lake sediments with different oxygen and organic carbon supply. *Limnology and Oceanography* **54** (2) 428–438.
- McCrackin ML, Elser JJ. 2010. Atmospheric nitrogen deposition influences denitrification and nitrous oxide production in lakes. *Ecology.* **91**:528–39.
- National River Flow Archive. 2012 www.ceh.ac.uk/data/nrfa/
- Van der Nat FJWA, de Brouwer JFC, Middelburg JJ & Laanbroek HJ (1997) Spatial distribution and inhibition by ammonium of methane oxidation in intertidal freshwater marshes. *Appl. Environ. Microbiol.* **63**: 4,734–4,740
- Roy R, Conrad R. 1999. Effect of methanogenic precursors (acetate, hydrogen, propionate) on the suppression of methane production by nitrate in anoxic rice field soil. *FEMS Microbiol Ecol.* **28**: 49–61.
- Saarnio, S., Winiwater, W., and Leitão, J. 2009. Methane release from wetlands and watercourses in Europe, *Atm. Env.*, **43**: 1421–1429
- Sanders IA, Heppell CM, Cotton JA, Wharton G, Hildrew AG, Trimmer M. 2007. Emission of methane from chalk streams has potential implications for agricultural practices. *Freshw Biol* **52**:1176–86.
- Schindler, DW. 2006 Recent advances in the understanding and management of eutrophication. *Limnol. Oceanogr* **51**: 356 - 363

Smith, V.H., Tilman, G.D., Nekola, J.C., 1999. Eutrophication: impacts of excess nutrient input on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution* **100**: 179– 196.

Spooner DR, Maher W. 2009. Benthic sediment composition and nutrient cycling in an intermittently closed and open lake lagoon. *J Mar Syst* **75**:33–45.

Stadmark J, Leonardson L. 2005. Emissions of greenhouse gases from ponds constructed for nitrogen removal. *Ecological Engineering* **25**:542-551

Stadmark J, Leonardson L. 2007. Greenhouse gas production in a pond sediment: Effects of temperature, nitrate, acetate and season.

Walter, K. M., Smith, L. C., and Chapin III, F. S. 2007. Methane bubbling from northern lakes: present and future contributions to the global methane budget, *Phil. Trans. Royal Soc.*, **365**: 1657–1676

Wilson HF, Xenopoulos MA. 2009. Effects of agricultural land use on the composition of fluvial dissolved organic matter. *Nat Geosci* **2**:37–41

Chapter 6. Paper IV

Seasonal temperature and nutrient controls of the production of the greenhouse gases N₂O, CO₂ and CH₄ in river sediments across the River Tay Catchment, Scotland

Seasonal temperature and nutrient controls of the production of the greenhouse gases N₂O, CO₂ and CH₄ in river sediments across the River Tay Catchment, Scotland

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Abstract

The production of the greenhouse gases CH₄, CO₂, and N₂O from river sediments sampled across a temperate river catchment showed strong spatial and seasonal changes, with the production of N₂O in particular enhanced by both nitrate and carbon additions to the sediments. We studied river sediments from four sites across the River Tay catchment (Scotland) spanning upland nutrient poor to lowland more nutrient rich conditions, in spring, summer and autumn. Gas production varied spatially, with highest production of N₂O, CH₄, and CO₂ at the lowland nutrient rich sites, and lowest production in the upland nutrient poor river site. Spatial variability in production was significantly positively related to river water concentrations of NO₃⁻ and NH₄⁺ and sediment organic matter content. These in turn were related to landuse in the catchment with both gas production and concentration of dissolved nutrients positively correlated with agricultural landuse.

Sediment slurries from both a nutrient rich site and nutrient poor site were incubated in potential denitrification rate experiments, and potential N₂O production rate experiments, which showed that sediments supported higher production rates when NO₃⁻ and temperature were non-limiting, and that the production rate was higher in a nutrient rich lowland river site compared to a nutrient poor upland site. These sediments were also used to perform nutrient addition experiments (addition of NO₃⁻ and carbon as glucose). Additions of NO₃⁻ significantly increased the production of N₂O at both sites, whilst carbon addition only had a small impact at the nutrient rich site, and no impact at the nutrient poor site suggesting that the production of GHG in these sediments are predominantly N limited. However, combined additions of C+N led to substantial

increases in N_2O production suggesting that at higher NO_3^- concentrations the sediments become carbon limited. Whilst CO_2 production increased with separate additions of C and N at the nutrient rich site, this did not occur at the nutrient poor site. Simultaneous additions of C and N did not impact on CO_2 production at either site. Considerable variability in CH_4 production made it unclear if C or N additions influenced production from sediment.

Introduction

Freshwaters are important sources of greenhouse gas (GHG), methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) and the production and emission of these gases from aquatic environments is the subject of a wide range of ongoing research. The contributions of aquatic environments such as streams and rivers to the budgets of CH_4 , CO_2 and N_2O are thought to be significant but highly uncertain (IPCC 2007). This uncertainty is increased by the existing evidence that suggests GHG fluxes from these aquatic environments are highly variable both on temporal and spatial scales.

CO₂ and CH₄:

Wetlands are known to be a major source of global CH_4 emissions contributing 20 – 40% of the estimated global output (Houghton et al., 2001). CH_4 production in lentic wetlands results from the decomposition of organic matter via the microbial process of methanogenesis in anaerobic sediments. However, much less is known about CH_4 production and emission in rivers, streams and lakes, probably because they are thought to be a far smaller source compared to lentic wetlands and have therefore received little study. Recent work, however, suggests that emissions could be strongly related to organic matter accumulation, with macrophytes trapping sediment and providing organic carbon for methanogenesis (Sanders et al., 2007).

Relatively little is known about the dynamics of CO_2 fluxes in aquatic systems, despite being of significant ecological significance within rivers and lakes, and connecting terrestrial carbon stores (such as peatland) to the atmosphere and ocean (Dawson and Smith, 2007). Moreover, it has been recently shown to be an important and significant component of regional carbon budgets (Billett et al., 2004; Teodoru et al., 2009).

CO_2 concentrations are controlled by a host of dynamic processes that vary both spatially

and seasonally encompassing both allochthonous inputs and autochthonous inputs/outputs. Autochthonous inputs include heterotrophically respired CO₂ from in stream mineralization of organic matter and autotrophic respiration. Outputs include photosynthetic uptake by biota in the water column and exchange with the atmosphere (Cole and Caraco, 2001; Finlay, 2003; Jones et al., 2003; Baker et al., 2008; Battin et al., 2008; Dawson et al., 2009).

N₂O:

The most important global source of N₂O is agriculture (Mosier et al., 1998), and emissions have been increasing because of an increase in animal production and nitrogen (N) fertilizer use. However, aquatic emissions are thought also to constitute a significant global source (Seitzinger et al., 2000; Mosier et al., 1998) fuelled by indirect emissions associated with microbial processing of N leached to rivers and streams from agricultural fields. These indirect emissions are among the most uncertain in the global N₂O budget (De Klein et al., 2007) and the methods used to model global emissions from aquatic systems have been under constant revision over the last 15 years (Ivens et al., 2011).

N₂O is produced as a by-product from the microbial transformation of ammonium (NH₄⁺) and nitrate (NO₃⁻) in nitrification and denitrification respectively. Nitrification oxidises NH₄⁺ to nitrites (NO₂⁻) and NO₃⁻ in aerobic conditions in sediments and in the water column. Denitrification is the anaerobic dissimilatory reduction of NO₃⁻ to N₂ and only occurs in anoxic environments, predominantly in sediments. Denitrification is known to be an important mechanism of N₂O production in aquatic systems (Seitzinger et al., 2000; Beaulieu et al., 2009) and has received intense focus in the literature (Pina-Ochoa and Alvarez-Cobelas, 2006) driven mainly by the perceived benefit of denitrification which removes NO₃⁻ from the water column and helps prevent eutrophication. However these studies have focused predominantly only on the level of NO₃⁻ removal, with only a few concerned about the production of N₂O as a by-product essentially making denitrification a pollution swapping process. On the whole the processing and production of N₂O within aquatic environments, especially river environments, is still highly uncertain.

In this study we aim to assess the spatial and seasonal variation in sediment CH₄, CO₂ and N₂O production rates over four sites spanning the River Tay catchment from upland nutrient poor to lowland nutrient rich. We also examine in more detail the production

rates from two sites, a nutrient poor upland river site and nutrient rich lowland river site in late summer and autumn. These experiments include analysing the impact of NO_3^- and carbon addition to sediment gas production as well as looking at the semi-potential denitrification rates, semi-potential N_2O production rates and N_2O yield in these contrasting sites.

Methods

Site description

The four sites used in this seasonal experimental study (Figure 1) were selected to match those in earlier field studies on gas saturation and emission in the Tay catchment (Chapter 4). Two of the sites are on the lowland tributaries River Isla and River Almond which can be described as nutrient rich in the context of the catchment. Two other sites were placed in the upland Tay catchment, which is nutrient poor, and in the lowland main stem river at Perth. The River Isla and another site in the upland Tay were used in more detailed sediment studies (Figure 1) to represent an lowland nutrient rich site with fine sediment and an upland nutrient poor site with fine sediment, respectively.

Seasonal Production Assays

We measured sediment CH_4 , CO_2 and N_2O production rates in the laboratory using incubations of sediment slurry sampled from the 4 sites in the River Tay in April, May, July and October 2010. Incubations were conducted at *in-situ* temperature with no additional amendments of nutrients to provide the best available estimate of *in-situ* gas production rates.

Sediment samples (0-10 cm depth) were collected from the same locations using a Plexiglas cylinder by hand from areas of sediment that were permanently underwater. The top 10 cm of sediment were collected from multiple cores, placed in an air tight sealable bucket and mixed on site to create a slurry. Water column temperature, pH and dissolved oxygen concentration (DO) were measured using a hand held device (Hach Quanta Probe). A 4-litre sample of surface water was taken and stored in a large plastic

flexible container. Both sediment and water were transported back to the laboratory in an ice-box and sediment was stored overnight at *in-situ* temperature in a temperature controlled room. The water samples were stored at 4 °C.

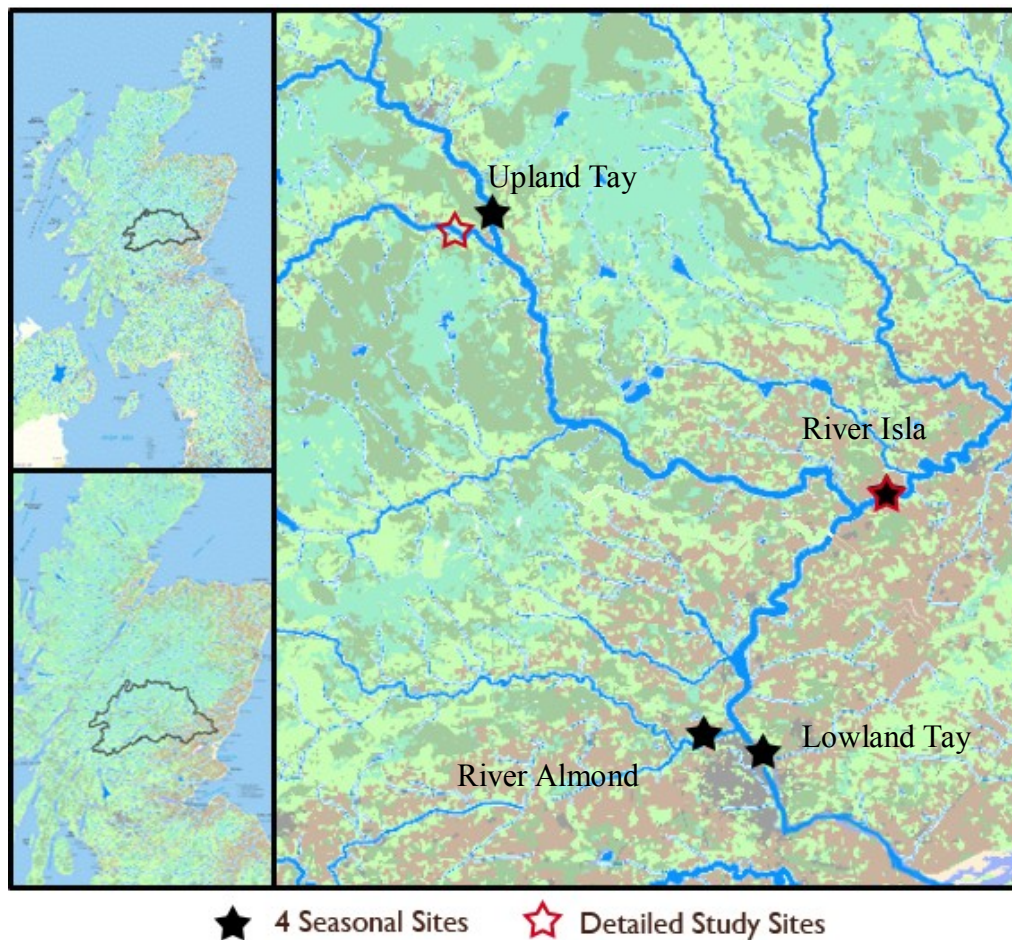


Figure 1: Map of Tay catchment showing sediment sampling sites, including the 4 seasonal sites and 2 detailed process study sites.

The day after collection, sediments were further mixed in each bucket for 3 minutes using a stirring rod. Subsamples were extracted quickly ensuring slurry remained well mixed. 50 ml of slurry was transferred into 300 ml glass jars in triplicate for each treatment in all the experiments. Two sets of triplicate samples were also extracted for the analysis of sediment properties. Samples were dried for 48 h at 105 °C in an oven to determine the dry weight (DW) and the % water content (% WC). One set of triplicate samples were then ashed in a muffle furnace for 4 hours at 475 °C to determine the loss on ignition (% LOI) which is a measure of the % of organic material in the sediment.

In each jar containing 50 ml sediment slurry, a further 100 ml of *in-situ* site water was added. The water was allowed to reach experimental temperature prior to addition to minimise temperature disturbance. Gas tight rubber septa with grease applied were then placed over the bottle openings to seal the bottles and allow for gas sampling. Two syringe needles with 3-way valves were placed through each septum to minimise potential gas loss through repeated piercings. Attached to one needle a length of rubber tubing extending to the base of the bottle allowed for purging of the sediment using N₂ (5 minute purge) and the other needle vented the displaced gas in the experimental bottle. This ensured that the sediments were anoxic. Immediately after purging, a 5 ml gas sample was extracted and again after 1 hour, 2 hours and 3 hours, using a gas tight syringe (Hamilton). After each headspace sampling, 5 ml of N₂ was added to the bottle headspace to maintain a constant pressure during the assay. The gas samples were then analysed within 1-5 hours using gas chromatography and gas concentration in the bottle calculated using the methods set out below.

Detailed Process Studies

In addition to the seasonal *in-situ* temperature assays, sediments were collected in September and November 2010 from two sites in the Tay catchment. These sites were chosen to represent a nutrient poor upland river site and nutrient rich (in the context of the River Tay) river site, and both comprised fine sediments where microbial production is likely to be highest (Garcia-Ruiz 1998). These sediments were incubated under a number of conditions. Firstly, a nutrient amended assay was conducted, to determine if NO₃⁻ or an organic carbon source was limiting gas production. Sediments assays were conducted as above, but in addition to a control set of slurries that had no amendments, additional slurries in triplicate were incubated with NO₃⁻ (as KNO₃ at 8 mg N L⁻¹), organic carbon (+C, as glucose at 10 mg C L⁻¹), and NO₃⁻ and carbon (+C+N). These nutrient amended assays were conducted at 20°C to provide optimal (in terms of the *in-situ* water temperature ranges typically found in the Tay catchment 0 – 20°C) conditions for microbial processes.

Additionally, semi-potential denitrification rates were determined where acetylene addition inhibits N₂O reductase allowing for a linear increase in N₂O, which can be used to calculate the rate of denitrification production at each site. Two sets of triplicate

slurries were used for the denitrification assay, with the basic procedure described above followed initially (2.2.1). The first set (SPDR) of sediment slurries was used to monitor the semi-potential denitrification rate where acetylene gas was introduced after purging to a final concentration of 10 % by volume. KNO₃ was added immediately after to both the SPDR set and the second set of slurries (control set or N₂O semi-potential production rate (N₂O_{S.P})) at 8 mg N l⁻¹ concentration, to stimulate the denitrification production of N₂O. The denitrification rate at laboratory temperature (20°C) can be limited by both NO₃⁻ concentration and carbon concentration and is described as semi-potential when carbon could be an additional limiting factor (Garnier et al., 2010).

After amendments were added, all slurry bottles were then shaken for 1 minute to ensure full equilibration of sediment, water and air. A gas sample was immediately extracted from each bottle using a gas tight syringe with further gas samples extracted after 1 hour and 2 hours to calculate the linear increase in N₂O and produce potential denitrification rates. Prior to each gas extraction slurry bottles were shaken vigorously for 1 minute to equilibrate gas between water and sediment slurry.

Analytical Procedure

N₂O and CH₄ were analysed on a HP5890 Series II gas chromatograph (Hewlett Packard (AgilentTechnologies) UK Ltd., Stockport, UK) with an electron capture detector (ECD) and flame ionisation detector (FID) with accuracy of 30 ppb for N₂O and 70 ppb for CH₄, respectively. CO₂ was measured simultaneously on another HP5890 Series II gas chromatograph fitted with a methaniser, facilitating the dual analysis of CH₄ and CO₂ by FID.

The gas concentration C_{gas} in (N₂O µg N L⁻¹, CH₄ µg C L⁻¹ and CO₂ mg C L⁻¹) the samples extracted from the slurry experiments was calculated using the following equation adapted from Garnier et al. (2010):

$$C_{gas} = \frac{1}{V_l} \left[\left(kRT S_{gas} \frac{V_l}{1000} \right) + \left(S_{gas} \frac{V_g}{1000} \right) \right] MW \times 10^6$$

Where *k* equals the solubility coefficient of the specific gas at experimental temperature (mol L⁻¹ atm⁻¹), *R* is the perfect gas constant (0.082057 atm L K⁻¹), *S_{gas}* is the concentration of gas derived from GC analysis (expressed in mol L⁻¹), *V_l* is the liquid volume of the experimental flask, *V_g* is the gas volume of the experimental flask (mL),

and MW is the molecule weight of either C or N in CH_4 (12.01 g mol^{-1}), CO_2 (44.01 g mol^{-1}) and N_2O (44 g mol^{-1}). Gas production for each experiment is estimated as the amount of gas in gas and water phases divided by experimental time and the sediment surface area of the experimental flask (0.0019 m^2) and expressed as per unit area and unit time ($\mu\text{g or mg m}^{-2} \text{ h}^{-1} / \mu\text{g or mg m}^{-2} \text{ d}^{-1}$).

