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Ciprofloxacin Effects On Nitrogen Cycling Processes In Freshwater Aquatic Sediments

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CIPROFLOXACIN EFFECTS ON NITROGEN CYCLING PROCESSES IN FRESHWATER AQUATIC SEDIMENTS

by

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Bachelor of Science (Honours), University of Waterloo, 1999

A thesis

presented to Ryerson University

in partial fulfillment of the

requirements for the degree of

Master of Applied Science

in the program of

Environmental Applied Science and Management

Toronto, Ontario, Canada, 2009

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ABSTRACT

Ciprofloxacin Effects on Nitrogen Cycling in Aquatic Sediments

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Four experiments were designed to assess the effect of ciprofloxacin (cipro) on the function of microbial populations in freshwater environments, specifically on nitrogen cycling. Cipro is a broad-spectrum fluoroquinolone antibiotic used in the treatment of both human and veterinary pathogenic diseases. Previous studies reported the presence of cipro in aquatic environments. This study investigates whether cipro has adverse effects on environmental bacteria which perform critical ecosystem processes associated with the nitrogen cycle. Microcosms containing sediment and synthetic lake water were amended with a series of environmentally relevant concentrations of cipro ranging from 0.5 to 2.5 mg cipro per kg of sediment. Nitrogen cycling processes including nitrification, denitrification and ammonification were measured using a combination of flux measurements (NH_4^+ , NO_3^- , NO_2^-), stable isotope techniques (^{15}N NO_3^- dilution) and changes in $\text{N}_2:\text{Ar}$ and $\text{O}_2:\text{Ar}$ using Membrane Isotope Mass Spectroscopy (MIMS). Results indicate that cipro has a dose-dependent effect on nitrification, while denitrification is dependent on nitrate availability and may be stimulated by cipro. Ammonification and respiration were not affected at these concentrations. Impacts on nitrification and denitrification are likely to be realized only at the highest concentrations measured in the environment. At the lower end of the environmentally relevant concentration range, observed impacts are not likely to be ecologically important, especially when averaged over an entire lake ecosystem.

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1.0 INTRODUCTION

1.1 Pharmaceuticals in the Environment

The growth of modern society has led to an associated increase in the use of pharmaceutical and personal care products (PPCPs) in humans as well as in agricultural and aquaculture activities (Daughton and Jones-Lepp, 2001).

In 2003 in the United States (US), prescription drug spending was US\$227.5 billion, comprising approximately 10% of national health care spending and was three times larger than in 1997 (US National Health Expenditures Tables, 2007). Canada's total drug expenditure has increased steadily from \$3.8 billion to \$16.7 billion between 1985 and 2001. In 2001, drug expenditure represented over 16% of total health care expenditure and was ranked third in drug expenditure per capita after the US and France (CIH, 2004). In 2007, Canadians spent \$665 million on oral antibiotics out of a total almost \$19 billion on prescription drugs (Morgan et al., 2008).

Furthermore, addition of antibiotics to animal feed is a significant non-medical use of antibiotics in North America for the purpose of growth promotion. The Union of Concerned Scientists estimates that approximately 11.2 million kilograms (kg) (24.6 million pounds) of antimicrobials are used for non-therapeutic uses in the US compared to 1.4 million kg (3 million pounds) in human medicine or between 70% and 85% of total antimicrobial use (Mellon et al., 2001). Administering antibiotics to livestock stimulates growth and accelerates the time taken to get the animal to market. The addition of 25 mg penicillin per pound of chicken feed saves 900 million kg (2 billion pounds) of feed per year (Madigan et al., 2000). These antibiotics are likely to inhibit organisms responsible for low grade infections and reduce intestinal epithelial inflammation thereby promoting nutrient uptake and improving feeding efficiency (Madigan et al., 2000). Veterinarians also prescribe human drugs to prevent disease or treat illness in a practice referred to as off-label use (Holtz, 2006).

Recent research has shown the prevalence of various pharmaceuticals entering the environment from water treatment plants, agricultural and aquaculture practices and landfills throughout the world (Daughton and Jones-Lepp, 2001). Richardson and Bowron (1985) reported and studied the presence and fate of various pharmaceuticals in a river downstream from homes and hospitals in North London. Hirsch et al. (1999) analyzed water samples collected from river water and sewage treatment plant effluents in Germany for 18 antibiotic substances and reported concentrations of antibiotics up to 6 µg/L. Zuccato et al. (2000) measured low levels of clofibrilic acid, diazepam and tylosin in Italian drinking water. Kolpin et al. (2002) conducted the first comprehensive study of 95 pharmaceuticals, hormones and other organic wastewater contaminants in 139 streams across 30 states in North America between 1999 and 2000 and reported 31 antibiotics with concentrations up to 1.9 µg/L. Jacobsen and Berglund (1988) and Coyne et al. (1994) reported high concentrations of the antibiotic oxytetracycline in sediments beneath fish farms.

PPCPs can have far-reaching, but often little considered, effects on the environment. Although not traditionally considered contaminants, pharmaceuticals are designed for a variety of uses and are intended to target a variety of organisms. Therefore, they may have unanticipated effects on non-target organisms. For example, avermectins (antiparasitic drugs used in agriculture and aquaculture) were very toxic to crustaceans with LC₅₀ values ranging from 0.0026 µg/L to 22 µg/L. Ivermectin has been measured in surface/subsurface runoff in concentrations less than 1.1 mg/L to 4.4 mg/L (Boxall et al., 2004). In comparison, the chronic and acute toxicity of phosmet (an organophosphate pesticide) ranged from 0.0056 mg/L (EC₅₀ for daphnids) to 0.07 mg/L (LC₅₀ for fish) (Boxall et al., 2004). This indicates a much higher toxicity of the pharmaceutical compared to the pesticide.