Temperature, DO/DO-sat and pH were measured *in-situ* using a Hydrolab Quanta Probe (Hach) at each sample location. A subsample of site water was filtered in the laboratory through a pre-combusted (500°C for 10 h) Whatman GF/F 47 mm diameter filter paper, using a Nalgene filtration unit and an electric vacuum pump, to obtain sub-samples for analysis of NO_3^- , NH_4^+ . The filtered sub-samples were transferred to small plastic sample tubes (Sterilin 50 ml high clarity polypropylene) and frozen until analysis. NO_3^- and NH_4^+ were analysed simultaneously on a Skalar San plus automated flow analyser using standard colorimetric methods. NO_3^- was determined using copper/cadmium reduction and sulphanilamide/NEDD reaction to form a red/purple colour whose absorbance was measured at 540 nm. NH_4^+ was determined using the hypochlorite reaction, to form chloroamine, reacting with nitroprusside and salicylate to form an idophenol dye whose absorbance was measured at 640 nm. DOC was analysed on a PPM LABTOC analyser using ultraviolet oxidation and infra-red gas analysis.

Statistics

All statistical analysis was carried out using Minitab (V16). Data was first tested for normality and transformations were applied if necessary. Impact of seasonal and added NO_3^- and carbon concentrations on GHG production was analysed with a one-way ANOVA and Tukeys family error rate. Relationships between production and water chemistry and sediment properties were tested with simple linear regression.

Results and Discussion

Seasonal Greenhouse Gas Production Rates

The results from the seasonal study of GHG production rates are displayed in Figure 2 and the accompanying field data and sediment properties in Table 1.

Table 1: Mean Properties of Water and Sediment from the seasonal sediment experiments

Site	Water Chemistry					Sediment Properties	
	T °C	DO Sat %	NO ₃ -N mg l ⁻¹	NH ₄ -N mg l ⁻¹	DOC mg l ⁻¹	Description	% LOI
Lowland Tay	12.2	94.5	0.60	0.02	4.16	Gravel/Sand	0.91
Upland Tay	12.6	94.8	0.15	0.01	5.57	Gravel/Sand	0.95
R.Isla	12.8	87.9	2.24	0.07	3.30	Silt	8.07
R.Almond	12.9	94.8	0.98	0.03	3.15	Sand/Silt	2.50

Isla							
Seasonal	°C	DO Sat	NO ₃ -N	NH ₄ -N	DOC	% LOI	
April	9.5		87	2.31	0.06	2.97	8.78
May	14.2		89	2.11	0.07	1.75	7.67
July	18.2		90	1.87	0.09	5.64	7.25
October	9.6		86	2.67	0.05	2.86	8.56

Almond							
	°C	DO Sat	NO ₃ -N	NH ₄ -N	DOC	% LOI	
April	9.9		94	1.05	0.05	2.88	3.9
May	14.2		95	0.87	0.01	2.39	3.5
July	18.1		97	0.78	0.03	3.95	0.8
October	9.7		93	1.23	0.03	3.37	1.78

Tay Lowland							
	°C	DO Sat	NO ₃ -N	NH ₄ -N	DOC	% LOI	
April	10		96	0.59	0.02	3.84	1.24
May	12		94	0.5	0.02	3.33	0.98
July	17		95	0.54	0.03	5.67	0.45
October	10		92	0.79	0.03	3.83	0.98

Tay Upland							
	°C	DO Sat	NO ₃ -N	NH ₄ -N	DOC	% LOI	
April	10.1		94	0.16	0.01	5.16	1.45
May	13.2		95	0.15	0.01	4.17	0.98
July	17.5		97	0.18	0.01	7.68	0.47
October	9.6		93	0.14	0.01	5.27	0.89

N₂O Production

Nitrous oxide production rates varied considerably both spatially through the catchment and seasonally ranging from 0 (where N₂O was being consumed rather than produced) to a maximum of 1.85 mg N m⁻² d⁻¹ (Figure 2). Highest rates of production were observed in the two lowland tributaries, with rates significantly lower at the lowland main-stem Tay site and even lower at the upland Tay site. This spatial pattern is similar to the spatial pattern in N₂O areal fluxes and dissolved concentration reported in Chapter 6. The seasonal patterns in N₂O production varied from site to site, however peak production rates were commonly observed in autumn. Our initial prediction that denitrification would be the primary mechanism in N₂O production in the assays would mean that production rates would correlate with the controlling factors of denitrification i.e. NO₃⁻ concentration and organic carbon availability (Seitzinger, 1988). The overall spatial variability in the mean N₂O production rates was positively related to water column NO₃⁻ ($p=0.035$), which has been observed in other studies (Garcia-Ruiz et al., 1999; Beaulieu et al., 2009). This corresponds with the findings of Chapter 4 where N₂O flux was correlated with NO₃⁻ concentration which both were correlated to agricultural landuse.

The secondary controlling factor in denitrification is carbon availability. Sediment N₂O production was not related to water column DOC concentrations but was positively related to sediment % organic matter, which initially suggests that carbon content of sediment is of greater importance to N₂O production than water column DOC. Other studies have come to similar conclusions (Pattinson et al., 1998; Royer et al., 2004; Wall et al., 2005; Beaulieu et al., 2009). Beaulieu et al., (2009) suggest that the lack of apparent relationship between DOC and N₂O production could be linked to overall availability of carbon in DOC, which is known to vary substantially (Kaplan and Newbold, 2003). Furthermore heterotrophic metabolism has been found to be mostly supported by a small fraction of highly labile carbon within the bulk DOC (Mayorga et al., 2005). The relationship between N₂O production and sediment organic matter content has also been observed elsewhere (Beaulieu et al., 2009; Inwood et al., 2005; Richardson et al., 2004). Even in fully oxic conditions aerobic respiration can create anoxic micro-sites in sediments (Fuss and Smock, 1996) promoting anaerobic processes such as denitrification. However, in fine grained sediments, oxygen concentration has a steep gradient with sediment depth, with oxygen often being depleted over the first few

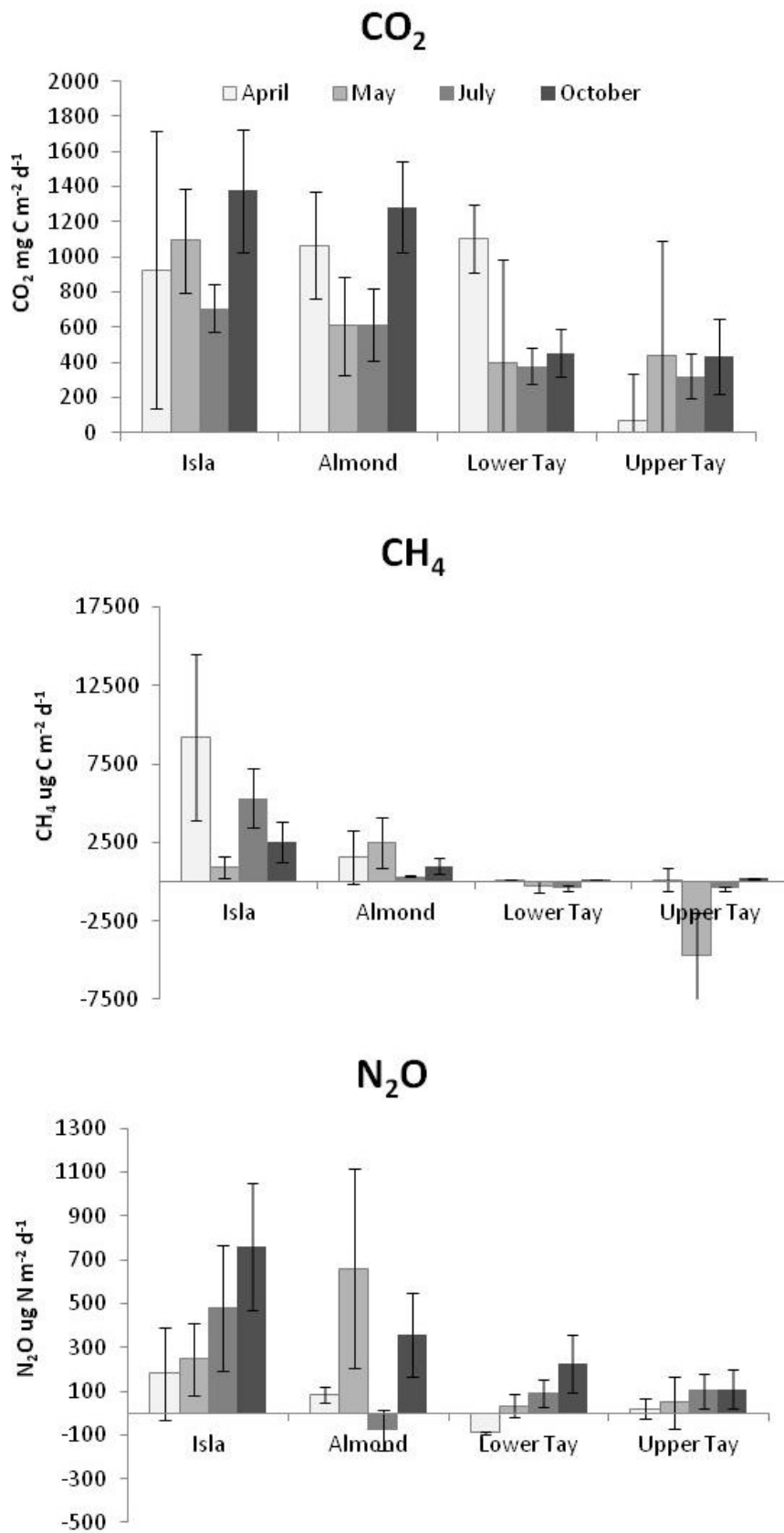


Figure (2): Results from the seasonal production assays (n=3), error bars equal ± SD

millimetres (Strauss et al., 2002) as found in the River Isla (unpublished data).

N₂O production rates also displayed substantial seasonal variation, and this variation was not the same at all sites. The relationship between N₂O production and NO₃⁻ concentrations was weaker seasonally, but in general maximum N₂O concentrations were reached at times of greatest NO₃⁻ concentration. Denitrification and N₂O production were also limited by temperature and O₂ levels which become important when the process rates are not limited by NO₃⁻.

CH₄ and CO₂

Carbon dioxide and methane production rates varied considerably both spatially and seasonally through the catchment with a range of 70.1 mg C m⁻² d⁻¹ to 1376 mg C m⁻² d⁻¹ for CO₂, and ranging from a consumption of -4.7 C m⁻² d⁻¹ to a production of 9.2 mg C m⁻² d⁻¹ for CH₄, which is within the range of values reported in the literature for various aquatic environments. For example, Trimmer et al., (2009) reported CO₂ production values considerably higher from a vegetated chalk river impacted by sediment with an annual mean efflux of 1395.4 mg C m⁻² d⁻¹ (4829 μmol m⁻² h⁻¹). Moreover, Sanders et al., (2007) reported substantially higher methane production in a chalk river in the UK with a depth integrated production rate of 216 mg C m⁻² d⁻¹.

Mean CO₂ production rates were higher in the lowland tributaries, River Isla and River Almond (mean 1026 mg C m⁻² d⁻¹, and 892 mg C m⁻² d⁻¹ respectively) compared to the lowland and upland river sites (584 mg C m⁻² d⁻¹, and 317 mg C m⁻² d⁻¹ respectively). Likewise, a similar relationship was found with CH₄ production rates, with mean production of 4510 mg C m⁻² d⁻¹, and 1358 mg C m⁻² d⁻¹ in the River Isla and Almond, and -129 mg C m⁻² d⁻¹ and -1214 mg C m⁻² d⁻¹ in the lowland Tay and upland Tay respectively.

Similarly to N₂O production, the spatial distribution of CO₂ and CH₄ production were positively related to stream water NO₃⁻ concentrations (p=0.002 and p=0.01 respectively) and properties of sediment including organic matter % (p=0.048). The type and quality of sediment is likely to play the greatest role in controlling CH₄ production, which requires both anoxic conditions and a supply of organic carbon, which were readily available in the sediments of the River Isla and seasonally in the River Almond.

The upland and lowland Tay sites however, which contained predominantly coarse sands and gravels intermixed with finer particles displayed little production and at times consumption of CH₄ was observed. This is in despite of the sediments being purged with N₂, suggesting assay bottles were either not fully anoxic, or that O₂ increased during the incubation. However, methane oxidation in river gravels has been observed elsewhere, with Trimmer et al., (2010) reporting significant oxidation in the River Lambourn, UK. This is likely to mean that the CH₄ production and consumption within river beds, is highly variable spatially and dependent on the quality and quantity of substrate. In high energy rivers like the Tay, significant CH₄ production is likely to be limited to key areas within the river channel such as backwaters where fine sediment and organic matter accumulation occurs, with the majority of the river bed providing ideal conditions for CH₄ oxidation in the sand and gravel rich river bed.

Impact of NO₃⁻ and Carbon on GHG production

The impact of carbon and NO₃⁻ additions were examined in a nutrient rich and nutrient poor site in September and November with the results of the experiment displayed in Figure 3. The experiment showed that N₂O production during late summer and autumn were NO₃⁻ limited and to various degrees carbon limited. Additions of NO₃⁻ significantly increased the production of N₂O at both sites, and although carbon addition increased production rate it was significantly lower than the rate observed with NO₃⁻ only amendment. This suggests that the sediments are predominantly N limited; however additions of C and N together significantly increased the N₂O production rate even further (approx. 2 -3 times that of NO₃⁻ only addition) suggesting at higher NO₃⁻ concentrations the sediment N₂O production becomes limited by organic carbon availability. Additionally, seasonal changes in properties of sediment and water appear to also play a part in N₂O production. Production rates of N₂O were lower in September than November in the River Isla sediment containing additions of NO₃⁻, but the opposite was true for the River Tay site. This suggests that there is an imbalance in sediment quality between September and November and that this relationship is not the same between the two sites. The results from the upper Tay site suggest that N₂O production is limited more by carbon in November than September with NO₃⁻ amendment leading to greater production in September. The opposite appears to be true for the River Isla, with greater limitation by carbon in September indicated by the greater production of N₂O in

November with the NO_3^- only amendment compared to September. Both sites are limited by NO_3^- , with greater limitation at the upper Tay site than the River Isla site. Whilst both sites are carbon limited, the River Isla site appears to have greater carbon limitation than the upper Tay, with higher production rates of N_2O in the Tay with NO_3^- only amendment compared to the Isla sediment.

The overall capacity to produce N_2O , after additions of both C and N, is greater in November than September in the River Isla, but greater in September in the upper River Tay. Moreover, the N_2O production with C+N amendment is only marginally higher in the River Isla compared to the upland Tay. This indicates that although the upland Tay site is generally NO_3^- starved, with concentration predominantly below $< 0.25 \text{ mg N l}^{-1}$, the sediment still contains the capacity for denitrification and this rate of denitrification is lower but still reasonably close to the equivalent at a NO_3^- rich site. The positive impact of carbon addition both separately and with NO_3^- suggests that highly labile DOC can enhance N_2O production, and although no relationship between N_2O and DOC was found in the seasonal study, it is possible a small labile fraction of water column DOC was an important energy source.

The impact of nutrient addition on CO_2 and CH_4 production is less obvious with considerable variability between treatments and sampling occasion. Non amended sediments produced more CO_2 in September than November in the upper Tay site but the opposite was found at the River Isla site. Moreover non amended production rates were higher in September in the upper Tay compared to the River Isla, but lower in November compared to the River Isla. Addition of NO_3^- and carbon separately increased CO_2 production in the upper Tay in November but not September. Likewise at the River Isla site, separate NO_3^- and carbon additions led to small increases in production both in September and November. However simultaneous addition of C+N had no impact on CO_2 production rates at either site.

Large variations in the CH_4 production rates made analysis of the impact of C and N difficult. Separate additions of NO_3^- and carbon had little statistical impact on production rates of CH_4 , although production rates were observed to increase with additions of both nutrients although the high standard deviation between replicate slurries made this observation unclear. Certainly in the upper Tay combined addition of

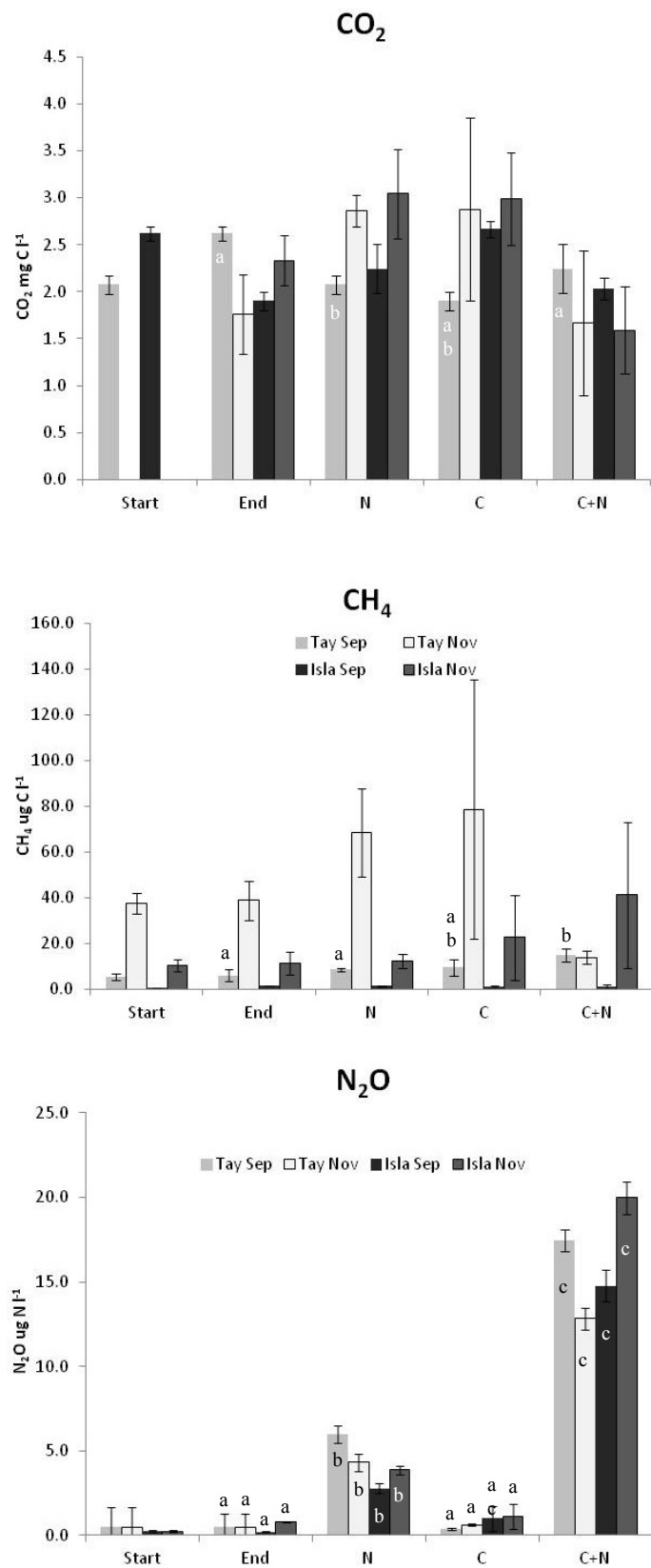


Figure 3: Nutrient amendment assay showing impact of NO_3^- , carbon (glucose), and carbon + NO_3^- . Error bars equal standard error of mean ($n=3$). Letters indicate significance of one-way ANOVA with Tukey Family Error rate ($p<0.05$)

C and N appeared to limit CH₄ production in November which was substantially reduced from the non amended sediments but this was not observed for separate additions of NO₃⁻ and C. However, in September in the upper Tay site the opposite was true with an increase in production of CH₄ with combined addition of C+N although once again large variation was observed between slurries. This was replicated in the Isla sediment in November but not in September. On the whole it remains unclear the full impact of C and N addition on CH₄ production due to the large variability.

Semi-Potential Denitrification Rate

Results from the semi-potential denitrification rate (SPDR) experiments are shown in Figure (4). In the River Isla sediments, which were incubated for SPDR in May, September and November, the overall variation was small, with denitrification rates between May and September reasonably similar with a small increase in November. SPDR was also measured for the upper Tay site in September and November, with denitrification rate remaining similar. Using the denitrification rate, N₂O yields can also be estimated by comparing denitrification rate with N₂O production rate (Weier et al., 1993). N₂O yield was evenly matched between sampling months and with only small variation between sites with yields from the upper Tay of 5.1 and 5.2 % and for the River Isla between 5.5 and 6.3 %. These are within the range reported in the literature of 0.1 – 6.0 % (Seitzinger 1988; Mulholland et al., 2008) but much lower than a recent study with a range of 0.01 to 239 % (Beaulieu et al., 2009). Variation between sites in N₂O yield is thought to an important factor in creating variation in N₂O production between different sites within river and streams (Beaulieu et al., 2009) other than that explained by substrate variability. It is possible that the small, but perhaps important variation of 1 % yield between the sites could explain the differences found between the sites in the other experiments.

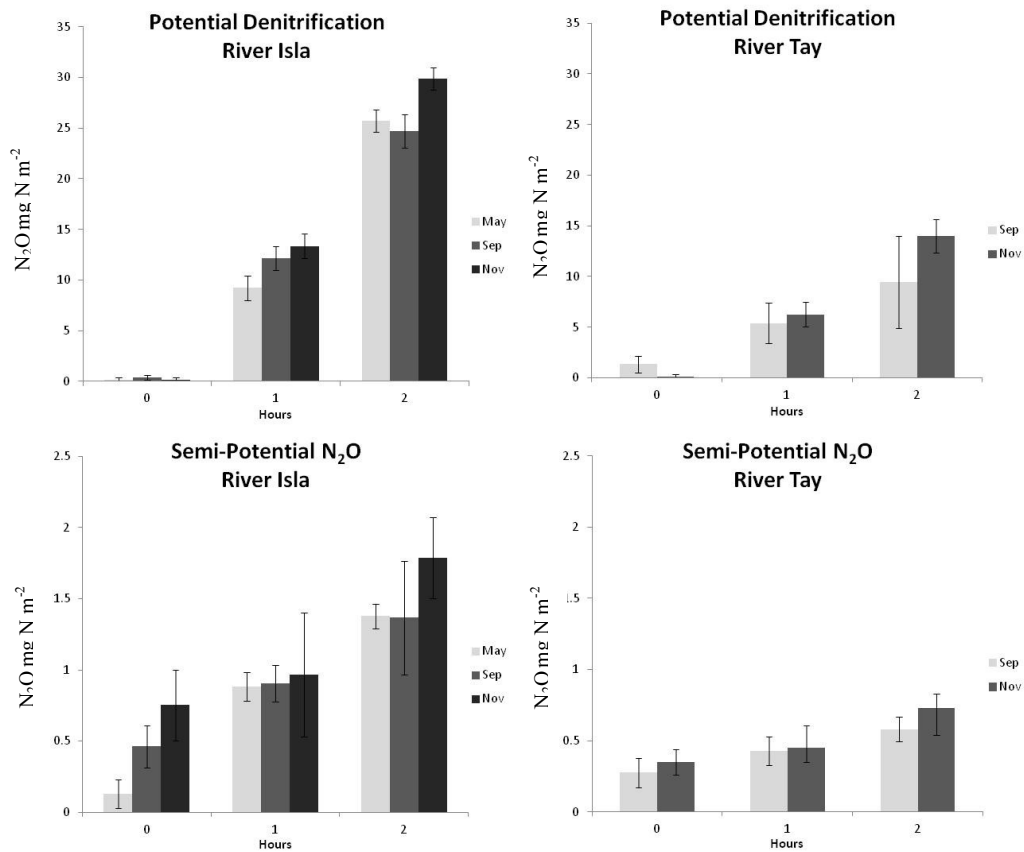


Figure 4: Semi-Potential Denitrification rates and Semi-Potential N₂O production rates in the River Isla and Upper River Tay. Error bars equal SD (n=3).

Experiment Overview

A key assumption of the SPDR and the estimated N₂O yield is that identical denitrification rates exist in both the control slurries and acetylene amended slurries which is important to calculate an accurate N₂O yield. A key problem with sediment slurry incubations is that slurries cannot be fully and reliably homogenised, and particles or clumps of organic material can be transferred to one slurry sub-sample and not others resulting in a potential bias in production rates (Arango et al., 2007). The denitrification rates experiments and seasonal rate experiments were designed to capture the initial rate of production, since confinement can alter reaction rates during incubation (Garnier et al., 2010). Moreover, whilst the slurry experiments were intended to replicate the broad *in-situ* conditions, they likely provide an environment for production that could possibly be higher than *in-situ* conditions. With ‘slurried’ sediments, carbon is approximately mixed to an even distribution providing an increase in carbon to areas of sediments that were initially lacking. Moreover the anoxic conditions induced by flushing with N₂,

which is commonly employed in sediment slurry experiments, likely enhances the sedimentary environment for certain microbial processes, such as denitrification.

Furthermore, the potential denitrification rates cannot be described as a true environmental rate as carbon could be a limiting factor, but the method does allow for analysis of the impact of sediment quality changes between sites in a river system (Garnier et al., 2010). Another problem, regardless of the specific denitrification estimation method, is the wide range of uncertainties in production rates (Garcia Ruiz et al., 1998; Groffman et al. 2006; Beaulieu, 2008, 2009; Garnier et al., 2010) which can also be true for N₂O *in-situ* production rates (Beaulieu, Arango and Tank, 2009; Garnier et al., 2010). For example, Beaulieu, Arango and Tank (2009) found substantial variation in replicate assays average 53% coefficient of variation for denitrification and 96 % for *in-situ* N₂O production assays. Variation was also significant in this study between replicates, with coefficient of variation for denitrification assays ranging from 6.2 – 62.4 % and for the seasonal production assays between 8.16 and 647 %, with a mean of 79.8 %. The large amount of variation highlights the uncertain nature of estimating gas production rates especially where the production rates are low.

However, despite certain deficiencies, the slurry technique does allow for a broad understanding of the sediment processing of GHGs in a river catchment. In the case of this study, it is clear that there are large spatial and temporal variations in the production rates through the river continuum. The spatial changes are likely to mainly driven by the relative ‘quality’ of sediment as a substrate for microbial production. For instance, fine grained sediment rich in organic matter is likely to be a greater source of GHGs than coarse sandy sediment with little organic content. Therefore the type and quality of sediment is likely to be a primary driver in gas production, with percentage organic matter and the overall lability of carbon an important controlling factor. This was highlighted by the nutrient addition experiments, with no statistical difference in production of CO₂ and CH₄ at either the nutrient poor upland site or nutrient rich lowland site that both contained fine sediments with relatively high percentage organic matter. N₂O production is controlled both by the sediment quality as well as the spatial changes in N concentrations driven by changes in landuse, highlighted by strong correlations with NO₃⁻ concentrations and % LOI. Therefore the sediment production of GHGs is likely driven by the physical processes that control sediment distribution and sedimentation in river catchments and delivery of dissolved nutrients.

References

- Arango CP, Tank JL, Schaller JL, Royer TV, Bernot MJ, David MB. 2007 Benthic organic carbon influences denitrification in streams with high nitrate concentration. *Freshw Biol.* **52**:1210–22.
- Baker A, Cumberland S, Hudson N. 2008. Dissolved and total organic and inorganic carbon in some British rivers. *Area* **40**: 117-127
- Battin, T. J., L. A. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, A. I. Packman, J. D. Newbold, and F. Sabater (2008), Biophysical controls on organic carbon fluxes in fluvial networks, *Nat. Geosci.*, **1**, 95– 100, doi:10.1038/ngeo101.
- Beaulieu, J.J., C.P. Arango, S.K. Hamilton, and J.L. Tank. 2008. The production and emission of nitrous oxide from headwater streams in the Midwestern USA. *Glob. Change Biol.* **14**:878–894.
- Beaulieu J.J, Arango C.P, Tank J.L. 2009. The effects of season and agriculture on nitrous oxide production in headwater streams. *J. Environ. Qual.* **38**: 637-646
- Billett MF, Palmer SM, Hope D, Deacon C, Storeton-West R, Hargreaves KJ, Flechard C, Fowler D. 2004. Linking land-atmospherestream carbon fluxes in a lowland peatland system. *Global Biogeochemical Cycles* **18**: GB1024, DOI:10.1029/2003GB002058.
- Cole JJ, Caraco NF. 2001. Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. *Marine and Freshwater Research* **52**: 101–110
- Dawson JJC, Smith P. 2007. Carbon losses from soil and its consequences for land-use management. *Science of the Total Environment* **382**: 165–190.
- Dawson JC. Soulsby C. Hrachowitz M. Speed M. Tetzlaff D. 2009. Seasonality of epCO₂ at different scales along an integrated river continuum within the Dee Basic, NE Scotland. *Hydrological Processes* **23**, 2929 – 2942
- De Klein C, Novoa RSA, Ogle S, Smith KA, Rochette P, Wirth TC, McConkey BG, Mosier A, Rypdal K, Walsh M et al.: Chapter 11: N₂O emissions from managed soils, and CO₂ emissions from lime and urea application. 2006 *IPCC Guidelines for National Greenhouse Gas Inventories*. 2007:1 54.
- Finlay JC. 2003. Controls of streamwater dissolved inorganic carbon dynamics in a forested watershed. *Biogeochemistry* **62**: 231–252
- Fuss, C.L., and L.A. Smock. 1996. Spatial and temporal variation of microbial respiration rates in a blackwater stream. *Freshwater Biol.* **36**:339–349.
- García-Ruiz R, Pattinson SN, Whitton BA. 1998. Kinetic parameters of denitrification in a river continuum. *Appl Environ Microbiol* **64**:2533–8
- García-Ruiz R, Pattinson SN, Whitton BA. 1999. Nitrous oxide production in the River Swale-Ouse, North-East England. *Wat.Res.* **33**:1231–37
- Garnier, J., Billen, G., Vilain, G., Martinez, A., Silvestre, M., Mounier, E., and Toche, F.

2009. Nitrous oxide (N₂O) in the Seine river and basin: observations and budgets, *Agr. Ecosyst. Environ.*, **133**: 223–233
- Garnier, J. A., Mounier, E. M., Laverman, A. M., and Billen, G. 2010. Potential denitrification and nitrous oxide production in the sediments of the Seine river drainage network (France), *J. Environ. Qual.*, **39**: 449–459
- Groffman PM, Altabet MA, Bohlke JK, Butterbach-Bahl K, David MB, Firestone MK, et al. 2006. Methods for measuring denitrification: diverse approaches to a difficult problem. *Ecol Appl* **16**:2091–122
- Houghton, R. A. and Hackler, J. L. 2001. Carbon flux to the atmosphere from land-use changes: 1850 to 1990. ORNL/CDIAC 131, NDP-050/R1. *Carbon Dioxide Information Analysis*
- Inwood, S.E., J.L. Tank, and M.J. Bernot. 2005. Patterns of denitrification associated with land use in nine midwestern headwater streams. *J. N. Am. Benthol. Soc.* **24**:227–245.
- IPCC. 2007. Summary for policymakers, in: *Climate Change, 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., Miller, H. L., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2-18, 2007.
- Ivens WPMF, Tysmans DJJ, Kroeze C, Lohr AJ, Van Wijnen J. 2011. Modelling global N₂O emissions from aquatic systems. *Current Opinion in Environmental Sustainability*.
- Jones JB Jr., Stanley EH, Mulholland PJ. 2003. Long-term decline in carbon dioxide supersaturation in rivers across the contiguous United States. *Geophysical Research Letters*
- Kaplan, L.A., and J.D. Newbold. 2003. The role of monomers in stream ecosystem metabolism. p. 97–119. In S.E.G. Findlay and R.L. Sinsabaugh (ed.) *Aquatic ecosystems: Interactivity of dissolved organic matter*. Academic Press, Amsterdam
- Mayorga, E. et al. Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers. *Nature* **436**: 538-541
- Klein CAM de, Novoa RSA, Ogle KA, Rochette P, Wirth TC, McConket BG, Mosier A, Rypdal K, Walsh M. 2007. Chapter 11: N₂O emissions from managed soils, and CO₂ emission from lime and urea application. In: *2006 IPCC guidelines for national greenhouse gas inventories*.
- Mosier, A., C. Kroeze, C. Nevison, O. Oenema, S. Seitzinger, and O. van Cleemput. 1998. Closing the global N₂O budget: Nitrous oxide emissions through the agricultural nitrogen cycle- OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. *Nutr. Cycling Agroecosyst.* **52**:225–248.
- Mulholland PJ, Helton AM, Poole GC, Hall Jr RO, Hamilton SK, Peterson BJ, et al. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature* **452**:202–6.
- Pattinson, S.N., Garcia-Ruiz, R., Whitton, B.A., 1998. Spatial and seasonal variation in denitrification in the Swale-Ouse system, a river continuum. *Science of the Total Environment* **210** (1–6), 289–305

- Pina-Ochoa E, Alvarez-Cobelas M. 2006. Denitrification in aquatic environments: a cross-system analysis. *Biogeochemistry* **81**:111–30.
- Richardson W.B., Strauss E.A., Bartsch L.A., Monroe E.M., Cavanaugh J.C., Vingum L. And Soballe D.M. 2004. Denitrification in the upper Mississippi River: rates, controls, and contribution to nitrate flux. *Can. J. Fish. Aquat. Sci.* **61**: 1102–1112.
- Royer T.V., Tank J.L. and David M.B. 2004. Transport and fate of nitrate in headwater agricultural streams in Illinois. *J. Environ. Qual.* **33**: 1296–1304.
- Sanders IA, Heppell CM, Cotton JA, Wharton G, Hildrew AG, Trimmer M.2007. Emission of methane from chalk streams has potential implications for agricultural practices. *Freshw Biol* **52**:1176–86.
- Seitzinger SP (1988) Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol Oceanogr* **33**:702–724.
- Seitzinger, S. P., C. Kroeze, AND R. Styles. 2000. Global distribution of N₂O emissions from freshwater and coastal marine systems: Natural emissions and anthropogenic effects. *Chemosphere—Global Change Science* **2**:267–279.
- Strauss, E.A., N.L. Mitchell, and G.A. Lamberti. 2002. Factors regulating nitrification in aquatic sediments: Effects of organic carbon, nitrogen availability, and pH. *Can. J. Fish. Aquat. Sci.* **59**:554–563.
- Teodoru CR, del Giorgio PA, Prairie YT, Camire M. 2009. Patterns in pCO₂ in boreal streams and rivers of northern Quebec, Canada. *Global Biogeochemical Cycles* **23**: GB2012, DOI:10.1029/2008GB003404.
- Trimmer M, Hildrew AG, Jackson MC, Pretty JL, Grey J. 2009. Evidence for the role of methane-derived carbon in a free-flowing, lowland river food web. *Limnol Oceanogr* **54**:1541–7
- Trimmer M, Maanoja S, Hildrew AG, Pretty JL, Grey J.2010. Potential carbon fixation via methane oxidation in well-oxygenated riverbed gravels. *Limnol Oceanogr*; **55**:560–8
- Wall LG, Tank JL, Royer TV, Bernot MJ. 2005. Spatial and temporal variability in sediment denitrification within an agriculturally influenced reservoir. *Biogeochemistry* **76**: 85-111
- Weier KL, Doran JW, Power JF, Walters DT. 1993. Denitrification and the Dinitrogen/Nitrous Oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Sci Soc Am J* **57**: 66-72.

7. Overview of Results and General Discussion:

This section of the thesis provides an overview of the results, drawing together the main conclusions in a discussion on the production and emission of GHGs in the River Tay catchment from source to sea.

7.1. Hotspots of emission of GHG's from source to sea in the River Tay catchment : Spatial Variation

A cross-system comparison of mean emission density (as CO₂ equivalents) from source to sea (including the 9 sites within the freshwater catchment and 10 sites within the estuary) are displayed in Fig. 1. The spatial variation in GHG saturation and emission density strongly reflected both the landuse within the freshwater catchment (Paper II) and the primary controlling factors within the Estuary (Paper I).

In the freshwater river, both saturations and flux densities of CO₂ and N₂O were highly positively correlated to landuse and nutrient load with greater saturations at the downstream lowland sites (e.g. Site 7: R. Isla, Fig. 1), where agricultural landuse becomes increasingly prevalent. This result is consistent with other studies on N₂O production and emission (Garcia-Ruiz et al., 1999, Beaulieu et al., 2009). Moreover, the link between N₂O and the proportion of agriculture landuse has been observed in groundwaters and drainage water with often exceptionally high N₂O concentrations recorded (Hasegawa et al., 2000; Hiscock et al., 2003) and although this has been observed to de-gas quickly (Reay et al., 2003), agricultural soils are still likely to provide a substantial input to river N₂O concentrations at the catchment scale.