Studies have shown reproductive behavioural changes at very low doses of pharmaceuticals such as anti-depressants. Carbamazepine, an antiepileptic and anti-depressant, was used to assess inhibition of *Chironomus riparius* emergence. *C. riparius* is a non-biting midge that serves as an important food source for many fish and other organisms and is frequently the most abundant

insect in freshwater ecosystems (Nentwig et.al, 2004). No acutely toxic effects from aquatic exposure were observed. However, a chronic effect (significantly reduced emergence of larvae) was observed at a sediment concentration of 1.25 mg/kg dry weight. At sediment concentrations of 2.5 mg/kg dry weight, more than 90% inhibition was observed followed by total inhibition and arrested development at higher concentrations (Nentwig et al., 2004).

Schulte-Oehlmann et al. (2004) conducted a study to assess the effects of synthetic steroids on the freshwater snail *Marisa cornuarietis*. Both 17 α -methyltestosterone (MT) and 17 α -ethinylestradiol (EE2) induced virilisation (i.e. development of male reproductive organs) in female *M. cornuarietis* (Schulte-Oehlmann et al., 2004). Similar results from a different study indicated that EE2 induced feminization of the freshwater amphipod *Hyalella azteca* (Vandenbergh et al., 2003).

Although pharmaceutical toxicity studies provide comprehensive pharmacological and clinical effects, little to no assessment is completed for ecotoxicity or ecosystem effects (Richardson and Bowron, 1985; Seiler, 2002; Hartemann, 2004), especially for chronic effects (Webb, 2004a). Even more troubling are the effects of continual life-cycle, multi-generational exposure to aquatic organisms. Antibiotics may increase the incidence of multi-resistant strains of bacteria especially in areas around aquaculture and agriculture operations (Midtvedt, 2004). Kandeler et al. (1995) reported a significant decrease in respiration, nitrogen mineralization and nitrification in soils exposed to heavy metals. Effects may occur so gradually as to be undetected leading to irreversible change that may instead be attributed to naturally occurring evolutionary change (Daughton and Ternes, 1999). Furthermore, although environmental concentrations of pharmaceuticals may be low, the additive or synergistic effects may be considerable (Daughton and Jones-Lepp, 2001).

For decades, pharmaceuticals have not been considered a problem because their concentrations were often too low to be measured and their effects were too small to be observed. With recent advances in technology, and increasing use of pharmaceuticals leading to increased prevalence in

the natural environment, this has become an emerging field of study, first in Europe and later in North America. Although preventing the use of pharmaceuticals is not an acceptable solution, their widespread presence in the environment could be reduced in order to mitigate those effects on non-target organisms, to avoid development of resistance in disease organisms and to prevent ecological toxicity and ecosystem effects.

1.1.1 Sources and Fate of Pharmaceuticals in the Environment

Pharmaceuticals can potentially enter all four major environmental compartments from a variety of routes: surface water (from waste water and overland runoff), soil (from sewage sludge, manure and biosolids application), sediment (from overland runoff and direct surface water application) and groundwater (through leaching and percolation) (Diaz-Cruz et al., 2003; Kümmerer, 2004).

Excretion from humans and animals, as well as disposal of unused inventory, are major sources of pharmaceuticals found in the environment. Disposal and discharge into the environment from domestic and industrial effluent and surface water runoff result in continuous input into the environment (Daughton and Ternes, 1999). Disposal of human and animal waste into the environment is usually by emission into water resources via wastewater treatment plants (WWTPs) (Bound and Voulvoulis, 2005). Generally, WWTPs are reactive, rather than proactive, to new environmental pollutants so there is often a lag period during which time new contaminants are emitted to the environment before they are perceived as a problem. As pharmaceuticals are not widely considered to be environmental contaminants, flushing into municipal WWTPs is considered a safe and effective method of disposal. Furthermore, institutional disposal methods, thought to be a significant source of pharmaceutical input into the environment, have their own set of practical considerations. In many parts of the US, strict disposal regulations mean that many institutions must flush away unused medications as the most effective means of disposal within the law (Daughton, 2003a).

A secondary route of exposure is the agricultural application of biosolids on land (Bound and Voulvoulis, 2005). From this point, pharmaceuticals may bind to soil, run off to surface water or groundwater or undergo biodegradation (Boxall et al., 2004).

Some pharmaceuticals are highly persistent and are not broken down in the environment. However, accumulation in the body is not generally a desired property for pharmaceuticals (Stuer-Lauridsen et al., 2000). In order to promote uptake in the body, the vast majority of pharmaceuticals are designed to be soluble in water (Kümmerer, 2001a). Boxall et al. (2004, 2004a) reported half-lives for various veterinary pharmaceuticals in soil, manure, sediment and surface water. In soil, half-lives ranged from less than 1 day for penicillin to greater than 1 year for emamectin benzoate (an avermectin antiparasitic drug) (Boxall et al., 2004a). Half-lives in manure were significantly less, ranging from a couple of days for tylosin (an antiparasitic drug) to greater than 3 months for some antibiotics (quinolones and tetracyclines) (Boxall et al., 2004). Half-lives in sediments are highly variable, ranging from less than 1 day to greater than 1 year for oxytetracycline. Half-lives for fluoroquinolones range from just over one month to almost one year (Boxall et al., 2004a).

In general, pharmaceuticals are less persistent in water than in soil because they are generally designed to be soluble to promote uptake in the gastrointestinal tract of treated organisms. Quinolones and tetracyclines that had half-lives greater than three months in manure have considerably shorter half-lives in water, ranging from less than one hour to three hours (Boxall et al., 2004a). Wastewater is generally the predominant transport pathway into the environment (Lishman et al., 2006) and the effects of pharmaceuticals are most often observed and studied in surface water.

Finally, the application of pharmaceuticals, especially antibiotics, in aquaculture through feed additives or direct application to surface water has an impact on benthic sediments in both marine and aquatic environments. According to Cabello (2006), industrial aquaculture worldwide has increased fourfold in the last 20 years. Antibiotics are administered in feed or as

baths or injections to prevent infection resulting from increased population density, crowding, lack of sanitary barriers and failure to isolate infected animals. Where aquaculture and agriculture are integrated, practices that use manure and other agricultural residues as fish feed are widespread (Cabello, 2006). Approximately 70 to 80% of medicated feed ends up in the environment (Hektoen et al., 1995). These practices allow residual quantities to build up in sediments altering microbial composition and exerting selection pressure (Cabello, 2006). Pharmaceuticals applied to water can enter sediments without undergoing any attenuation processes which may lead to locally high concentrations (Kümmerer, 2004a). These drugs may be either degraded or released back into the overlying water (Hirsch et al., 1999).