The spatial controls of fluxes and saturation of CH₄ on the other hand appear more complex, with saturations generally higher in the upper river (Site 3: 2185 %) than the lower river (Site 9: 915%), with the exception of the high fluxes in the lowland tributary River Isla (6649 %). The high fluxes in the upper river most likely relate to higher inputs from surrounding terrestrial soils, with the influence of peatlands and peaty soils particularly important in upland catchments (Jones and Mulholland 1998 etc) and the upper Tay catchment has significant areas of peatland (Paper II). Higher CO₂ saturations were also expected in the upland river sites, linked with soil water inputs from peaty soils, as found in other studies in upland environments (Hope et al., 2004).

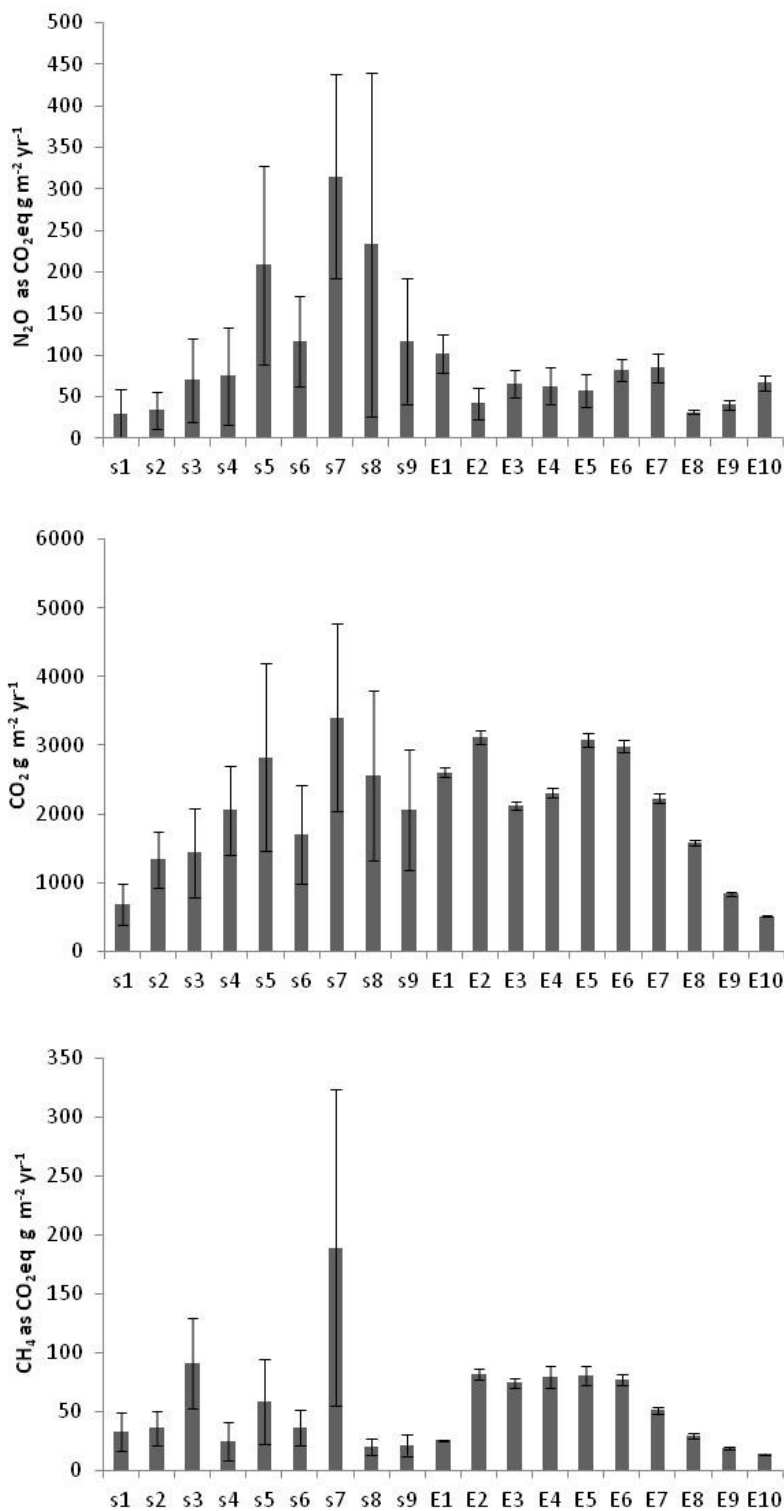


Figure 1. Mean (\pm standard deviation) emission density of N_2O , CO_2 and CH_4 from source to sea in the River Tay catchment reported in CO_2 equivalents ($n= 276$). Site nomenclature as in Chapters 3 and 4 i.e. sites S1-S9 are freshwater and E1-E10 are in the estuary. Flux densities for the estuary are reported as the mean from the 4 parameterizations used in Paper I.

Instead CO₂ increased through the main-stem river, influenced perhaps by high saturations in the main lowland tributaries River Almond and River Isla. However it is likely that the spatial coverage of sampling sites in the upper catchment does not truly capture the potential flux hotspots of CO₂ and CH₄. These hotspots are likely to be higher in the catchment in smaller rivers and streams where peaty organic soils dominate the terrestrial zone. Soil water inputs in these small upland catchments are known to be supersaturated in CO₂ and CH₄. Once in the stream or river this quickly degasses with emission decreasing with distance from source (Hope et al., 2004)

Figure 1 highlights the importance of hotspots in emission of GHG in the freshwater Tay catchment with Loch of Clunie (S5) and the lowland tributaries (S7 and S8) having substantially higher flux rates of N₂O and CO₂. Whilst the upper river (S3) had important fluxes of CH₄, the loch site (S5) and River Isla (S7) were also important hotspots of emission for this gas.

The spatial variability of GHG concentrations and fluxes in the estuary were likely to be predominantly controlled by the balance between lateral inputs (from tidal flushing of surrounding intertidal areas) *in-situ* microbial production/consumption (both in the water column and bed sediments) and physical mixing/loss processes. The importance of each of these varied spatially and temporally. In the upper estuary, tidal flushing and *in-situ* production were probably the greatest source for increases in GHG. However in the maximum turbidity zone in the middle estuary, where strong hydrodynamics are prevalent and sediment is re-suspended, GHG concentrations varied substantially. Whilst CH₄ was, on occasion, dramatically reduced in this section of the estuary, most likely through intense microbial oxidation, both CO₂ and N₂O saturations showed peaks indicating increased microbial processing which is a common feature in many other macro-tidal estuaries (Borges and Abril 2011).

Although in Figure 1 N₂O flux density does not increase substantially through the estuary compared to the main river sites, this may in fact be a product of the significant morphological and hydrological changes that are taking place in the estuary as surface area substantially increases and river water is diluted by sea water. Results from Paper I indicate that the amount of N₂O emitted to the atmosphere is 10 times greater in the middle estuary (887.8 kg N yr⁻¹; sites E5, E6 and E7) compared to the upper estuary/river (84.7 kg N yr⁻¹; sites E1,E2,E3,E4) despite little change in the flux density (Figure 1). This indicates that the middle estuary/maximum turbidity zone is both a hotspot of production and emission of CO₂ and N₂O in the estuary and the catchment on

the whole. Another flux hot spot occurred towards the mouth of the estuary (E10; Figure 1), where flux density and emission of N₂O increased substantially compared to sites immediately prior (E8 and E9 Figure 1). This may be the result of further N processing, fuelled by nutrient pollution from the city of Dundee which has been noted to impact on water quality in the area (SEPA unpublished data).

7.2 Hotspots of emission of GHG's from source to sea in the River Tay catchment : Temporal and Seasonal Variation

Seasonal variation in the river:

Seasonal CO₂ saturations and emissions across the catchment were in general highly variable but with peaks in spring/early summer and in late summer and autumn. Results from other studies from rivers and streams have been varied, with some exhibiting CO₂ concentrations highest in late autumn and winter when photosynthesis is reduced (Neal et al., 1998; Dawson et al., 2001; 2009), or in the summer linked to low discharge and high DOC concentrations (Raymond et al., 2000) or with no clear seasonal patterns (Neal et al., 1998; Dawson et al., 2004). The seasonal patterns across the Tay are likely to result from the changing balance between heterotrophy and autotrophy in the system. Seasonal peaks in spring and early summer in the freshwater catchment likely relate to temperature related increases in microbial production and respiration. The observed reduction through the summer could result from increases in autotrophic processing with CO₂ reduced through uptake for photosynthesis. Following cooling and shorter day lengths in autumn, photosynthesis and primary production reduces, and this leads to the autumn CO₂ peaks, possibly fuelled by increasing loading from run-off from the catchment linked to higher rainfall.

Methane saturation also varied considerably across the catchment, but in general values were higher in early summer and autumn. In the upper catchment this is likely to be linked to changes in temporal changes in flow paths in the upland soils linked with temperature related seasonal production increases. The large peaks in autumn most likely result from changes in rainfall inducing flushing of peaty soils, exporting soil waters supersaturated in CH₄ to the upland rivers.

Nitrous oxide saturation and emission in the freshwater catchment generally showed

peaks in early spring, and late summer/autumn across the freshwater catchment with the peaks most pronounced in the lowland sites affected by agriculture. Peaks in spring and autumn are likely controlled by surface water and floodwater flushing from agricultural soils, that often do not contain crops in early spring (which is also the key season for fertiliser application) and autumn, washing both dissolved and particulate nutrients (N, P, OM) into river catchments (Kortelainen et al., 1997). This possibly stimulates in-stream production of N₂O when ambient temperatures are not limiting. However, agricultural soils are also known to be super-saturated in N₂O, especially in spring after winter thawing (Syväsalo et al., 2004) and through late summer/autumn (Robinson et al., 1998; Cole and Caraco 2001).

Seasonal variation in the estuary:

The Tay estuary shows similar seasonal patterns in gas saturations compared with other European estuaries, reflecting common controls of production and loss processes (Borges and Abril 2011). Methane showed the greatest seasonality with saturations peaking in summer in the freshwater upper estuary. The summer peaks were likely related to temperature related controls on production both in bed sediments but also in intertidal muds and marshes. Tidal flushing of these inter-tidal areas is most likely responsible for the increases in CH₄ in the upper estuary. Short term temporal analysis showed that CH₄ concentration increased with the falling tide as tidal floodwater ebbed from the marshy banks of the upper estuary. Concentrations of CO₂ and N₂O were also generally higher in the upper to middle estuary in summer, although seasonality was less pronounced compared to CH₄. However, increases in these two gases are likely to be controlled by not only tidal flushing and *in-situ* benthic production, but also production in the maximum turbidity zone. These areas are known to have intense microbial activity, linked to resuspension of sediment and increases in nutrient levels, with many estuaries displaying peaks in CO₂ and N₂O in this mid-region (Barnes and Upstill-Goddard, 2011)

7.3 Greenhouse gas production in the freshwater River Tay Catchment:

To further understanding of the controls on GHG saturation and emission across the catchment a number of laboratory experiment were conducted to ascertain if production

was ongoing in the sediments in both in the river and study lakes, also to see if the production varied seasonally, and if production rates were affected by supply of nutrients.

7.3.1 Production in Loch Sediments

The production of the greenhouse gases CH₄, CO₂ and N₂O in a temperate, lowland lake shows strong seasonal changes with the production, of N₂O, in particular, also impacted by both NO₃⁻ and carbon concentrations in the sediments (Chapter 5: Paper III). Sediments were collected during spring, summer and late autumn from a small Scottish loch in the River Tay catchment (Loch of Clunie) and incubated in the laboratory at in-situ temperature. Gas production varied seasonally, with greatest production of CH₄ in the summer, highly correlated to temperature. CO₂ production was greatest in summer and in autumn, with lowest production rates in spring. N₂O had highest production rates in spring and summer with no detectable production in late autumn. Semi-potential denitrification rate experiments and semi-potential N₂O production rate experiments showed that sediments supported higher production rates when both NO₃⁻ and temperature were raised to non-limiting levels.

Nitrate additions to the spring sediment had a strong impact on N₂O production, but no effect on either CH₄ or CO₂. Carbon additions, as glucose, to the summer sediments had a strong impact on CH₄ and CO₂ production but little impact on N₂O production. However, additions of both glucose and NO₃⁻ led to a substantial increase in N₂O production, but little additive effect on CH₄ and CO₂ production compared to the glucose only addition.

Sediments were also collected and incubated from a chain of lochs (including Loch of Clunie) in late autumn along a known nutrient gradient (Chapter 5). CO₂ production was observed to increase along with water column nutrient concentrations. However there was no statistically significant increase in production of either N₂O or CH₄. This may have been due to relatively low temperatures limiting production. Potential denitrification experiments (Chapter 5: Paper III) showed N₂O production increased along the loch chain highlighting the importance of nutrient loading from catchment agriculture to GHG production in lochs.

7.3.2 Production in River Sediments

Spatial and Temporal Variability in GHG production

N₂O

Sediments were collected from four sites across the freshwater river and incubated at *in-situ* temperature. N₂O production rates (Paper IV) varied considerably both spatially through the catchment and seasonally ranging from 0 to a maximum of 1.85 mg N m⁻² d⁻¹, which is within the range reported in a literature review for rivers, lakes and estuaries (0.02 - 3.36 mg N m⁻² d⁻¹) (Seitzinger, 1988). However, these were considerably lower than the maxima reported in a comprehensive study of rivers in the USA with Beaulieu et al., (2009) reporting a range of ~0.01 to 77 mg N m⁻² d⁻¹ with a mean annual production rate exceeding 0.96 mg N m⁻² d⁻¹. This likely reflects the difference in nutrient status with many of the sites studied in Beaulieu et al., (2009) having high NO₃⁻ availability, whereas the opposite was true for the sites along the River Tay.

Spatially, N₂O production rates were higher in the lowland tributaries compared to the main river-stem sites in the upper and lower River Tay. We predicted that spatial changes in N₂O production would correlate with the variables that control denitrification which includes NO₃⁻, and organic carbon availability. In line with this prediction, NO₃⁻ concentration was significantly related to N₂O production which is consistent with studies on other river systems (Garcie-Ruiz et al., 1999; Beaulieu et al., 2009). Accordingly both the NO₃⁻ concentrations and N₂O production rates were significantly correlated to the proportion of catchment agriculture which increased downstream, and was substantially higher in the lowland tributaries. However whilst % organic carbon within sediments was significantly related to N₂O production, DOC within stream water was not, suggesting that organic carbon concentrations within sediments are of greater importance than that dissolved in the water column, which may not be so labile.

N₂O production rates also displayed substantial seasonal variation, and this variation was not the same at all sites. The relationship between N₂O production and NO₃⁻ concentrations was weaker seasonally, but in general maximum N₂O production was reached at corresponding times of high NO₃⁻ concentration. Denitrification and N₂O production were also limited by temperature and O₂ levels which become important when the process rates are not limited by NO₃⁻. This may explain why higher N₂O production rates are observed in summer rather than in spring in the River Isla, with

similar NO_3^- concentrations temperature became the limiting factor. Other studies have found N_2O production to be highly connected to seasonal changes in temperature, with greater production in the summer months (García-Ruiz, Pattinson and Whitton 1998). However, seasonal changes in sediment quality are likely also to be a key driver in these production rates with the quantity and quality of organic matter an important controlling factor as found in N_2O production rates in a range of streams in the U.S (Inwood, Tank and Bermot, 2005).

CH_4 and CO_2

Carbon dioxide production rates varied considerably both spatially and seasonally through the catchment with a range of $70.1 \text{ mg C m}^{-2} \text{ d}^{-1}$ to $1376 \text{ mg C m}^{-2} \text{ d}^{-1}$. Few studies have measured the direct flux of CO_2 from river sediments, with the majority of studies inferring carbon respiration indirectly from changes in oxygen concentrations (Johnson et al., 2010). Recently, Trimmer et al., (2009) reported CO_2 production values considerably higher from a vegetated chalk river impacted by sediment with an annual mean efflux of $1395.4 \text{ mg C m}^{-2} \text{ d}^{-1}$ ($4829 \text{ } \mu\text{mol m}^{-2} \text{ h}^{-1}$). Mean CO_2 production rates were higher in the lowland tributaries, River Isla and River Almond (mean $1026 \text{ mg C m}^{-2} \text{ d}^{-1}$, and $892 \text{ mg C m}^{-2} \text{ d}^{-1}$ respectively) compared to the lowland and upland river sites ($584 \text{ mg C m}^{-2} \text{ d}^{-1}$, and $317 \text{ mg C m}^{-2} \text{ d}^{-1}$ respectively).

Methane production rates also varied considerably both spatially and seasonally, ranging from a consumption of $-4.7 \text{ C m}^{-2} \text{ d}^{-1}$ to a production of $9.2 \text{ mg C m}^{-2} \text{ d}^{-1}$ for CH_4 . There are few production rates in the literature to compare those from the Tay but recently Sanders et al., (2007) reported substantially higher CH_4 production rates in a chalk river with a depth integrated production rate of $216 \text{ mg C m}^{-2} \text{ d}^{-1}$. A similar spatial relationship to CO_2 was found with CH_4 production rates, with mean production of $4510 \text{ mg C m}^{-2} \text{ d}^{-1}$, and $1358 \text{ mg C m}^{-2} \text{ d}^{-1}$ in the River Isla and Almond, and $-129 \text{ mg C m}^{-2} \text{ d}^{-1}$ and $-1214 \text{ mg C m}^{-2} \text{ d}^{-1}$ in the lowland Tay and upland Tay respectively.

CO_2 and CH_4 production were positively related to stream water NO_3^- concentrations and properties of sediment, including % organic matter. The type and quality of sediment is likely to play the greatest role in controlling CH_4 production, which requires both anoxic conditions and a supply of organic carbon, which were readily available in the

sediments of the River Isla and seasonally in the River Almond. The upland and lowland Tay sites however, which contained predominantly coarse sands and gravels intermixed with finer particles displayed little production and at times consumption of CH₄ was observed. However, methane oxidation in river gravels has been observed elsewhere, with Trimmer et al. (2010) reporting significant oxidation in the River Lambourn, UK. CH₄ production and consumption within river beds is highly variable spatially and most likely dependent on the quality and quantity of substrate. In high energy rivers, like the Tay, significant CH₄ production may be limited to key areas within the river channel such as backwaters where fine sediment and organic matter accumulation occurs, with the majority of the main river bed providing ideal conditions for CH₄ oxidation in the sand and gravel rich river bed. For example, Trimmer et al., (2010) found significant production of CH₄ in the sediment accumulation areas in the lee of rooted macrophytes or in marginal depositional areas, but the gravel zones of the river were acting as a significant sink for CH₄ (Sanders et al., 2007; Trimmer et al., 2010).

Controls of GHG production and Potential Production Rates

In addition to the investigation of spatial and temporal production rates of GHG's from the River Tay, further experiments were conducted from sediments from two locations within the River Tay catchment, both of which contained fine grained sediment, but with one site being in the nutrient poor upland river (upland Tay sediment), and the other a nutrient rich lowland river (River Isla sediment). This was primarily to ascertain if there was a difference in GHG processing between the two sites, and also whether the controls differed, with the hypothesis being that the upland sediments would be inherently less able to produce N₂O and possibly CO₂, even when nutrient concentrations and temperature were both raised. The results indicate that semi-potential denitrification rates were higher in the River Isla sediment than the upland Tay sediment both in September and November. Furthermore, semi-potential rates of N₂O production were also greater in the River Isla sediment than the Upland Tay sediment.

The difference between semi-potential denitrification rate and semi-potential N₂O production rate is known as the N₂O yield (Weier et al., 1993) and is known to be an important factor in contributing to variance in N₂O production rates from denitrification. In general, higher yields were found in the River Isla which varied from 5.5 to 6.3 % compared to 5.1 -5.2 % in the upper Tay.

These results on the whole indicate that the sediments in the River Isla function more effectively in relation to the production of N_2O . Since organic matter content was similar between the two sites it is unlikely to drive the difference in production rates. Alternative explanations include greater sediment pore-water concentrations of N increasing the production rates in the River Isla, although NO_3^- concentrations in the experiments were set high to minimise this possibility. Alternatively, differences in the type and quality of organic carbon availability could be responsible for fuelling greater production rates, or even possibly distinct differences in the established microbial communities that exist at the two sites.

The impact of carbon and NO_3^- additions to sediment GHG production from the two sites was also studied to ascertain if the influence of C and N addition was the same between sites and months. Additions of NO_3^- significantly increased the production of N_2O at both sites, and although carbon addition increased production rate it was significantly lower than the rate observed with NO_3^- amendment. This suggests that the sediments are predominantly N limited; however additions of C and N together significantly increased the production rate even further (approx. 2 -3 times that of NO_3^- only addition) suggesting at higher NO_3^- concentrations the sediment N_2O production becomes limited by organic carbon lability. Beaulieu et al., (2009) also found that both NO_3^- and carbon limit N_2O production in river sediments in a nutrient amendment experiment. Furthermore, they found the degree of NO_3^- limitation was not associated with ambient stream water NO_3^- concentration.

Seasonal changes in properties of sediment and water also appear to play a part in N_2O production. Production rates of N_2O were lower in September than November in the River Isla sediment containing additions of NO_3^- , but the opposite was true for the River Tay site. This suggests that there is an imbalance in sediment quality between September and November and that this relationship is not the same between the two sites. The overall capacity to produce N_2O , after additions of both C and N, is greater in November than September in the River Isla, but greater in September in the upper River Tay. The positive impact of carbon addition both separately and with NO_3^- suggests that highly labile DOC can enhance N_2O production, and although no relationship between N_2O and DOC was found in the seasonal study, it is possible a small labile fraction of water column DOC was an important energy source. Moreover, the large impact of C+N addition in the upper Tay site in September, greater than the River Isla production rate

with C+N, challenges the previous assertion that the upper Tay sites may be less suited to N₂O production than the River Isla, indicating the lability of carbon within the sediments is likely to be an important controlling factor.

The impact of nutrient addition on CO₂ and CH₄ production is less obvious with considerable more variability between treatments and sampling occasion. This was especially the case with CH₄ production with the results indicating on the whole that production rates were not influenced by concentrations of additional C and N, although substantial variation between replicates made it impossible to fully understand the CH₄ processing. Whilst the non-amended rates of CO₂ production were significantly different between sites, higher in the River Isla sediment the impact of NO₃⁻ and carbon was less clear. Whilst separate additions of C and N increased CO₂ production rates in the River Isla in September and November this was not observed at the upper Tay site with an increase in November but not September. However, additions of C and N together resulted in no significant change in production from the control production rates either in the River Isla sediments or the upland Tay sediments.

7.3.3 Sources of GHG in the River Tay and relation between GHG production and emission

The patterns in GHG production at the hotspot locations in the Tay catchment (agricultural rivers, and lochs) matched reasonably well the temporal changes in gas saturations and fluxes. However, whilst the slurry experiments were intended to replicate the broad *in-situ* conditions, they likely provide an environment for production that could possibly be higher than *in-situ* conditions. With ‘slurried’ sediments, carbon is approximately mixed to an even distribution providing an increase in carbon to areas of sediments that were initially lacking. Moreover the anoxic conditions induced by flushing with N₂, which is commonly employed in sediment slurry experiments, likely enhances the sedimentary environment for certain microbial processes, such as denitrification. Whilst it is known that there is a steep oxygen gradient in the fine sediments, both in Loch of Clunie and in some of the river sites (unpublished data), it is not known if this exists in the coarser sands and gravels that are interspersed with finer sediments. Therefore, other processes that require oxygenated conditions could have

been limited, potentially meaning that experiments could have under/over estimated production rates, for example missing out on nitrification of NH_4^+ and subsequent production of N_2O , or decreases in CH_4 linked to oxidation processes in gravels.

Disparity between production and emission has been observed elsewhere with Beaulieu et al., (2009) finding production rates measured from slurry assays 120% of the aerial emissions from the same rivers. The production rates of all three gases were lower than the emission rates in the rivers, especially in the lowland main-stem and upper main-stem Tay sites. On the whole this is because for much of the River catchment in-stream production is only one element that controls the dissolved concentration of GHG's, with input of groundwaters, soil waters and *in-situ* consumption and loss processes also important. The results from the main-stem river highlight that *in-situ* production of gases are very low. This likely highlights that for much of the main-stem river, that either there is very little production of CH_4 or N_2O or that the production is spatially highly variable, limited to hotspot locations with fine sediment accumulation. The majority of the main stem river has a rocky bed with pockets of sands and gravels interspersed, with the mean water velocity remaining high throughout the main river (Paper II). The concentrations of gases within the main-stem river are therefore likely to be highly influenced by the main tributaries and inputs from ground and soil waters. For example the concentrations of GHG, especially CH_4 and N_2O , at Perth are likely influenced by the input of gas rich waters from the River Isla and River Almond.

In the upper catchment allochthonous sources are likely to dominate river GHGs sources, with supersaturated soil waters likely to play a strong role in controlling GHG concentrations as found in other studies (Dawson et al., 2009). The strength of the allochthonous source in the upper catchment is dependent of on the terrestrial-river connectivity, i.e. the volume and GHG saturation of terrestrial waters compared to that in the surface waters (Griffiths et al., 2007). Progressively downstream through the river continuum, the importance of allochthonous derived GHGs is likely to be reduced, as surrounding terrestrial soils become more freely drained and less responsive. For example, Dawson et al., (2009) found that CO_2 concentrations decreased through the River Dee catchment (NE Scotland) linked to changes in the temporal and spatial connectivity of the terrestrial system and increases in autotrophic processing.

In the lowland tributaries, the broad increases in emission are associated with increases in *in-situ* production in bed sediments. Exports of carbon (POC, DOC) from upstream

become particularly important in driving in-stream production rates and combined with increased sediment accumulation help fuel the establishment of biotic communities both in the bed sediment and water column (Dawson et al., 2009). One of the primary drivers of in-stream production of GHGs is the type and quality of sediment as a substrate for microbial processes. This is broadly controlled by the changing river environment, where classically sedimentation increases down through the river continuum as water velocity reduces. Allied to this is the spatial and temporal delivery of organic carbon, with the quantity and lability of carbon being an important controlling factor in GHG production especially in the bed sediments. N₂O production is also controlled by the concentration in dissolved N, which also increases downstream as the proportion of agriculture in the catchment increases as found in other rivers (Stutter et al., 2008).

The organic matter content found in the suspended particulate matter transported through river catchments has been largely ignored as a source of carbon to the atmosphere. However it has been recognised as an important source of energy in estuaries for heterotrophic metabolism (Cole et al., 2007). Moreover in rivers, it has been recognised that both the quality and source of POC and DOC changes through river catchments, with important implications for driving in-stream processing though heterotrophic decomposition. Dawson et al., (2012) found that through the River Dee continuum, that an increasing percentage of total carbon of suspended particulate matter was respired as CO₂-C, from 0.49 % in the upland peat-dominated site to 3.20 % in the lowland river. This highlights that it is not only the bed sediments that are important in controlling GHG production, and that in the Tay respiration of carbon associated with POC may an important link in the cycling of C and N.

7.4.1 Flux of nitrogen from the River Tay: a wider perspective

The total N₂O flux was calculated using an approximate measure of total freshwater area in the Tay catchment (110.3 km²) (NRFA, 2012: Landcover map 2000) which is on par with the size of the Tay estuary (115 km²). The upper freshwater catchment holds a number of large lochs (Loch Tay, Loch Tummel, Loch Rannoch) linked with the River Tay, which significantly adds to the total freshwater area. For example, Loch Tay by itself is 23 km long with an approximate total area of 26.39 km². The mean emission density for the 9 sites were then multiplied by the total freshwater area and this is reported in Tables 1 as Tay^a. This is contrasted with a second emission total that takes

Table (1) Emission of N₂O from the Tay and other estuaries and rivers from the UK and globally.

	Area (km ²)			Amount of N ₂ O emitted (kg N yr ⁻¹)			Emission (water area)	Percentage		References
	River	Estuary	Total	River	Estuary	Total	kg N km ² yr ⁻¹	River	Estuary	
Tay ^a	110	115	230.0	31410	6785	38195	166	82.2	17.8	This Study
Tay ^b	110	115	230.0	14718	1282	15112	66	92.0	8.0	This Study
Colne	0.39	4.81	5.2	211	41049	41259	7935	0.5	99.5	Dong et al., 2004
Stour	0.87	24.4	25.3	619	8389	9009	356	6.9	93.1	Dong et al., 2004
Orwell	0.38	17.4	17.8	281	25828	26110	1464	1.1	98.9	Dong et al.,2004
Deben	0.16	9.27	9.44	103	5628	5731	607	1.8	98.2	Dong et al.,2004
Trent	14.2	-	14.2	13748	-	-	968	12.5	-	Dong et al.,2004
Ouse	10.5	-	10.5	6632	-	-	633	6.1	-	Dong et al.,2004
Humber	-	277	301 ^{**}	-	89193	109571 ^{**}	364 ^{**}	-	81.4	Dong et al.,2004
Conwy	1.13	4.97	6.1	317	1013	1330	218	31.3	68.7	Dong et al.,2004
Scheldt	-	269	-	-	178141	-	662	-	-	Barnes & Upstill Goddard 2011
Elbe	-	224	-	-	76346	-	341	-	-	Barnes & Upstill Goddard 2011
Ems	-	162	-	-	127244	-	786	-	-	Barnes & Upstill Goddard 2011
Gironde	-	442	-	-	114519	-	259	-	-	Barnes & Upstill Goddard 2011
Loire	-	41	-	-	6108	-	149	-	-	De Wilde and De Bie 2000
Douro	-	2.4	-	-	1845	-	768	-	-	Barnes & Upstill Goddard 2011
Seine	283	-	-	100000	-	-	353	-	-	Garnier et al., 2009

^{**} Trent, Ouse and Humber a = mean emission density multiplied by total area (for river and estuary) b= emissions the sum of sub-compartments: (upland and lowland river/loch); (estuary site compartments)

into account the spatial variability of N₂O emission densities throughout the catchment and the influence of the large lakes in the upper catchment (Table 1; Tay^b; see chapter 4 for detailed methods). It is assumed that the mean emission density for each section holds true for all freshwater in the section, and with the limited spatial coverage in the upper catchment this may still be an over estimate of total fluxes.

The total emission estimates were then compared to total emissions estimates from the estuary and other rivers and estuaries from the UK and globally. Comparisons between river and estuary indicate unusually that the dominate source of total emission from water in the Tay catchment is sourced from freshwaters. This contrasts to findings from Dong et al 2004 who found from a comprehensive study in a number of English and Welsh catchments, that estuaries dominated the total estimated emission. This was especially the case in intensely nutrified catchments such as the Humber, but less so in oligotrophic catchments such as the Conwy (Table 1). However, the catchments study by Dong et al., 2004 were also dominated by the estuaries in terms of total water area. To put this into perspective, emissions were also calculated in terms of total emission per unit of water area. This indicates that emission density was substantially lower in the Tay compared to the wide range of rivers and estuaries reported in Table 1. For example to highlight this contrast in emissions, Dong et al., 2004 reported a total estimated emission from the River Colne of $\sim 41300 \text{ kg N yr}^{-1}$, with the highest estimate from the river Tay being $\sim 38200 \text{ kg N yr}^{-1}$, however there is a stark difference in total water area in the two catchments, with the River Tay a factor of 40 larger than the River Colne. Comparing the estimated emission from the Tay estuary to others in the UK and Europe highlights that the Tay is generally a small source of N₂O compared to other hyper-nutrified estuaries. For example the Scheldt was estimated to emit up to $\sim 178000 \text{ kg N yr}^{-1}$ compared to only $\sim 1282 \text{ kg N yr}^{-1}$ from the Tay estuary. The Tay may provide a perspective or benchmark on N₂O fluxes from relatively undisturbed river basins. These types of catchments are infrequently studied, so the Tay results may be particularly useful for scaling up budgets for the UK or Europe as a whole as a representative of more nutrient poor catchments.

To explore fluxes of nitrogen further in the Tay catchment, a basic budget of fluxes was constructed, comparing estimated fluxes from the terrestrial catchment with that of dissolved nitrogen and estimated N₂O emission (Table 2). N₂O emission from the

various land uses found in the Tay catchment (land use data taken from NRFA 2012) were calculated based on recommended fertiliser inputs (Fertiliser Manual – rb209) for arable and grassland landuse, with a conversion factor of 1% N₂O per kg of N fertiliser (IPCC, 2006). Emissions rates of N₂O for forest and natural areas were taken from Skiba et al., (2012).

In total 503.3 10³ kg N-N₂O yr⁻¹ is estimated to be emitted in the terrestrial zone of the catchment with 61.1 % emitted from grasslands, 18.4 % from forest, 8.9 % from arable land and a further 10.9 % from natural/semi-natural land uses. The total emissions from freshwaters in the catchment (~ 10.60 10³ kg N-N₂O yr⁻¹) represent 2.1 % of total emissions from the terrestrial zone in the catchment, highlighting that overall freshwaters in the Tay contribute significantly less than terrestrial soils.

Table 2. Gas emissions for a) terrestrial emission separated by landuse b) indirect emissions and dissolved flux from surface water in the catchment

a) Direct Emissions from catchment soils/landuse				b) Indirect Emissions and dissolved flux from freshwater			
	Area km ²	Flux kg N ha ⁻¹ yr ⁻¹	Emission 10 ³ kg N- N ₂ O yr ⁻¹	Area km ²	Flux kg N ha ¹ yr ⁻¹	Emission 10 ³ kg N N ₂ O yr ⁻¹	DIN Flux 10 ³ kg N yr ⁻¹
Forest	806	1.148**	92.6	110	1.66	10.60	3685
Natural	2408	0.228**	54.9				
Arable	344	1.300*	44.6				
Grassland	1245	2.500*	311.2				
Tot.	4803	-	503.3				

*N₂O emission based on 1 % of the N fertiliser input which was set at 270 kg N ha⁻¹ yr⁻¹ for improved grasslands and 130 kg N ha⁻¹ yr⁻¹. ** Skiba et al.,(2012)

The resulting data was also used to compare fluxes of N₂O with current IPCC methodologies to calculate indirect emission of N₂O. For this it is assumed that total N dissolved flux down the river equated to the total flux at site 9 (DIN=3685 tonnes of N yr⁻¹) and that this represented N leaching in the catchment. The current IPCC methodology to calculate indirect N₂O fluxes from nitrogen leaching and runoff in rivers states that 0.0025 kg N₂O-N is produced per kg N leached (0.25 %); which in the River Tay equates to 10.8 tonnes of N₂O-N per 3684 tonnes of DIN-N. However based on the emission flux densities in this study, the total emission (based on method Tay^b: Table 1) is estimated to be 14.71 tonnes of N₂O-N, equating to 0.39 % of dissolved inorganic N flux. Although higher than the current IPCC methodology, it is still lower than a recent

wide ranging study by Beaulieu et al., (2010) with their tracer study finding 0.75 % of dissolved inorganic N inputs to rivers is converted to N₂O. Since total leaching and runoff of N to the river is likely to be higher than the export at site 9 (since this only represents the fraction of dissolved DIN that has not undergone transformation), the estimate of 0.39 % is likely to be an over estimate.

7.4.2 Flux of carbon from the River Tay: a wider perspective

In order to set the findings of this study into a wider context, the flux densities of CO₂ were also compared to the flux densities reported in other rivers and estuaries both in the United Kingdom and globally (Table 3). The mean flux density of CO₂ (0.36 kg C m⁻² yr⁻¹) in the Tay estuary is substantially lower than those of some other UK and European estuaries. For example, Frankignoulle et al., (1998) reported higher mean flux densities (area weighted) in the Thames (1.10 kg C m⁻² yr⁻¹) as well as elsewhere in Europe, Douro (1.05 10 kg C m⁻² yr⁻¹). Abril et al., 2003 estimated high emissions of CO₂ (2.83 kg C m⁻² yr⁻¹) from the Loire estuary, but later work by Bozec et al., 2011 reported a significantly lower emission density (0.38 kg C m⁻² yr⁻¹) similar to the values found in the Tay. Overall, the flux density found in the Tay estuary is generally within the lower range but similar to other estuaries such as the Tamar (UK), Gironde (FR), Rhine (DE) and York River (US) (Table 3).

Values are also reported in terms of total emission, however a number of varying factors add difficulty in directly comparing total emissions between studies. For example, it is unclear how the total emission values from other studies were calculated; i.e. whether mean values of flux density for the whole estuary were used to calculate total emission. Results from this study highlight the danger in using mean flux density values for calculating total emissions with estuary mean values of flux density significantly over-estimating total flux. For example, by summing the total emissions in individual compartments (upper, middle, and lower) of the estuary resulted in a total emission of only 22% of the total emission calculated using the estuary mean flux density (Paper 1). This problem is created by high flux densities in the upper estuary (in the case of the Tay estuary) combined with significantly smaller total water surface area which results in substantially lower total emissions despite the high emission densities.