Although some pharmaceuticals are in fact as persistent in the environment as persistent organic pollutants (POPs), a great deal more pharmaceuticals take on a “pseudo-persistent” quality as a result of this continual replenishment which effectively compensates for any transformation and removal that may occur in the environment (Daughton and Ternes, 1999). This “pseudo-persistence” resulting from continuous input into the environment can be considered to cause ongoing pollution and potentially lead to chronic toxicity. Specifically, non-target organisms in soil and water may be affected by continuous, sub-therapeutic levels of pharmaceuticals (Daughton, 2003b). These effects have only recently begun to be documented and assessed.

While it seems that indirect exposure to pharmaceuticals via drinking water is not a significant threat to humans (Kolpin et al., 2002; Heberer, 2002), indirect risks to humans include increased microbial resistance to disease treatment, potential uptake through consumption of treated foods (e.g. grain, animals) (Boxall, 2004) and the implications of unanticipated effects on other organisms in lakes, rivers, streams and soils for ecosystem health.

1.1.2 Fluoroquinolones

Fluoroquinolone antibiotics are a subset of quinolone antibiotics, a class of widely prescribed broad-spectrum antibacterials. They are totally synthetic compounds, active against many gram-positive and some gram-negative pathogenic bacteria and licensed as broad-spectrum antibiotics for human (ciprofloxacin, norfloxacin, ofloxacin, levofloxacin) and veterinary use (enrofloxacin, danofloxacin) (Alder et al., 2004).

Fluoroquinolone antibiotics (FQs) are a relatively new class of antibiotic compound. Our understanding of their environmental behaviour, fate and effects is still limited compared to our knowledge of traditional environmental contaminants such as metals, hydrocarbons and pesticides. In 1962, nalidixic acid was isolated as a by-product in the synthesis of chloroquine. Two years later, nalidixic acid was in general practice for treatment of urinary tract infections. Modifications to the basic structure of nalidixic acid yielded new derivatives including oxolinic acid, piperamic acid and piromidic acid. These are considered the first-generation quinolones. The first fluoroquinolone, flumequine, was synthesised in 1973. The synthesis of norfloxacin in 1980 included two fundamental structural modifications: a fluorine atom at C6 and an aliphatic cyclic amine (piperazine N-alkylpiperazine) at C7. Absorption of FQs is much higher and metabolism much lower than compared to the first generation of quinolones (Martorell et al., 1993).

FQs are rapidly degraded in surface water by ultraviolet light but are insensitive to hydrolysis and temperature (Sections 1.2.3 and 1.2.4). Several authors have shown that FQs partition to organic matter, both within the water column and in sediments. FQs are of environmental concern because of their tendency to sorb to sediments and therefore persist in the environment (Section 1.2.5) and, together with their unfavourable toxicity profile (Section 1.2.6) (Golet et al., 2002) may affect microbial populations in sediments (Sections 1.2.7 and 1.2.8).

Ciprofloxacin (cipro) is the most widely prescribed FQ followed by ofloxacin (Pico and Andreu, 2007). The global value of cipro sales exceeded US\$1.3 billion by November 1999. Ciprofloxacin was listed as number 32 among the most prescribed medications in 2001 and represented about 50% of the prescriptions of quinolones in Canada in 2000 and 2001 (Basic and Clinical Pharmacology as cited in Pico and Andreu, 2007). Reflecting the relative rates of prescription of quinolone antimicrobials in Canada, ciprofloxacin, norfloxacin, and ofloxacin have been detected in WWTP effluents, with ciprofloxacin and ofloxacin most frequently detected (Miao et al., 2004).

After being administered, pharmaceuticals are subject to modification through metabolic processes. These include hydroxylation, cleavage or glucuronation by enzymes produced in the liver to promote water solubility and excretion. However, a significant portion of the original, unmetabolized drug will leave via urine or feces. These metabolites may have significant residual activity or even be transformed back into the original active drug (Hirsch et al., 1999). Therefore, the presence of any given pharmaceutical in wastewater is dependent on dosage and metabolism (hydrolysis, oxidation, reduction, conjugation reactions) (Cunningham, 2004). Many pharmaceuticals are only partially metabolized before excretion from humans and animals, and continue to be broken down during the transport process (Kümmerer, 2001a). Metabolism rates of FQs are variable. Approximately 10% of enrofloxacin is excreted unmetabolized in animals (Vancutsum et al., 1990 as cited in Halling-Sorensen et al., 1998). Humans excrete unmetabolized ciprofloxacin at a rate of 45 to 62% and norfloxacin at a rate of 20 to 40% in urine. In feces, 15 to 25% of ciprofloxacin is excreted unmetabolized and approximately 28% of norfloxacin is excreted unmetabolized (Kuhlmann et al., 1997).

Furthermore, Golet et al. (2003) found that FQs are stable during methanogenesis and biodegradation is of minor importance compared to sorption during tertiary sewage treatment using activated sludge. Therefore, FQs enter the environment through both solid and aqueous matrices largely unmetabolized, and then persist in the organic matrix of sludge.

1.1.3 Mode of Action

FQs inhibit many gram-positive and some gram-negative bacteria. FQs interfere with bacterial DNA synthesis by inhibiting the enzymes Topoisomerase II (DNA gyrase) in gram-negative and Topoisomerase IV in gram-positive bacteria. The inhibition of these enzymes prevents growth and eventually results in death of the bacterial cells (Hooper, 2001). DNA topoisomerases have been isolated from a variety of different organisms including viral (Shuman and Moss, 1987), prokaryotic and eukaryotic sources (Reece et al., 1991; Huang, 1994) indicating that FQs may inhibit cell growth in other organisms.

1.1.4 Environmental Occurrence

FQs were first measured in North American surface waters in 2002 in the ng/L range (Kolpin et al., 2002). In Europe, quinolones and FQs have been detected in sediments and fauna in the µg/g range by various authors and in soil and sediments at varying concentrations depending on the physical and chemical characteristics of the matrix (Bjorklund et al., 1991; Samuelsen et al., 1992; Kolpin et al., 2002; Golet et al., 2003; Metcalfe et al., 2004; Nakata et al., 2005). Bjorklund et al. (1991) reported concentrations of oxolinic acid in sediments ranging from <0.05 to 0.2 mg/kg in five days after treatment in sediments beneath fish farms in the Baltic Sea off the south-west coast of Finland. Samuelsen (1992) reported the half-life of oxolinic acid in the sediments beneath a fish farm off the coast of Norway was 48 days. Nakata et al. (2005) investigated quinolones in wastewater effluents and rivers/lakes in the US and Canada and reported ofloxacin concentrations of 204 ng/L and 100 ng/L in secondary and tertiary WWTP effluents, respectively. Mass flow calculations put total river discharge at 4.8 g cipro per day of discharge from a single WWTP.

Metcalfe et al. (2004) reported FQs in surface waters around WWTPs in 5 Canadian cities. Concentrations of FQs were measured in the nanogram per Litre range in effluents discharged from the WWTPs. In the surface waters around the same WWTPs, concentrations ranged from below detection limits to greater than 500 ng/L indicating some attenuation processes, likely

dilution, were occurring. The median concentration of cipro was 0.011 µg/L with a maximum measured concentration of 0.015 µg/L. Samples were collected from the Otonabee River, Hamilton Harbour, Detroit River and Little River in Ontario (Metcalf et al., 2004).

In a Swiss study, cipro comprised almost 50% of the total FQ mass flow entering the WWTP (93 g/d) with concentrations ranging from 233 to 568 ng/L. Final effluent cipro concentrations ranged from 62 to 106 ng/L (Golet et al., 2002).

1.1.5 Fate in Surface Water

In general, microbial degradation of antibiotics is slower in surface water compared to WWTPs due to lower density and diversity of bacteria. FQs are insensitive to hydrolysis and temperature change relative to degradation rates of other antibiotics such as penicillin. However, photodegradation plays an important role in degradation of FQs in surface water (Alexy et al., 2004).

FQs that had half-lives greater than three months in manure have considerably shorter half-lives in surface water, ranging from less than one hour to three hours (Boxall et al., 2004a). Halling-Sorensen (2000) reported the half-life of ciprofloxacin ranged from 1.6 to 2.5 days in two bioreactors with activated sludge although there was no accounting for sorption or photodegradation.

1.1.6 Photodegradation

Photodegradation in the photic zone of surface waters may result in significant degradation as FQs are degraded by UV light (Alexy et al., 2004). Photodegradation is important because the process eliminates FQs whereas sorption simply partitions them into another compartment exposing different organisms and possibly creating a source for future release. As light intensity in surface waters is dependent on climate and latitude, photodegradation is likely to be a variable fate mechanism for different geographic locations at different times of the year.

Mesotrophic mid-latitude light intensity

Cardoza et al., (2005), evaluated the rate of 250 µg/L ¹⁴C-ciprofloxacin disappearance in surface waters under different light, dissolved and particulate organic carbon (DOC and POC) conditions to determine when photodegradation vs. adsorption dominates fate. The authors conducted three laboratory scale and one field scale experiment under conditions of mesotrophic mid-latitude light intensity. Rapid photodegradation (half-life of approximately 1.5 hours) occurred with numerous photodegradation products when POC levels were low. However, photodegradation was significantly reduced when moderate amounts of POC were present in the water column. Soluble ciprofloxacin disappearance rates were accelerated and no degradation products were detected at moderate POC levels relative to low POC levels.

The influence of DOC on ciprofloxacin fate was negligible. In the dark, prior to light exposure, no significant disappearance was observed. After light was provided, ciprofloxacin disappeared rapidly with a typical half-life of 4.6 ± 0.4 h. No significant difference in half-life was observed for DOC ranging from 5.36 to 38.13 mg C/L (Cardoza et al., 2005).

The final lab scale experiment indicated that elevated POC resulted in rapid disappearance of soluble phase ciprofloxacin after 15 minutes, regardless of light and DOC conditions.

Ciprofloxacin sorption to POC was also substantially higher at lower pH (pH 3.0 compared to pH 7.8) by almost 1 order of magnitude (Cardoza et al., 2005).

A lower ciprofloxacin concentration of 25 µg/L was used for the mesocosm study which consisted of six tanks deployed in a protected reservoir. The tanks were microbially seeded using sediment trays and covered to insulate against rapid changes in air temperature. Three of the six tanks were covered with black PVC lids. DOC and POC concentrations were low and secondary nutrient levels were representative of lower mesotrophic levels. Ciprofloxacin concentrations were monitored over 12 days at which point the lids were removed. In the open tanks, ciprofloxacin dropped rapidly and was not detectable after the first 5 hours. In the covered

tanks, ciprofloxacin was relatively persistent over 120 hours. The lids were removed after 290 hours to obtain more data. First order half-lives were between 0.97 and 1.14 hours in the covered tanks (Cardoza et al., 2005).

The authors concluded that both mechanisms (photodegradation and adsorption) are conditionally significant to ciprofloxacin fate in surface waters, and that determining the fate of ciprofloxacin adsorbed onto solids is an important aspect of its ultimate fate (Cardoza et al., 2005).

Effect of pH and particle size

Belden et al., (2007), investigated the effect of fine and coarse particulate matter (FPOM and CPOM) on sorption of ciprofloxacin at environmentally relevant pHs of 5 and 7.5 (above and below the lower pKa value of 5.9). FPOM consisted of sugar maple leaves processed by *Hyalella azteca* and filtered to less than 63 μm . CPOM consisted of leaves cut into 1.1 cm diameter discs. Soil collected from a fallow field was used as control sediment. The sorption coefficient (K_d) is the ratio of a substance's total equilibrium concentrations in the sorbed phase and in the solution (Schwarzenbach et al., 1993). K_d for CPOM was an order of magnitude lower than partition coefficients for sediment and FPOM. The effect of pH was most significant in sediments where lower pH led to greater sorption because, above pKa the compound ionizes, although this was minimal compared to matrix effects.