This problem is compounded with the uncertainties created by using gas transfer rate parameterizations. For example, the discrepancy between emission calculations for the Loire estuary, arise mainly from using different methods to calculate k and a lack of temporal coverage in the earlier work by Abril et al., 2003. The estimations of total emission for the Tay estuary are similarly uncertain with the means of each parameterization ranging from 12148 – 45178 tonnes C yr⁻¹. However even the highest estimation is still considerably lower than those of the majority of European estuaries.

At the global scale emission of CO₂ from estuaries is an important component of the carbon system, with Borges and Abril (2011) estimating emission from estuaries equalling that for rivers and streams. Furthermore estuaries account for 19 % of the sink of CO₂ from the open ocean despite having a much reduced surface area. Borges and Abril (2011) also found that total emission of CO₂ from estuaries compared well to the estimated processing of total organic carbon exported into estuaries.

In comparison to CO₂ and N₂O, CH₄ has been less studied in estuaries and rivers, with few estimations of flux emissions. Middleburg et al., (2002) studied the temporal and spatial distribution of CH₄ in 9 European estuaries, but only reported a mean flux density for the complete study (0.57 g C m⁻² yr⁻¹) comparing well to the Tay (1.02 g C m⁻² yr⁻¹). Other studies include those from the United States with De Angelis and Scranton (1993) reporting fluxes of 1.53 g C m⁻² yr⁻¹ from the Hudson and De Angelis and Lilley (1987) reporting fluxes of 0.79 g C m⁻² yr⁻¹ from the Yaquina Salmon and Alsea. However globally, CH₄ emissions from estuaries does not play a significant part in global budgets, accounting for <1% of total CH₄ global emissions (Borges and Abril 2011).

The emission densities of CO₂ found in the freshwater river are also compared to other rivers in Table 3 but on the whole estimation or directly measured fluxes of CO₂ are sparse in the literature. The range of emission densities fit well with the ranges found in other studies, with similar ranges found in other UK rivers such as the River Tees (0.00-0.97 kg C m² yr⁻¹) and River Coquet (1.99 kg C m² yr⁻¹) (Worall and Burt 2005) and some European rivers such as the Danish Havelse (2.14 kg C m² yr⁻¹) (Sand-Jensen and Staehr 2012). However, flux density was substantially lower than found in smaller streams influenced by peaty soils such as the Brocky Burn (0.12-22.40 kg C m² yr⁻¹) (Hope et al., 2001) and small streams in northern Europe, Temmesjoki (0.59 – 7.04 kg C m² yr⁻¹) (Sand-Jensen and Staehr 2012).

Table 3. Global CO₂ emission densities and total emissions from estuaries and rivers

		Area km ²	Mean Emission Density (Range) kg C m ⁻² yr ⁻¹	Total Emission t C yr ⁻¹	References
a)Estuaries	Tay Estuary (UK)	120	0.36	25282	This study
	Douro (PT)	2	1.05	6	Frankignoulle et al., 1998
	Elbe (DE)	224	0.79	208050	Frankignoulle et al., 1998
	Ems (DE)	162	0.83	113150	Frankignoulle et al., 1998
	Gironde (FR)	442	0.42	163520	Frankignoulle et al., 1998
	Loire (FR)	41	2.83	-	Abril et al., 2003
	Loire (FR)	41	0.38	38000	Bozec et al., 2012
	Rhine (NL)	71	0.45	31025	Frankignoulle et al., 1998
	Scheldt	269	1.69	169117	Frankignoulle et al., 1998
	Tamar (UK)	39.6	0.44	4927.5	Frankignoulle et al., 1998
	Thames (UK)	47.5	1.10	226300	Frankignoulle et al., 1998
	York River (US)	-	0.27	-	Raymond et al., 2000
	Parker River (US)	-	0.05	-	Raymon and Hopkinson 2003
b)Rivers	Tay (UK)	-	0.55 (0.01-2.00)	-	This study
	Entire USA	-	(0.88-4.00)	-	Butman & Raymond, 2011
	Havelse (DK)	-	2.14	-	Sand-Jensen and Staehr 2012
	Temmesjoki (FI)	-	----- (0.59-7.04)	-	Sand-Jensen and Staehr 2012
	Brocky Burn (UK)	-	----- (0.12-22.40)	-	Hope et al. 2001
	Pøle (DK)	-	2.90 (0.59-7.04)	-	Sand-Jensen and Staehr 2012
	R.Tees (UK)	-	----- (0.00-0.97)	-	Worall and Burt (2005)
	R.Coquet (UK)	-	----- (0.00-1.99)	-	Worall and Burt (2005)
	R.Swale (UK)	-	----- (0.59-5.86)	-	Neal et al., 1998

The results from this study highlight the importance of CO₂ evasion from freshwaters relative to other fluxes of carbon within catchments. For example, recent studies have found that freshwaters on the whole play a major part in the net carbon balance in river catchments on regional and global scales. For example, at the global scale, freshwater processing of carbon is estimated to be in the region of 18 g C m⁻² catchment area yr⁻¹ (Battin et al., 2009) accounting for up to 60 % of total terrestrial NEE. Regional scale studies have also accounted for carbon export, evasion and sedimentation (6-19 g C m⁻² yr⁻¹) equalling between 6 and 40 % of NEE (Jonsson et al., 2007; Buffman et al., 2011).

A basic carbon budget for the Tay catchment was constructed (Table 4) and the terrestrial carbon budgets were estimated based on the results from a wide ranging synthesis of carbon balances in croplands (Ciais et al., 2010) grassland (Ciais et al., (2010b), forest (Luyssaert et al., 2010) and natural landuses (Heinemeyer et al., 2010) in the 25 member states of the European Union (EU-25) during 1990-2005. The methods used to calculate freshwater flux are detailed in Chapter 4.

The freshwater flux (both dissolved export and atmospheric emission of C) accounted for up to 38 % of the terrestrial carbon sink and evasion of CO₂ dominated the freshwater flux (46 %) of carbon. Estimated CO₂ evasion accounted for 17.2 % of the terrestrial net sink of C.

Table 4. a) terrestrial emission separated by landuse b) indirect emissions from fresh surface water in the catchment

a) Sinks/Sources from catchment landuse				b) Fluxes in the river		
	Area	Flux	Emission		Dissolved Flux	Emission
	km ²	g C m ² yr ⁻¹	10 ³ kg C yr ⁻¹		10 ³ kg C yr ⁻¹	10 ³ kg C yr ⁻¹
Croplands	807	17*	5840	CO ₂	2967.7	36944 CO ₂
Grassland	344	-74**	-92122	CH ₄	2.88	109 CH ₄
Forest	1245	-75***	-60496	DOC	19793	
Natural	2408	-28****	-67422	TdC	44068	
Total	4802	-160	-214199	Total	44068	

* Ciais et al., (2010) ** Cias et al., (2010b) *** Luyssaert et al., (2010) **** Heinemeyer et al., (2010)

These estimates are in line with those from other studies. For example, Wallin et al., (2012) estimated fluvial C loss accounted for 34 % of the terrestrial C uptake in a

Swedish stream catchment. Despite the relative simplicity of the constructed carbon budget, it still highlights the significant role CO₂ emission plays in the catchment carbon cycle. Moreover, the emission estimations in the study could either underestimate or over-estimate total emission depending on a number of crucial factors. Firstly, the estimated emission from the large lochs in the catchment, could play a greater role in overall emission totals but these are not fully covered within the sampling sites of this study. Secondly, the low order streams in the upland catchment are also not included. These sites are likely to be flux hotspots of CO₂, as found in other upland studies in the UK and globally (Dawson, Bakewell and Billet 2000; Hope et al., 2004). Further complexity is added when factoring in the estimations of total area of surface water. Recently, Benstead & Leigh (2012) has suggested stream surface area has been globally underestimated, which has resulted in the estimates of GHG emissions also being significantly underestimated. The figures used for water surface area in this study are also likely to be a significant underestimate.

7.5 Significance of the study and future research recommendations

This thesis provides data on the variability in production and emission of GHG across a temperate, relatively unpolluted river system from source to sea. There has, in general, been a lack of data of GHG dynamics from rivers and estuaries, particularly those that remain relatively unpolluted. The River Tay is of regional significance in terms of the UK as it has the largest mean discharge of freshwater to the North Sea. Few studies have examined processing of the three major GHGs, and even less over a complete river catchment. Much of the current research, especially in estuaries, focuses on those that are heavily impacted with nutrient pollution. However, many estuaries and rivers in northern Europe are relatively nutrient poor, and it is, therefore, important to understand the cycling of GHG in these less impacted aquatic environments and their potential contribution to regional and global GHG budgets.

Unsurprisingly, N₂O flux densities across the catchment were relatively low especially across the main river and in the estuary. This, however, not only reflects the relatively low N loading in the catchment but also the turbulent and dynamic nature of the River Tay on the whole, with the lowland river in general described as ‘sediment starved’.

Many of the other studies of N₂O emission from rivers, both in the UK and globally have taken place in N rich, and sediment rich lowland rivers that exhibit greater N₂O production and emission (Dong et al., 2004). This study not only helps balance this discrepancy, but also sheds light on the spatial changes in emission even in a river system that is broadly speaking classed as unpolluted. Specific hotspots of production and emission were found within the catchment highlighting the importance of aquatic water type, morphology and management on top of nutrient loading in controlling the habitat to facilitate not only the production of N₂O but also of CO₂ and CH₄. These hot spots in emission density coincided with hotspots in production in river and lake sediments with the highest emission density and production in the lowland tributary sites. The production rates, particularly of N₂O, were correlated to NO₃⁻ concentrations and sediment organic carbon content, which in turn are correlated to agricultural landuse. This suggests that proportion of agricultural landuse indirectly stimulates N₂O production in the catchment by providing a source of NO₃⁻. Currently considerable uncertainty exists over the magnitude of N₂O yield in rivers and streams, and how these may vary spatially within a catchment (Beaulieu et al., 2009). More research is needed to understand the impacts of an increased nutrient load on different sediment types within rivers, importantly looking to assess how the biodegradability of organic matter and suboxic conditions relate to N₂O yield from denitrification across river catchments. Additionally, future research incorporating pore water chemistry as an additional measurement could help predict N₂O production rates within sediments.

In general little is known about the production rates of CH₄ in river systems with few published studies (Trimmer et al., 2012) with Sanders et al., (2007) providing one of the first accounts of seasonal production in a river bed. The results from this study suggest CH₄ production varies considerably across the catchment with substantially higher production rates in lowland tributaries with organic matter content of sediments being an important controlling factor. Although the relatively simple production assays used in the sediment provide a good overall picture of the potential production, it is impossible to infer from them how much gas would be transferred from sediment to water, and then to the atmosphere. Recent research has highlight that well oxygenated areas of sediment may be zones of CH₄ oxidation (Trimmer et al., 2010). More research is needed in rivers using a combination of *in-situ* benthic chambers and more detailed laboratory studies to understand the full cycling of CH₄ in river sediments where production and consumption may exist side by side. Moreover, CH₄ ebullition and transport through aquatic plants is

known to be an important emission pathway (Sanders et al., 2007) in many aquatic environments therefore future research could focus on understanding the flux of CH₄ from sediment to water/atmosphere. For example, CH₄ is known to accumulate in freshwater sediments (Trimmer et al., 2012), so understanding the influence of sediment disturbance during flood events could prove helpful in understanding the temporal variability in export and emission of CH₄ from river systems.

The study has shown that concentration and flux varies substantially in the River Tay estuary, with the results reported important in the overall context of placing estuary emissions in global GHG budgets. On the whole, there has been a tendency to study N₂O in nutrient rich estuaries across Europe and the UK. Recently, this bias has begun to be addressed with a comprehensive study by Barnes and Upstill-Goddard, 2011, across a range of estuaries in the UK, which included a single ‘spot’ survey study in the Tay estuary, which concluded that nutrient poor estuaries are not substantial sources of N₂O. The results from this study add further weight to this conclusion, indicating that although there is significant seasonal variation, the overall emission is still relatively low compared to the more polluted estuaries in the southern UK. The estuary study on the whole provides much needed seasonal and spatial emission rates from a macro-tidal low nutrient estuary to help address the bias in flux data towards nutrient rich estuaries which, if considered in isolation, would result in substantial overestimation of the contribution of estuarine waters to N₂O regional and global budgets. Whilst Paper I did not have the scope to fully address the controls of N₂O production within the estuary, it is clear that there are strong spatial changes in the saturation and emission of N₂O through the estuarine environment and future work should examine the relation between spatial variability both in dissolved concentrations and in production in sediments and the water column, with emphasis placed on trying to understand the short term temporal changes in N₂O production and emission sources across tidal cycles.

7.6 Conclusions

Based on the results of this thesis the following conclusions were made:

- o Overall greenhouse gas dynamics of River Tay are defined by the broad changes in the catchment.

- o Lowland lakes, and rivers dominated by agriculture landuse are emission hotspots of GHGs in the catchment, with GHGs both supplied from gas rich soil waters and enhanced *in-situ* production.
- o Significant production and emission takes place in the estuary with a high degree of variability, both spatially, seasonally and across tidal cycles.
- o The sediments of the River Tay and small lochs are the main freshwater sources of the GHGs CH₄, CO₂ and N₂O. NO₃⁻ concentrations were an important controlling factor both in seasonal rate experiments and in nutrient amendment experiments, and overall NO₃⁻ was positively correlated with agricultural landuse in the catchment.
- o The River Tay catchment on the whole (river, lake and estuary) is a smaller source of N₂O compared to other rivers and estuaries in the UK and Europe that are more heavily impacted by nutrient pollution.
- o The Tay on the whole, appears not to be a substantial source of either CH₄ or CO₂, with estuary fluxes in the middle range of those reported elsewhere in the UK and Europe.

The main conclusion from this thesis is that river catchments like the Tay, contain hotspots of GHG production and emission, which is broadly defined by seasonal changes in temperature, nutrient loads and available substrates for microbial processes.

References:

Abril, G. and Iversen, N. 2002 Methane dynamics in a shallow non-tidal estuary (Randers Fjord, Denmark), *Mar. Ecol.-Prog. Ser.* **230**: 171-181

Abril, G., Etcheber, H., Delille, B., Frankignoulle, M., Borges, A.V., 2003. Carbonate dissolution in the turbid and eutrophic Loire estuary. *Marine Ecology-Progress Series* **259**: 129-138.

Abril, G. and Borges, A. V. 2004. Carbon dioxide and methane emissions from estuaries, in: *Greenhouse Gas Emissions - Fluxes and Processes: Hydroelectric Reservoirs and Natural Environments*, edited by Tremblay, A., Varfalvy, L., Roehm, C. and Garneau, M., *Springer-Verlag*, 187-207

- Abril, G., Riou, S.A., Etcheber, H., Frankignoulle, M., de Wit, R. and Middelburg, J. J. 2000. Transient, tidal time-scale, nitrogen transformations in an estuarine turbidity maximum–fluid mud system (the Gironde, South-west France), *Estuar. Coast. Shelf S.*, **50**: 703–715
- Aitkenhead-Peterson, J.A., McDowell, W.H. & Neff, J.C. (2003). Sources, Production, and Regulation of Allochthonous Dissolved Organic Matter Inputs to Surface Waters. In: Findlay, S.E.G., *et al.* (Eds.) *Aquatic Ecosystems, Interactivity of Dissolved Organic Matter*. pp. 25-70. San Diego: Academic Press. ISBN 0-12-256371-9.
- Aufdenkampe, A. K., et al. (2011), Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere, *Front. Ecol. Environ*, **9**, 53–60, doi:10.1890/100014.
- Balls, P.1992. Nutrient behaviour in two contrasting Scottish estuaries, the Forth and Tay, *Oceanol. Acta*, **15**: 261–277
- Bange, H.W., Bartell, U.H., Rapsomanikis, S., Andrea, M.O., 1994. Methane in the Baltic and North Seas and a reassessment of the marine emissions of methane. *Global Biogeochemical Cycles* **8**: 465–480
- Barnes, J. and Owens, N. J. P.1998 Denitrification and nitrous oxide concentrations in the Humber estuary, UK, and adjacent coastal zones, *Mar. Poll. Bull.*, **37**: 247–260
- Barnes, J. and Upstill-Goddard, R. C.2011. N₂O seasonal distributions and air-sea exchange in UK estuaries: Implications for the tropospheric N₂O source from European coastal waters. *J. Geophys. Res.* **116**: G01006
- Bastviken D., Cole J., Pace M. & Tranvik L. (2004). Methane emissions from lakes: dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochemical Cycles*, **18**
- Bastviken D., Tranvik L.J., Downing J.A., Crill P.M. & Enrich-Prast A. 2011. Freshwater methane emissions offset the continental carbon sink. *Science* **331**: 50
- Battin et al., 2008 Controls on organic carbon fluxes in fluvial networks. *nature geoscience* **V1**, February
- Battin, T. J. et al. 2009 The boundless carbon cycle. *Nature Geosci.* **2**: 598 600
- Beaulieu, J.J., C.P. Arango, S.K. Hamilton, and J.L. Tank. 2008. The production and emission of nitrous oxide from headwater streams in the Midwestern USA. *Glob. Change Biol.* **14**:878–894.
- Beaulieu J.J, Arango C.P, Tank J.L. 2009. The effects of season and agriculture on nitrous oxide production in headwater streams. *J. Environ. Qual.* **38**: 637-646
- Beaulieu, J. J., et al. 2010 Nitrous oxide emission from denitrification in stream and river networks, *Proc. Natl. Acad. Sci. U. S. A.*, **108**: 214–219
- Berner, R.A. & Kothavala, Z. (2001). GEOCARB III: A revised model of atmospheric CO₂ over phanerozoic time. *American Journal of Science* **301(2)**, 182-204.
- Billett MF, Palmer SM, Hope D, Deacon C, Storeton-West R, Hargreaves

- KJ, Flechard C, Fowler D. 2004. Linking land-atmosphere-stream carbon fluxes in a lowland peatland system. *Global Biogeochemical Cycles* **18**: GB1024, DOI:10.1029/2003GB002058.
- Billett, M. F. and Moore, T. R.:2008. Supersaturation and evasion of CO₂ and CH₄ in surface waters at Mer Bleue peatland, Canada. *Hydrol. Process.*, **22**: 2044–2054
- Borges, A. V. and Abril, G.2011. Carbon dioxide and methane dynamics in estuaries. In: Wolanski, E. Mcluskay D.S.(eds.) *Treatise on Estuarine and Coastal Science*. Waltham: Academic Press., **5**, 119–161
- Borges, A. V., Delille, B., Schiettecatte, L.-S., Gazeau, F., Abril, G. and Frankignoulle, M.2004. Gas transfer velocities of CO₂ in three European estuaries (Randers Fjord, Scheldt, and Thames). *Limnol. Oceanogr.*, **49**: 1630-1641
- Borges, A.V., Vanderborcht, J.-P., Schiettecatte, L.-S., Gazeau, F., Ferron-Smith, S., Delille, B., Frankignoulle, M., 2004b. Variability of the gas transfer velocity of CO₂ in a macrotidal estuary (the Scheldt). *Estuaries* **27**: 593–603
- Boyer EW, Howarth RW, Galloway JN, Dentener FJ .2006. Riverine nitrogen export from the continents to the coasts. *Global Biogeochemical Cycles*, **20**: GB1S91, doi: 10.1029/2005GB002537.
- Bozec Y, Cariou T, Mace E, Morin P, Thuillier D, Vernet M. 2012. Season dynamics of air-sea CO₂ fluxes in the inner and outer Loire Estuary (NW Europe). *Estuarine, Coastal and Shelf Science*. 100L 58-71
- Brunet F, Gaiero D, Probst J L, Depetris P J, Gauthier Lafaye F and Stille P 2005 δ¹³C tracing of dissolved inorganic carbon sources in Patagonian rivers (Argentina) *Hydrological Processes* **19**: 3321–44
- Buffam I., Turner M. G., Desai A. R. *et al.* (2011) Integrating aquatic and terrestrial components to construct a complete carbon budget for a north temperate lake district. *Global Change Biology*, **17**, 1193-1211
- Burgin, A.J., Hamilton, S.K., 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Frontiers in Ecology and the Environment* **5**: 89–96
- Butman, D., and P. A. Raymond. 2011. Significant efflux of carbon dioxide from streams and rivers in the United States, *Nat. Geosci.*, **4**: 839–842
- Ciais P, Watterbach M, Vuichard N et al. 2010a The European carbon balance. Part 2: croplands. *Global Change Biology*, **16**: 1409–1428.
- Ciais P, Soussana JF, Vuichard N, Luyssaert S, Don A, Janssens IA, Piao SL, Dechow R, Lathière J, Maignan F, Wattenbach M, Smith P, Ammann C, Freibauer A, Schulze ED, the CARBOEUROPE Synthesis Team. 2010b. The greenhouse gas balance of European grasslands. *Biogeosci Discuss* **7**:5997–6050
- Casper, P., 1992. Methane production in lakes of different trophic state. *Arch.*

Hydrobiol. Beih. Ergebn. Limnol. **37**: 149–154.