The authors concluded that further studies are required to investigate specific changes in organic material such as increased oxidation and surface area that may lead to increased sorption. Furthermore, greater binding to FPOM may prolong the environmental half-life of FQs and species within that ecosystem niche such as stream invertebrates that utilize FPOM as a food source may be more susceptible to contamination by FQs (Belden et al., 2007).

1.1.7 Fate in Sediments

Studies have shown that sorption of FQs to organic matter is an important removal process in wastewater treatment (Giger et al., 2003; Golet et al., 2003; Kümmerer et al., 2001). Golet et al. (2003) reported that the cipro concentrations in sewage sludge (3.1 mg/kg) from a Swiss WWTP were over four orders of magnitude higher than raw sewage (427 ng/L). Cipro concentrations in treated tertiary effluent were 71 ng/L (Golet et al., 2003). Lindberg et al. (2007) reported cipro concentrations ranging from 1.4 to 1.7 mg/kg in digested dewatered sewage sludge. There is little data on bioavailability of FQs but similar sorption mechanisms may result in FQ binding and sequestration in benthic sediments. Córdova-Kreylos and Snow (2007) published cipro sorption in sediments at $\log K_d = 2.90$ to 4.27 which was similar to previous studies by Golet et al. (2003) ($\log K_d = 4.29$, pH = 7.5 - 8.4) and Giger et al. (2003) ($\log K_d = 2.62$, pH = 6.5). The octanol-water partition coefficient, $\log K_{oc}$, was positively correlated with clay content but not with organic carbon content (Córdova-Kreylos and Snow, 2007). These data are somewhat contradictory to the information published in the previous studies by Belden et al., (2007) and Cardoza et al., (2005).

Benthic sediments are critical for nutrient cycling processes and as habitat for organisms. They are also often a sink for environmental contaminants and may therefore also provide a source via resuspension and bioturbation by benthic organisms. Approximately 70 to 80% of medicated feed ends up in the environment (Hektoen et al., 1995). Rapid sedimentation is typical at many aquaculture operations because debris consisting of feces and uneaten food accumulates beneath the fish enclosures, where sediments containing persistent pharmaceuticals may quickly build up (Boxall et al., 2005). Similarly, FQs released from WWTPs or feed lots may partition onto organic matter and settle, transferring the contaminant to sediments.

Half-lives of FQs in sediments vary between studies from just over one month for oxolinic acid to almost one year for flumequine, oxolinic acid and sarafloxacin (Boxall et al., 2004a).

Hektoen et al. (1995) investigated the persistence of oxolinic acid (1st generation quinolone), flumequine (1st FQ) and sarafloxacin and other antibiotics at 4 depth intervals from 1-7 cm from

sediment cores and found that, despite FQ sorption to sediments, FQs in sediments continued to be toxic to algae after approximately 180 days. Half-lives of oxolinic acid ranged from 151 days at surface to greater than 300 days at depths of 5 to 7 cm. Half-lives of flumequine ranged from 60 to greater than 300 days. Half-lives of sarafloxacin were similarly highly variable. While there was no discussion on possible FQ bioavailability, top layer residues depurated more rapidly and bottom layers were near initial concentrations suggesting leaching not degradation was causing redistribution (Hektoen et al., 1995).

1.1.8 Toxicity Effects of Fluoroquinolones

Table 1-1 Summary of results of previous studies of FQ toxicity on various organisms

FQ	Organism	EC₅₀	Reference
Cipro	<i>M. aeruginosa</i>	5 µg/L	Halling-Sorensen et al.(2000)
Cipro	<i>Microcystis</i>	17 µg/L	Robinson et al. (2005)
Cipro	<i>Lemna minor</i>	203 µg/L	Robinson et al. (2005)
Cipro	<i>P.subcapita</i>	18.7 mg/L	Robinson et al. (2005)
Ofloxacin	<i>Vibrio fischeri</i>	13.59 µg/L	Backhaus et al. (2000)
Flumequine	<i>Vibrio fischeri</i>	19.02 µg/L	Backhaus et al. (2000)
Flumequine	<i>Microcystis</i>	159 µg/L	Holten-Lutzhof et al. (1999)
Flumequine	<i>S. capricornutum</i>	5.0 mg/L	Holten-Lutzhof et al. (1999)
Flumequine	<i>R. salina</i>	18 mg/L	Holten-Lutzhof et al. (1999)
Norfloxacin	<i>Vibrio fischeri</i>	22.0 µg/L	Backhaus et al. (2000)
Nalidixic acid	<i>Vibrio fischeri</i>	199.95 µg/L	Backhaus et al. (2000)
Oxolinic acid	<i>Microcystis</i>	180 µg/L	Holten-Lutzhof et al. (1999)
Oxolinic acid	<i>Daphnia magna</i>	5.9 mg/L	Wollenberger et al. (2000)
Oxolinic acid	<i>R. salina</i>	10 mg/L	Holten-Lutzhof et al. (1999)
Oxolinic acid	<i>S. capricornutum</i>	24 mg/L	Holten-Lutzhof et al. (1999)
Sarafloxacin	<i>R. salina</i>	15 µg/L	Holten-Lutzhof et al. (1999)
Sarafloxacin	<i>S. capricornutum</i>	16 mg/L	Holten-Lutzhof et al. (1999)

Due to the challenges of FQ toxicity testing, the results have been highly variable.

Robinson et al., (2005), investigated the effects of 7 FQs on *Pseudokirchneriella subcapita* (formerly *Selenastrum capricornutum*), *Microcystis aeruginosa*, algal species for two divisions Chlorophyceae and Cyanophyceae, duckweed (*Lemna minor*), *Daphnia magna* and fathead minnow (*Pimephales promelas*). The data show that the cyanobacteria *Microcystis aeruginosa* is about two orders of magnitude more sensitive to six out of seven FQs with a median EC₅₀ of 49 ug/L compared to a much higher value for *P.subcapita* (median EC₅₀ 7,400 ug/L). The authors reported chlorosis of new duckweed (*Lemna minor*) fronds produced during the course of their experiment and it was the second most sensitive organism with median EC₅₀ at 106 ug/L. One explanation posed was that chloroplasts are prokaryotic in nature and may have similar metabolic pathways as cyanobacteria and may therefore be susceptible to the same mode of action. However, no such observations were made in *P. subcapita* so it is unclear why that organism is less sensitive (Robinson et al., 2005).

The same study briefly discussed the stability of FQs during the 7-day daily static renewal tests and found that lower concentrations were less stable and showed larger declines at the end of the tests. There was some indication that this may be due to adsorption onto algal cells but the study also did not account for the effects of photodegradation. Therefore, these EC₅₀ values likely underestimate toxicity because they were based on concentrations at the start of the test. The authors concluded that future research should examine the effects of FQs on aquatic bacterial communities such as microbial resistance and disruption of nutrient cycles (Robinson et al., 2005).

Backhaus and Grimme (1999) demonstrated inhibition in function (bioluminescence) of *Vibrio fischeri* (marine bacteria) as a result of antibiotic exposure. EC₅₀ ranged from 13.5 µg/L for ofloxacin to 0.206 mg/L for nalidixic acid (Backhaus and Grimme, 1999). A later study completed by the same authors examined the effects of FQs on *V. fischeri* and reported EC₅₀ concentrations ranging from 13.59 µg/L for ofloxacin to 1.02 mg/L for piperidic acid (Backhaus

and Grimme, 2000). Backhaus and Grimme concluded that the impact of antibiotics on ecosystem functions such as nitrification processes should be investigated (Backhaus and Grimme, 2000).

Froehner et al. (2000) reported that the standardized (bioluminescence) short-term acute toxicity test (30 minutes) underestimated toxicity of nalidixic acid to *Vibrio fischeri* (i.e. no toxicity effects detected) but two longer term tests conducted over 7 hours and 24 hours predicted delayed toxicity to within a factor of 2. The EC_{50} for the 7-hour growth inhibition test was 0.12 mg/L (0.5 μ mol/L) and the EC_{50} for the 24-hour bioluminescence inhibition assay was 0.21 mg/L (0.9 μ mol/L). As the mechanism of action for FQs involves inhibition of enzymes, effects are more likely to be demonstrated during the longer-term tests (Froehner et al., 2000). Given that the process of nitrogen cycling takes longer than 30 minutes, effects should be measured over several hours to days.

Wollenberger et al. (2000), conducted acute and chronic toxicity tests of various antibiotics including oxolinic acid on *Daphnia magna*. The reported 24-hour and 48-hour EC_{50} values were 5.9 mg/L and 4.6 mg/L, respectively. The authors found that toxicity values for bacteria were two to three orders of magnitude lower than toxicity values for higher trophic levels such as crustaceans. In the reproductive test with oxolinic acid, no test organisms survived for the test duration at oxolinic acid concentrations greater than 0.75 mg/L. This is significant because oxolinic acid is used commonly in fish farming (Wollenberger et al., 2000).

1.1.9 Ecosystem Impacts of Fluoroquinolones

One question that has not been answered is what effect FQs may have on nutrient cycling mediated by microorganisms. Both cyanobacteria and green algae display sensitivity to, and shifts in population dynamics in the presence of, antibiotics (Harass et al., 1985). Effects on algae have been observed at low doses and are comparable with similar industrial chemicals labelled as toxic in the aquatic environment (Stuer-Lauridsen et al., 2000). These effects may

occur so gradually as to be undetected leading to irreversible change that may instead be attributed to naturally occurring evolutionary change (Daughton and Ternes, 1999). Algae form the basis of the food chain and small decreases in algal populations such as *Chlorella* spp. and *Pseudokirchneriella* (formerly *Selenastrum*) spp. caused by antibiotics such as metronidazole may have significant effects on ecosystem balance (Lanzky and Halling-Sorensen, 1997; Alexy et al., 2004).

Inhibition of bacteria by antibiotics may also affect biogeochemical cycles (e.g. carbon, nitrogen, sulphur and phosphorus) as well as decomposition of organic matter. Due to their function and widespread presence, microorganisms can be environmentally relevant indicators of pollution. Adaptation of a community through replacement of sensitive species with resistant organisms may result in significant ecological effects (van Beelen and Doelman, 1997). Long-term chronic exposure to low concentrations may cause gradual shifts in community structure and gradual changes in community function.

1.1.10 Effects on Community Composition and Function

Knapp et al. (2005), assessed both the fate and effects of enrofloxacin in a field mesocosm (surface water) and monitored microbial community changes using DGGE, enrofloxacin disappearance and ciprofloxacin formation using LC/MS. Variable light conditions were used to simulate different portions of the water column. This was essentially a follow up to the study by Cardoza et al. (2005), mentioned previously in Section 1.1.6. The study concluded that the microbial community was not significantly affected either in terms of diversity or apparent resistance.

Al-Ahmad et al., (1999), investigated cipro biodegradation rate using growth inhibition, colony forming units (CFUs) and a Closed Bottle Test (CBT). High inhibition of *Pseudomonas putida* was observed in the growth inhibition test but only a weak effect in the CFU monitoring and none in the CBT. In the presence of cipro, bacteria still grew and formed visible colonies within

the incubation period specified for the test. However, these colonies might have been smaller than those formed on agar without cipro if growth was inhibited. The CFU test does not consider colony size. Furthermore, none of the compounds was readily biodegradable. The authors attributed these results to inactivation of the compound by sorption (i.e. low bioavailability of the compounds). Kümmerer et al. (2001), investigated the biodegradability, and hence the elimination of genotoxicity, of cipro and ofloxacin using growth inhibition, CFUs and a CBT. According to the results of the CBT, neither of the FQs was considered readily biodegradable over a 40 day period. Although growth inhibition was present over 8 days for cipro and 11 days for ofloxacin, the inhibition disappeared. 50% growth inhibition for cipro occurred at 80 µg/L and at 10 µg/L for ofloxacin. The CFU results indicated only a minority of the aquatic species monitored were inhibited. The authors noted that these tests are more relevant to the most numerable species and further studies are required to assess the effects of these compounds on whole population dynamics. These tests and the test by Knapp et al. (2005) indicated little effect of FQs on the assessed microbial community, but they did not address effects on specific guilds or clades of microorganisms, or on community function.