Casper P.S., Maberly S.C., Hall G.H. & Finlay B.J. 2000. Fluxes of methane and carbon dioxide from a small productive lake to the atmosphere. *Biogeochemistry* **49**: 1-19.

Chapin, F. S., et al. (2005), Role of land-surface changes in Arctic summer warming, *Science*, 310, 657–660, doi:10.1126/science.1117368

Chapman, P.J., Edwards, A.C., Reynolds, B., Neal, C., 1999. The nitrogen composition of streams draining grassland and forested catchments: influence of afforestation on the nitrogen cycle in upland ecosystems. Impact of land-use change on nutrient loads from diffuse sources (Proceedings of IUGG 99 Symposium, HS3, Birmingham, July 1999). *IAHS 25711*: 17–26.

Clark, J. F., Schlosser, P., Simpson, H. J. Stute, M., Wanninkhof, R. and Ho, D. T. 1995 Relationship between gas transfer velocities and wind speeds in the tidal Hudson River determined by the dual tracer technique, in: *Air–Water Gas Transfer: Selected Papers from the Third International Symposium on Air-Water Gas Transfer*, 785–800

Clough, T.J. Buckthought, L.E. Kelliher FM. Sherlock, RR. 2007. Diurnal fluctuations of dissolved nitrous oxide (N₂O) concentrations and estimates of N₂O emissions from a spring-fed river: implications for IPCC methodology. *Global Change Biology*. **13**: 1016–1027

Cole JJ, Caraco NF (2001) Emissions of nitrous oxide (N₂O) from a tidal, freshwater river, the Hudson River, New York. *Environmental Science and Technology*, **35**: 991–996

Cole, J. J. et al. 2007 Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems* **10**: 171-184

Davidson EA, Seitzinger S. 2006. The enigma of progress in denitrification research. *Ecol Appl* **16**:2057–63.

Dawson, J.J.C, Bakewell U.C, Billett, M.F 2001 Is in-stream processing an important control on spatial changes in carbon fluxes in headwater catchments? *The Science of the Total Environment* **265**: 153-167

Dawson, J.J.C., Billett, M.F., Neal, C., Hill, S., 2002. A comparison of particulate, dissolved and gaseous carbon in two contrasting upland streams in the UK. *Journal of Hydrology* **257**: 226–246.

Dawson, J. J. C., Billett M.F, Hope D, Palmer S.M, and Deacon C.M. 2004. Sources and sinks of aquatic carbon in a peatland stream continuum, *Biogeochemistry*, **70(1)**: 71-92.

Dawson J.J.C. Soulsby C. Hrachowitz M. Speed M. Tetzlaff D. 2009. Seasonality of epCO₂ at different scales along an integrated river continuum within the Dee Basin, NE Scotland. *Hydrological Processes* **23**: 2929 – 2942

Dawson J.J.C, Adhikari Y.R, Soulsby C, Stutter M.I. 2012. The biogeochemical reactivity of suspended particulate matter at nested sites in the Dee basin, NE Scotland.

434: 159-170

De Angelis, M.A., Lilley, M.D., 1987. Methane in surface waters of Oregon estuaries and rivers. *Limnology and Oceanography* **32**: 716–722.

De Angelis, M. A. and Scranton, M. I. 1993. Fate of methane in the Hudson River and estuary. *Global Biogeochem. Cy.*, **7**: 509–523, 1993.

Demars B.O.L, Manson J.R 2012. Temperature dependence of stream aeration coefficients and the effect of water turbulence: A critical review. *Water Research* **47**: 1 - 15

De Wilde, H. P. J., and M. J. M. De Bie (2000), Nitrous oxide in the Schelde estuary: Production by nitrification and emission to the atmosphere, *Mar. Chem.*, **69**: 203–216

Dong, L. F., Nedwell, D. B., Colbeck, I. and Finch, J. 2004. Nitrous oxide emission from some English and Welsh rivers and estuaries, *Water Air Soil Poll.* **4**: 127–134

Downing, J. A., et al. 2006. The global abundance and size distribution of lakes, ponds, and impoundments, *Limnol. Oceanogr.*, **51**, 2388– 2397.

Evans, M. & Warburton, J. 2001 Transport and dispersal of organic debris (peat blocks) in upland fluvial systems. *Earth Surf. Process. Landforms* **26**, 1087–1102.

Evans, M. & Warburton, J. 2005 Sediment budget for an eroding peat-moorland catchment in northern England. *Earth Surf. Process. Landforms* **30**, 557–577.

Ferrón, S., Ortega, T., Gomez-Parra, A. and Forja, J. M. 2007. Seasonal study of dissolved CH₄, CO₂ and N₂O in a shallow tidal system of the bay of Cadiz (SW Spain). *J. Marine Syst.* **66**: 244-257

Frankignoulle, M., Abril, G., Borges, A., Bourge, I., Canon, C., Delille, B., Libert, E., Théate, J.-M., 1998. Carbon dioxide emission from European estuaries. *Science* **282**: 434-436.

García-Ruiz R, Pattinson SN, Whitton BA. 1998. Kinetic parameters of denitrification in a river continuum. *Appl Environ Microbiol* **64**:2533–8

García-Ruiz R, Pattinson SN, Whitton BA. 1999. Nitrous oxide production in the River Swale-Ouse, North-East England. *Wat.Res.* **33**:1231–37

Garnier, J., Billen, G., Vilain, G., Martinez, A., Silvestre, M., Mounier, E., and Toche, F. 2009. Nitrous oxide (N₂O) in the Seine river and basin: observations and budgets, *Agr. Ecosyst. Environ.* **133**: 223–233

Germon, J.C. 1985. Microbiology of denitrification and other processes involving the .

Griffiths J, Nutter J, Binley A, Crook N, Young A, Pates J. 2007. Variability of dissolved CO₂ in the Pang and Lambourn chalk rivers. *Hydrology and Earth System Sciences* **11**: 328–339.

- Groffman PM, Altabet MA, Bohlke JK, Butterbach-Bahl K, David MB, Firestone MK, et al. 2006. Methods for measuring denitrification: diverse approaches to a difficult problem. *Ecol Appl.* **16**:2091–122
- Hasegawa K, Hanaki K, Matsuo T, Hidaka S. 2000. Nitrous oxide from the agricultural water system contaminated with high nitrogen. *Chemosphere - Global Change Sci* **2**: 335-345
- Heathwaite, A.L., Dils, R.M., 2000. Characterising phosphorus loss in surface and subsurface hydrological pathways. *Science of the Total Environment* **251–252**: 523–538
- Heinemeyer A, Croft S, Garnett MH, Gloor E, Holden J, Lomas MR, Ineson P. 2010. The MILLENNIA peat cohort model: predicting past, present and future soil C budgets and fluxes under changing climates in peatlands. *Climate Research* **45**: 207–226
- Hiscock KM, Bateman AS, Mühlherr IH, Fukada T, Dennis PF. 2003. Indirect emissions of nitrous oxide from regional aquifer in the United Kingdom. *Environ Sci Technol* **37**: 3507-3512.
- Hope, D., Billett, M.F. and Cresser, M.S., 1994. A review of the export of carbon in river water: fluxes and processes. *Environ. Poll.* **84**: 301-324
- Hope, D., Billett, M.F., Milne, R., Brown, T.A.W., 1997b. Exports of organic carbon in British rivers. *Hydrological Processes* **11**, 325-344.
- Hope, D., Palmer, S.M., Billett, M.F., Dawson, J.J.C., 2001. Carbon dioxide and methane evasion from a peatland stream. *Limnology and Oceanography* **46**: 847–857.
- Hope D, Palmer S, Billett MF, Dawson JJC. 2004. Variations in dissolved CO₂ and CH₄ in a first-order stream and catchment: an investigation of soil–stream linkages. *Hydrological Processes* **18**: 3255–3275.
- Howarth, R.W., Sharpley, A., Walker, D., 2002. Sources of nutrient pollution to coastal waters in the United States: implications for achieving coastal water quality goals. *Estuaries* **25**: 656– 676.
- Huttunen, J. T., Väisänen, T., Heikkinen, M., Hellsten, S., Nykänen, H., Nenonen, O., and Martikainen, P. J. 2002. Exchange of CO₂, CH₄ and N₂O between the atmosphere and two northern boreal ponds with catchments dominated by peatlands or forests, *Plant. Soil*, **242**: 137–146
- Huttunen J.T., Nykänen H., Turunen J. & Martikainen P.J. 2003. Methane emissions from natural peatlands in the northern boreal zone in Finland, Fennoscandia. *Atmos. Environ.* **37**: 147-151.
- Inwood, S.E., J.L. Tank, and M.J. Bernot. 2005. Patterns of denitrification associated with land use in nine midwestern headwater streams. *J. N. Am. Benthol. Soc.* **24**:227–45.
- IPCC 2006. IPCC Guidelines for national greenhouse gas inventories; prepared by the nations gas inventories programme. Japan: IPPC; 2006

- Jones, B. J., and P. J. Mulholland. 1998a. Methane input and evasion in a hardwood forest stream: Effects of subsurface flow from shallow and deep pathways. *Limnol. Oceanogr.* **43**: 1243–1250.
- Jones, B. J., and P. J. Mulholland. 1998b. Carbon dioxide variation in a hardwood forest stream: An integrative measure of whole catchment soil respiration. *Ecosystems* **1**: 183–196
- Jones JB, Mulholland PJ. 1998c. Methane input and evasion in a hardwood forest stream: effect of subsurface flow from shallow and deep pathways. *Limnology and Oceanography* **43**: 1243–1250.
- Johnson MS, Billett MF, Dinsmore K.J, Wallin M, Dyson K.E, Jassal R.S. 2010. Direct and continuous measurement of dissolved carbon dioxide in freshwater aquatic systems method and applications. *Ecohydrology* **3**:68–78.
- Jonsson A., Algesten G., Bergström A. K. *et al.* (2007) Integrating aquatic carbon fluxes in a boreal catchment carbon budget. *Journal of Hydrology*, **334**, 141-150
- Juutinen S., Rantakari M., Kortelainen P., Huttunen J.T., Larmola t., Alm J., Silvola J. & Martikainen P.J. 2009 Methane dynamics in different boreal lake types. *Biogeosciences*, **6**, 209–223.
- Kankaala, P., J. Huotari, E. Peltomaa, T. Saloranta, and A. Ojala. 2006. Methanotrophic activity in relation to methane efflux and total heterotrophic bacterial production in a stratified, humic, boreal lake. *Limnol. Oceanogr.*, **51**, 1195–1204.
- Kiene, R.P., 1991. Production and consumption of methane in aquatic systems. In: Rogers, J.E., Whitman, W.B. (Eds.), *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides and Halomethanes*. *American Society for Microbiology* 111–146.
- Kleber, M. & Johnson, M.G. (2010). *Advances in Understanding the Molecular Structure of Soil Organic Matter: Implications for Interactions in the Environment*. *Advances in Agronomy* **106** 77-142
- Kling GW, Kipphut GW, Miller MC. 1991. Arctic lakes and streams as gas conduits to the atmosphere: implications for tundra carbon budgets. *Science* **25**: 298–301.
- Knowles R (1982) Denitrification. *Microbiol Rev* **46**:43–70
- Kortelainen P, Saukkonen S, Mattsson T. 1997. Leaching of nitrogen from forested catchments in Finland. *Global Biogeochem Cy* **11**: 627 638
- Kortelainen P., Rantakari M., Huttunen J.T., Mattsson T., Alm J., Juutinen S., Larmola T., Silvola J. & Martikainen P.J. 2006. Sediment respiration and lake trophic state are important predictors of large CO₂ evasion from small boreal lakes. *Glob. Change Biol.* **12**: 1554- 1567.
- Kremer, J.N., Nixon, S.W., Buckley, B., Roques, P., 2003b. Technical note: conditions for using the floating chamber method to estimate air–water gas exchange. *Estuaries* **26**:

985–990.

Kroeze C, Dumont E, Seitzinger SP (2005) New estimates of global emissions of N₂O from rivers and estuaries. *Environmental Sciences* **2**: 159–165

Liss, P. S. and Merlivat, L. 1986. Air-sea exchange rates: introduction and synthesis,. in: (The Role of Air-Sea Exchange in Geochemical Cycling, edited by: Buat-Ménard, P., Reidel, Dordrecht, 113-127

Liss, P. S. and Slater, P. G. 1974. Flux of gases across the air-sea interface. *Nature* **247**: 181–184

Luysaert S, Ciais P, Piao SL et al. 2010. The European carbon balance. Part 3: forests. *Global Change Biology*, **16**: 1429–1450

Martienssen, M., and R. Schops. 1999. Population dynamics of denitrifying bacteria in a model biocommunity. *Wat. Res.* **33**:639-646.

Middelburg JJ, Klaver G, Nieuwenhuize J, Wielemaker A, de Haas W & van der Nat JFWA. 1996. Organic matter mineralization in intertidal sediments along an estuarine gradient. *Mar. Ecol. Prog. Ser.* **132**: 157–168

Middelburg, J. J., Nieuwenhuize, J., Iversen, N., Høgh, N., de Wilde, H., Helder, W., Seifert, R. and Christof, O. 2002. Methane distribution in European tidal estuaries, *Biogeochemistry*, **59**: 95-119

Monaghan MT, Thomas SA, Minshall GW. 2001. The influence of filter-feeding benthic macro-invertebrates on the transport and deposition of particulate organic matter and diatoms in two streams. *Limnology and Oceanography* **46**: 1091–1099.

Mulholland PJ, Helton AM, Poole GC, Hall Jr RO, Hamilton SK, Peterson BJ, et al. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature*. **452**:202–6.

National River Flow Archive. 2012 www.ceh.ac.uk/data/nrfa/

Nassehi, V. and Williams, D.J.A. 1987. A mathematical model for salt intrusion in the Tay Estuary. *Proceedings of the Royal Society of Edinburgh* **92**: 285-297.

Neal, C., Harrow, M., Williams, R. J. 1998. Dissolved carbon dioxide and oxygen in the River Thames: spring–summer 1997, *Science of the Total Environment* **210**: 205–217

Neal, C., Robson, A.J., 2000. A summary of river water quality data collected within the Land Ocean Interaction Study: core data for eastern UK rivers draining to the North Sea. *The Science of the Total Environment* **251/252**: 585-665

Neal, C., Reynolds, B., Neal, M., Hill, L., Wickham, H., Pugh, B., 2003. Nitrogen in rainfall, cloud water, throughfall, stemflow, stream water and groundwater for the Plynlimon catchments of mid-Wales. *Sci. Tot. Environ.* **314–316**: 121–151.

OSPAR: Eutrophication Assessment Reports: Tay Estuary, OSPAR Eutrophication

Committee, 2006.

Pfenning, K.S. and P.B. McMahon. 1996. Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate rich riverbed sediments. *Journal of Hydrology* **187**: 283-295.

Poth, M., Focht, D.D., 1985. Nitrogen-15 kinetic analysis of nitrous oxide production by *Nitrosomonas europaea*: an examination of nitrifier denitrification. *Applied and Environmental Microbiology* **49**: 1134–1141.

Raymond, P.A., Bauer, J.E., Cole, J.J., 2000. Atmospheric CO₂ evasion, dissolved inorganic carbon production, and net heterotrophy in the York River estuary. *Limnology and Oceanography* **45** (8): 1707–1717.

Raymond, P. A. and Cole, J. J. 2001. Gas exchange in rivers and estuaries: choosing a gas transfer velocity, *Estuaries* **24**: 312-317

Raymond, P.A., Hopkinson, C.S., 2003. Ecosystem modulation of dissolved carbon age in a temperate marsh-dominated estuary. *Ecosystems* **6**: 694–705.

Reay DS, Smith KA, Edwards AC. 2003. Nitrous oxide emission from agricultural drainage waters. *Global Change Biology*. **9**: 195–203.

Revsbech NP, Jacobsen JP, Nielsen LP. 2005. Nitrogen transformations in microenvironments of river beds and riparian zones. *Ecological Engineering* **24**:447–455.

Richey J E. 2005. Global river carbon biogeochemistry in Anderson M G ed *Encyclopedia of hydrological sciences* Wiley, London chapter 184

Robinson, A. D., Nedwell, D. B., Harrison, R. M. and Ogilvie, B. D.: 1998, 'Hypernutrified estuaries as sources of N₂O emission to the atmosphere: The estuary of the River Colne, Essex, UK', *Mar. Ecol. Prog. Ser.* **164**, 591–571

Sanders IA, Heppell CM, Cotton JA, Wharton G, Hildrew AG, Trimmer M. 2007. Emission of methane from chalk streams has potential implications for agricultural practices. *Freshw Biol* **52**:1176–86.

Sand-Jensen K, Staehr P.A. 2012. CO₂ dynamics along Danish lowland streams: water-air gradients, piston velocities and evasion rates. *Biogeochemistry*. **111**:615-628

Schlesinger, W.H. (1977). Carbon Balance in Terrestrial Detritus. *Annual Review of Ecology and Systematics* **8**(1) 51-81.

Schaller, M.F., Wright, J.D. & Kent, D.V. 2011. Atmospheric PCO₂ Perturbations Associated with the Central Atlantic Magmatic Province. *Science* **331**: 1404-1409.

Scranton, M.I., McShane, K., 1991. Methane fluxes in the southern North Sea: the role of European rivers. *Continental Shelf Research* **11**: 37–52

- Seitzinger SP. 1990. Denitrification in aquatic sediments. In: Revsbech NP, Sørensen J, editors. Denitrification in soil and sediment. New York: Plenum Press; 1990. p. 301–22.
- Seitzinger SP. 1988 Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol Oceanogr* **33**:702–724.
- Skiba U, Cresser MS. 1991. Seasonal changes in soil atmospheric CO₂ concentrations in two upland catchments and associated changes in river water chemistry. *Chemical Ecology* **7**: 217–225.
- Skiba U, Jones S.K, Dragosits U, Drewer J, Fowler D, Rees R.M, Pappa V.A, Cardenas L, Chadwick D, Yumulki S, Manning A.J. 2012. UK emissions of the greenhouse gas nitrous oxide. *Phil. Trans. R. Soc. B* **367**: 1175-1185
- Stutter M.I, Langan S.J, Cooper R.J. 2008. Spatial and temporal dynamics of stream water particulate and dissolved N, P and C forms along a catchment transect, NE Scotland. *Journal of Hydrology*. **350**: 187-202
- Svensson, J.M., and L. Leonardson. 1996. Effects of bioturbation by tube-dwelling chironomid larvae on oxygen uptake and denitrification in eutrophic lake sediments. *Freshwater Biology* **35**:289-300.
- Syväsalo E, Regina K, Pihlatie M, Esala M. 2004. Emissions of nitrous oxide from boreal agricultural clay and loamy sand soils. *Nutr Cycl Agroecosyst* **69**: 155-165.
- Tuominen L, Heinänen A, Kuparinen J, Nielsen LP. 1998. Spatial and temporal variability of denitrification in the sediments of the northern Baltic Proper. *Mar Ecol - Prog Ser* **172**: 13-24.
- Tranvik L.J., Downing J.A., Cotner J.B., Loiselle S.A., Striegl R.G., Ballatore T.J., Dillon P., Finlay K., Fortino K., Knoll L.B., Kortelainen P.L., Kutser T., Larsen S., Laurion I., Leech D.M., McCallister S.L., McKnight D.M., Melack J.M., Overholt E., Porter J.A., Prairie Y., Renwick W.H., Roland F., Sherman B.S., Schindler D.W., Sobek S., Tremblay A., Vanni M.J., Verschoor A.M., von Wachenfeldt E. & Weyhenmeyer G.A. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol. Oceanogr.* **54**: 2298-2314.
- Trimmer M, Hildrew AG, Jackson MC, Pretty JL, Grey J. 2009. Evidence for the role of methane-derived carbon in a free-flowing, lowland river food web. *Limnol Oceanogr* **54**:1541–7
- Trimmer M, Maanoja S, Hildrew AG, Pretty JL, Grey J. 2010. Potential carbon fixation via methane oxidation in well-oxygenated riverbed gravels. *Limnol Oceanogr.* **55**:560–8
- Trimmer M, Grey J, Heppell C.M, Hildree A.G, Lansdown K, Stahl H, Yvon-Durocher G. 2012. River bed carbon and nitrogen cycling: state of play and some new directions. *Science of the Total Environment.* **434**:143-158
- Upstill-Goddard, R. C., Barnes, J., Frost, T., Punshon, S. and Owens, N. J. P.2000. Methane in the southern North Sea: low-salinity inputs, estuarine removal, and

atmospheric flux, *Global Biogeochem. Cy.*, **14**: 1205-1217

Van der Nat F.-J. & Middelburg J.J. 2000. Methane emission from tidal freshwater marshes. *Biogeochemistry* **49**: 103-121

Wade, A.J, Neal, C, Whitehead. P.J, Flynn, N.J.2005. Modelling nitrogen fluxes from the land to the coastal zone in European systems: a perspective from the INCA project. *Journal of Hydrology* **304**: 413–429

Wallin M.B, Grabs T, Buffam I, Laudon H, Agren A, Oquist G, Bishop K. 2013. Evasion of CO₂ from streams – The dominant component of the carbon export through the aquatic conduit in a boreal landscape. *Global Change Biology* **19**: 785-797

Wanninkhof, R., 1992. Relationship between wind speed and gas exchange over the ocean. *Journal of Geophysical Research* **97**: 7373–7382

Weier KL, Doran JW, Power JF, Walters DT. 1993. Denitrification and the Dinitrogen/Nitrous Oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Sci Soc Am J* **57**: 66-72.

Whalen S.C. 2005 Biogeochemistry of methane exchange between natural wetlands and the atmosphere. *Environmental Engineering Science*, **22**, 73–94.

Whitehead PG, Crossman J. 2012. Macronutrient cycles and climate change: key science areas and international perspective. *Sci Total Environ* **434**

Williams, D.J.A. and West, J.R. 1975. Salinity distribution in the Tay Estuary. *Proceedings of the Royal Society of Edinburgh (B)* **75**: 29-39.

Wollheim WM, Vorosmarty CJ, Bouwman AF, Green P, Harrison J, Linder E, Peterson BJ, Seitzinger SP, Syvitski JPM (2008) Global N removal by freshwater aquatic systems using a spatially distributed, within-basin approach. *Global Biogeochem Cycles* **22**

World Meteorological Organization (WMO) (2011) The State of Greenhouse Gases in the Atmosphere Based on Global Observations through 2010. World Meteorological Organization Greenhouse Gases Bulletin No. 7. World Meteorological Organization. <http://www.wmo.int/gaw>

Worall F, Burt T (2005) Reconstructing long-terms records of dissolved CO₂. *Hydrological Process* **19**:1791–1806

Wrage N, Velthof GL, van Beusichem ML, Oenema O. 2001. Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology and Biochemistry*, **33**: 1723–1732.

Appendix A

Calculating Henry Constant (solubility coefficient)

Temperature and salinity dependence of Kh was calculated using the following formulations given by Weiss (1970), Weiss and Price (1980) and Weisenburg and Guisassa (1979) for CO₂, N₂O and CH₄ respectively:

$$\ln K_o = A_1 + A_2 (100/T) + A_3 \ln (T/100) + S [B_1 + B_2 (T/100) + B_3 (T/100)^2]$$

Where: A₁, A₂, A₃, B₁, B₂, B₃ constants, T is temp in K, S is salinity

Table (A1) Constants for Calculations of solubility coefficients using the above equation

Gas	A ₁	A ₂	A ₃	B ₁	B ₂	B ₃	Ref.
CO ₂	-58.0931	90.5069	22.294	0.027766	0.025888	0.0050578	1
CH ₄	-68.8862	101.4956	28.7314	-0.076146	0.043970	-0.0068672	2
N ₂ O	-59.5965	97.3066	24.1406	-0.058420	0.033193	-0.0051313	3

¹Weiss (1974) ²Wiesenburg and Guisasso (1979) ³Weiss and Price (1980)

Table (A2) Solubility Coefficients for N₂O mol L⁻¹ atm⁻¹

Temp	Salinity							
	0	5	10	15	20	25	30	35
1	0.0569	0.0552	0.0536	0.0520	0.0505	0.0490	0.0476	0.0461
2	0.0546	0.0530	0.0515	0.0500	0.0485	0.0471	0.0457	0.0444
3	0.0524	0.0509	0.0494	0.0480	0.0466	0.0453	0.0440	0.0427
4	0.0504	0.0489	0.0475	0.0462	0.0448	0.0436	0.0423	0.0411
5	0.0485	0.0471	0.0457	0.0444	0.0432	0.0419	0.0407	0.0396
6	0.0466	0.0453	0.0440	0.0428	0.0416	0.0404	0.0392	0.0381
7	0.0449	0.0436	0.0424	0.0412	0.0400	0.0389	0.0378	0.0368
8	0.0432	0.0420	0.0408	0.0397	0.0386	0.0375	0.0365	0.0355
9	0.0417	0.0405	0.0394	0.0383	0.0372	0.0362	0.0352	0.0342
10	0.0402	0.0391	0.0380	0.0369	0.0359	0.0349	0.0340	0.0330
11	0.0388	0.0377	0.0367	0.0357	0.0347	0.0337	0.0328	0.0319
12	0.0374	0.0364	0.0354	0.0344	0.0335	0.0326	0.0317	0.0309
13	0.0361	0.0352	0.0342	0.0333	0.0324	0.0315	0.0307	0.0299
14	0.0349	0.0340	0.0331	0.0322	0.0313	0.0305	0.0297	0.0289
15	0.0338	0.0329	0.0320	0.0311	0.0303	0.0295	0.0287	0.0280
16	0.0327	0.0318	0.0310	0.0301	0.0293	0.0286	0.0278	0.0271
17	0.0316	0.0308	0.0300	0.0292	0.0284	0.0277	0.0270	0.0262
18	0.0306	0.0298	0.0290	0.0283	0.0275	0.0268	0.0261	0.0254
19	0.0297	0.0289	0.0281	0.0274	0.0267	0.0260	0.0253	0.0247
20	0.0287	0.0280	0.0273	0.0266	0.0259	0.0252	0.0246	0.0240

Table (A3) Solubility Coefficients for CO₂ mol L⁻¹ atm⁻¹

Temp	Salinity							
	0	5	10	15	20	25	30	35
1	0.0698	0.0522	0.0397	0.0309	0.0245	0.0199	0.0165	0.0140
2	0.0694	0.0519	0.0395	0.0307	0.0244	0.0198	0.0164	0.0139
3	0.0691	0.0516	0.0393	0.0306	0.0243	0.0197	0.0163	0.0139
4	0.0687	0.0514	0.0391	0.0304	0.0242	0.0196	0.0163	0.0138
5	0.0684	0.0511	0.0390	0.0303	0.0241	0.0195	0.0162	0.0138
6	0.0680	0.0508	0.0388	0.0301	0.0239	0.0194	0.0161	0.0137
7	0.0677	0.0506	0.0386	0.0300	0.0238	0.0193	0.0161	0.0137
8	0.0673	0.0503	0.0384	0.0299	0.0237	0.0193	0.0160	0.0136
9	0.0670	0.0501	0.0382	0.0297	0.0236	0.0192	0.0159	0.0135
10	0.0666	0.0498	0.0380	0.0296	0.0235	0.0191	0.0159	0.0135
11	0.0663	0.0496	0.0378	0.0294	0.0234	0.0190	0.0158	0.0134
12	0.0659	0.0493	0.0376	0.0293	0.0233	0.0189	0.0157	0.0134
13	0.0656	0.0491	0.0374	0.0291	0.0232	0.0188	0.0157	0.0133
14	0.0652	0.0488	0.0372	0.0290	0.0231	0.0188	0.0156	0.0133
15	0.0649	0.0486	0.0371	0.0289	0.0230	0.0187	0.0155	0.0132
16	0.0646	0.0483	0.0369	0.0287	0.0229	0.0186	0.0155	0.0132
17	0.0642	0.0481	0.0367	0.0286	0.0227	0.0185	0.0154	0.0131
18	0.0639	0.0478	0.0365	0.0284	0.0226	0.0184	0.0153	0.0131
19	0.0636	0.0476	0.0363	0.0283	0.0225	0.0183	0.0153	0.0130
20	0.0632	0.0473	0.0361	0.0282	0.0224	0.0183	0.0152	0.0130

Table (A4) Solubility Coefficients for CH₄ mol L⁻¹ atm⁻¹

Temp	Salinity							
	0	5	10	15	20	25	30	35
1	0.0026	0.0021	0.0016	0.0013	0.0010	0.0008	0.0007	0.0006
2	0.0026	0.0021	0.0016	0.0013	0.0010	0.0008	0.0007	0.0006
3	0.0026	0.0021	0.0016	0.0012	0.0010	0.0008	0.0007	0.0006
4	0.0026	0.0021	0.0016	0.0012	0.0010	0.0008	0.0007	0.0006
5	0.0026	0.0020	0.0016	0.0012	0.0010	0.0008	0.0007	0.0006
6	0.0026	0.0020	0.0016	0.0012	0.0010	0.0008	0.0007	0.0006
7	0.0026	0.0020	0.0015	0.0012	0.0010	0.0008	0.0007	0.0005
8	0.0026	0.0020	0.0015	0.0012	0.0010	0.0008	0.0007	0.0005
9	0.0026	0.0020	0.0015	0.0012	0.0010	0.0008	0.0006	0.0005
10	0.0026	0.0020	0.0015	0.0012	0.0010	0.0008	0.0006	0.0005
11	0.0026	0.0020	0.0015	0.0012	0.0010	0.0008	0.0006	0.0005
12	0.0026	0.0019	0.0015	0.0012	0.0009	0.0008	0.0006	0.0005
13	0.0026	0.0019	0.0015	0.0012	0.0009	0.0008	0.0006	0.0005
14	0.0026	0.0019	0.0015	0.0012	0.0009	0.0008	0.0006	0.0005
15	0.0025	0.0019	0.0015	0.0012	0.0009	0.0008	0.0006	0.0005
16	0.0025	0.0019	0.0015	0.0011	0.0009	0.0008	0.0006	0.0005
17	0.0024	0.0019	0.0014	0.0011	0.0009	0.0007	0.0006	0.0005
18	0.0024	0.0019	0.0014	0.0011	0.0009	0.0007	0.0006	0.0005
19	0.0024	0.0019	0.0014	0.0011	0.0009	0.0007	0.0006	0.0005
20	0.0024	0.0018	0.0014	0.0011	0.0009	0.0007	0.0006	0.0005

Appendix B: SEPA monitoring data for the River Tay catchment

Table (B1) Mean \pm Standard deviation of SEPA monitoring data between 1987-2008 (site numbers correspond to site numbers in this thesis)

	9	8	7	6	4	3	2	1
Temperature	9.78 \pm 4.94	9.32 \pm 4.53	8.76 \pm 4.82	9.24 \pm 4.78	8.96 \pm 4.26	9.48 \pm 4.83	9.13 \pm 4.02	9.49 \pm 3.80
S.S	3.26 \pm 3.35	4.27 \pm 6.30	5.22 \pm 6.11	2.60 \pm 3.29	2.06 \pm 1.68	1.83 \pm 2.82	1.46 \pm 1.89	1.04 \pm 0.69
pH	7.46 \pm 0.33	7.65 \pm 0.35	7.58 \pm 0.26	7.34 \pm 0.31	7.39 \pm 0.25	7.28 \pm 0.38	7.31 \pm 0.36	7.27 \pm 0.31
Alkalinity	19.06 \pm 4.93	35.52 \pm 12.6	42.64 \pm 13.4	12.00 \pm 3.81	23.58 \pm 7.11	10.10 \pm 3.30	11.49 \pm 3.17	9.85 \pm 2.90
Hardness	30.36 \pm 7.54	53.32 \pm 13.9	67.34 \pm 18.0	20.35 \pm 6.66	30.01 \pm 5.77	17.44 \pm 5.01	18.63 \pm 5.05	13.63 \pm 3.75
DO mg	11.31 \pm 1.18	11.55 \pm 1.26	10.90 \pm 1.41	11.02 \pm 1.25	10.72 \pm 1.24	11.13 \pm 1.24	11.39 \pm 0.99	11.12 \pm 0.97
DO sat	99.31 \pm 5.60	100.00 \pm 7.96	92.59 \pm 5.51	94.93 \pm 3.69	92.34 \pm 4.69	96.37 \pm 3.98	98.45 \pm 5.79	97.13 \pm 5.12
BOD	1.41 \pm 0.67	1.60 \pm 0.82	1.53 \pm 0.65	1.34 \pm 0.61	1.18 \pm 0.48	1.22 \pm 0.53	1.14 \pm 0.56	0.96 \pm 0.55
TOC	4.02 \pm 0.94	3.92 \pm 2.69	4.80 \pm 2.14	4.31 \pm 1.20	4.61 \pm 1.39	4.54 \pm 1.28	3.63 \pm 1.23	3.22 \pm 0.59
Ammonia	0.03 \pm 0.03	0.04 \pm 0.04	0.06 \pm 0.04	0.03 \pm 0.03	0.03 \pm 0.03	0.03 \pm 0.03	0.02 \pm 0.02	0.02 \pm 0.02
TON	0.63 \pm 0.34	1.29 \pm 0.51	2.09 \pm 0.90	0.20 \pm 0.16	0.27 \pm 0.20	0.13 \pm 0.14	0.18 \pm 0.15	0.14 \pm 0.16
Nitrite	0.00 \pm 0.00	0.01 \pm 0.01	0.02 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Nitrate	0.63 \pm 0.35	1.32 \pm 0.51	2.11 \pm 0.85	0.21 \pm 0.15	0.31 \pm 0.14	0.14 \pm 0.13	0.18 \pm 0.15	0.08 \pm 0.02
SRP	0.02 \pm 0.02	0.03 \pm 0.03	0.05 \pm 0.04	0.01 \pm 0.02	0.02 \pm 0.02	0.01 \pm 0.02	0.01 \pm 0.02	0.01 \pm 0.01
TP	0.02 \pm 0.02	0.04 \pm 0.03	0.08 \pm 0.06	0.01 \pm 0.00	0.01 \pm 0.01	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00
Chloride	7.27 \pm 1.64	8.71 \pm 2.71	13.41 \pm 5.17	5.87 \pm 1.47	6.63 \pm 1.34	5.67 \pm 1.31	5.68 \pm 1.12	5.32 \pm 1.00
Sulphate	4.11 \pm 1.33	7.80 \pm 1.92	9.93 \pm 2.52	2.60 \pm 1.14	6.23 \pm 0.88	2.24 \pm 1.00	2.46 \pm 0.76	1.41 \pm 0.56