Benthic Macroinvertebrates

Maul et al. (2006), examined the effect of chronic exposure to cipro on stream microbial community level physiological profiles (CLPP) and growth indices of detritivorous amphipods and caddisflies. Reduced respiration in leaf-associated microbial communities was observed for samples exposed to 100 µg/L over 12 days. However, no effects were observed at a concentration of 1.0 µg/L or 10 µg/L and no effects were observed on detritivores. These results suggest that cipro may affect leaf-associated microbial communities but other factors such as adsorption and microbial redundancy prevented detection of any effects on detritovore processing of leaf material. The authors did not attempt to account for concentrations of cipro adsorbed to organic matter and concluded that future research should evaluate the implications of cipro partitioning to organic material as well as more sensitive stream microbes and microbially mediated ecosystem processes such as nitrogen cycling.

1.1.11 Effects on Nitrification

FQs have a broad spectrum of activity and are, therefore, likely to affect the entire sludge bacterial community including gram-negative bacteria responsible for nitrification. This may result in a shift in microbial population which in turn may affect the activity of the sludge such as a reduction in nitrification capacity. Nitrification is an important process in nitrogen biogeochemistry that seems to be controlled in many environments by relatively few species of slow-growing chemoautotrophic bacteria. However, there is growing awareness of the important role archaea play in nitrification in some environments, notably marine and soil habitats (Francis et al., 2007). Archaea use various reductive pathways, both assimilatory (nitrate assimilation and nitrogen fixation) and dissimilatory (nitrate respiration and denitrification). However, these processes are less well known than the prokaryotic processes of nitrogen metabolism (Cabello et al., 2004). Furthermore, there is very little understanding of the role of archaea in freshwater sediment nitrification, or of the sensitivity of these archaea to environmental stressors, and the generalized sensitivity of nitrification to environmental stressors may have a different root cause than the historic explanation of low bacterial diversity and functional redundancy. The low diversity makes this group of organisms, and hence the process of nitrification, particularly vulnerable to toxic chemicals.

Halling-Sorensen (2001) investigated the toxicity of antibacterial agents on environmentally relevant bacteria using activated sludge, specifically inhibitory effects on growth and nitrification. The effects of ten antibacterial compounds including oxolinic acid on growth inhibition and nitrification were assessed. In general, antibacterials with a broad spectrum of activity were more potent than narrow-spectrum antibacterials. Growth inhibition of activated sludge bacteria was assessed over 4 to 6 hours using a standardized ISO test and also using the pour plate method over 48 hours. EC_{50} for oxolinic acid was 0.1 mg/L and 0.064 mg/L, respectively. The effect of oxolinic acid on the nitrification rate of activated sludge bacteria was also assessed and oxolinic acid was found to stimulate nitrification. However, when growth inhibition of *Nitrosomonas europaea* was studied using the pour-plate method, the EC_{50} value was 1.0 mg/L (Halling-Sorensen, 2001).

The author was unable to account for the increased sensitivity during the pour-plate tests or for the stimulatory effect of oxolinic acid on nitrification. An additional challenge was the difficulty assessing nitrification inhibition rates. Finally, the author was not able to explain why the EC₅₀ values for growth inhibition using *N. europaea* were higher for most compounds including oxolinic acid compared to the values for activated sludge bacteria growth inhibition.

The author concluded that the EC₅₀ values were in the µg/L range for most compounds and that more work should be done to assess the effects on nitrogen cycling because of the difficulties associated with available methods.

Inhibition of denitrification by FQ in marine environments

Costanzo et al. (2005), evaluated the effects of short-term exposure to antibiotics, including cipro and norfloxacin, on denitrification rates in sewage effluent and downstream from a sewage discharge in Brisbane, Australia. The results of the study indicated no significant difference between treatments and control. However, this may be explained by the effect of seawater cations (Ca²⁺ and Mg²⁺) inhibiting the action of cipro, and suggests a need to test the effects of FQs on denitrification in freshwater.

1.1.12 Summary of FQs the Environment

FQs are synthetic antibiotics and are structurally variable within their class. They are active against both gram-positive and gram-negative bacteria and are approved for both human and veterinary use. FQs are generally only partially metabolized and enter the environment through waste water, application of biosolids and through direct application to surface waters during aquaculture. FQs have been measured in surface water, sediments and soils. Fate mechanisms are dominated by photodegradation and partitioning to organic matter. FQ antibiotics are rapidly degraded in surface water by UV light but are insensitive to hydrolysis and temperature. FQs partition to organic matter, both within the water column and in sediments. This results in a

short half-life in water but persistence in sediments. Therefore, they may affect microbial populations in sediments.

Environmental effects of FQs include acute and chronic toxicity, possible alterations of energy cycling and resulting food chain effects and antimicrobial resistance. Based on the usage, environmental occurrence and toxicity of FQs, there are a number of knowledge gaps which, if addressed, would improve our understanding of FQ behaviour and effects in the environment. Few studies have assessed the effects of FQs on benthic organisms or entire benthic microbial communities at environmentally relevant concentrations. Effects on ecosystem processes such as nutrient cycling are not well understood. Although standardized tests are generally not long enough or sensitive enough to capture effects on less numerous microbial populations such as nitrifying microorganisms, reliable non-standardized methodologies have not been developed.

1.2 The Nitrogen Cycle

The nitrogen cycle is a complex biogeochemical cycle comprising terrestrial, atmospheric and aquatic components. Nitrogen is an essential element found in amino acids, proteins, nucleic acids and chlorophyll. The earth's atmosphere is comprised of approximately 78 % nitrogen gas (N_2), of which approximately 99.636 % is in the form of the ^{14}N isotope and 0.364 % is in the form of the ^{15}N isotope (De Laeter et al., 2003). Plants, algae and bacteria can take up inorganic nitrogen as either nitrate (NO_3^-) or ammonium (NH_4^+). However, few processes convert molecular nitrogen (N_2) to inorganic nitrogen because it is relatively unreactive due to a thermodynamically stable triple bond ($N \equiv N$) (Madigan et al., 2000).

Although abundant in the atmosphere, nitrogen is often a limiting nutrient for plant growth. Very few organisms can cycle nitrogen through its inorganic forms (NO_3^- , NO_2^- , NH_3 , NH_4^+). Most inorganic nitrogen is biochemically cycled by specialized microorganisms (Bianchi, 2007). Humans, like all multicellular animals, obtain nitrogen only from dietary sources so the environmental stability of primary producers is essential to human survival. An environmental

stressor such as an antibiotic targeting prokaryotic organisms could alter or uncouple nitrogen cycling processes in local environments, affecting the processing and availability of nitrogen to eukaryotic organisms.

1.2.1 Nitrogen Cycle Processes

The five main processes which make up the nitrogen cycle are nitrification, denitrification, ammonification, assimilation and nitrogen fixation (Figure 1-1). Mineralization is the production of inorganic nitrogen from organic nitrogen and includes ammonification.

Immobilization is the reverse; the incorporation of inorganic nitrogen to organic nitrogen forms and includes uptake, assimilation and denitrification. While other processes such as anammox are important in some habitats, the five highlighted processes represent the most important in terms of energy and material (N) flow through aquatic ecosystems. This series of experiments was designed to assess what, if any, effects cipro has on each of three of the processes in the nitrogen cycle: nitrification, denitrification and ammonification (mineralization)/DNRA.

Respiration is the oxidation of organic matter to produce energy and, although not part of the nitrogen cycle, directly affects nitrification and denitrification as a result of O₂ distribution and inorganic nitrogen availability. Also, because most of ammonification comprises mineralization of organic compounds to ammonia, respiration was used as a proxy measure for ammonification.

Figure 1-1: The Nitrogen Cycle

Adapted from Madigan et al., 2000; Herbert, 1999

Nitrification NH_4^+ to NO_2^- to NO_3^- (oxidation)

Nitrification is the microbially mediated oxidation of ammonium to nitrite and nitrate. Nitrifying bacteria are virtually ubiquitous in soil and water and may be especially abundant in ammonia-rich environments such as surface waters downstream from sewage treatment facilities (Madigan et al., 2000).

Nitrification occurs in a two step process (ammonia oxidation and nitrite oxidation):

(Bianchi, 2007)

Nitrifying bacteria consume oxygen and oxidize NH_4^+ generated by the decomposition of organic matter (mineralization of organic nitrogen-containing compounds) to produce NO_2^- and NO_3^- . Nitrification in aquatic sediments is associated with a small and phylogenetically constrained group of bacteria (Ward, 2007). Nitrifying bacteria comprise two separate groups of predominantly gram-negative organisms: ammonia-oxidizing bacteria and nitrosifiers (*Nitrosomonas*, *Nitrosococcus*, *Nitrospira*, *Nitrosolobus* and *Nitrosovibrio*) and nitrite-oxidizing bacteria (*Nitrobacter*, *Nitrospina*, *Nitrococcus* and *Nitrospira*). Most nitrifying bacteria are obligate aerobic chemolithotrophs (autotrophs) which derive energy solely from the oxidation of reduced inorganic nitrogen compounds (ammonium and nitrite) and use CO_2 as their carbon source to create organic compounds (Madigan et al., 2000; Jaffe, 1992). More specifically, ammonia-oxidizing bacteria are considered to be strictly autotrophic while nitrite-oxidizing bacteria have limited heterotrophic potential (Ward, 2007).

Nitrification is oxygen-dependent and typically occurs in circulating or flowing surface waters and near-surface soils and sediments. The activity of these nitrifying bacteria in sediments is particularly sensitive to the absence of dissolved oxygen (Bianchi, 2007). Nitrification is therefore also dependent on light which stimulates photosynthesis and diffusion processes which affect O_2 distribution (Risgaard-Petersen et al., 1994).

Denitrification **NO_3^- to $\text{N}_2 + \text{NO} + \text{NO}_2$ (gas)**

The process of nitrate or nitrite reduction to nitrogen gas (N_2 , NO or N_2O) returns nitrogen to the atmosphere and is known as denitrification or dissimilatory reduction of nitrate or nitrite.

Denitrifying bacteria (e.g., *Thiobacillus denitrificans*, *Paracoccus denitrificans*, *Pseudomonas denitrificans*) are predominantly gram-negative, facultative anaerobes which derive energy from the anaerobic oxidation of organic compounds, (Jaffe, 1992) using nitrate as an alternative electron acceptor to oxygen during respiration.

(Bianchi, 2007)

When oxygen is present, aerobic respiration will occur even if nitrate is available. Conventional denitrification ability is widespread in bacteria (Ward, 2007). Some denitrifying bacteria will also reduce other electron acceptors (iron, manganese, sulphate, sulphide) and can also grow by fermentation (methanogenesis). Denitrifiers are a very phylogenetically diverse group of organisms. There are 17 genera of facultative anaerobic bacteria which can utilize nitrate as an oxidizing agent (Jaffe, 2000). Therefore, denitrifying bacteria are metabolically diverse with respect to generating energy (Madigan et al., 2000) and this diversity may offer reduced sensitivity to chemicals.

Denitrification in surface waters can be supported by nitrate supplied either by diffusion from overlying water (direct denitrification) or by nitrification in the sediments (coupled denitrification) (Rysgaard et al., 1994). Sediments are often an important source of dissolved inorganic nitrogen for denitrification (Soonmo, Gardner and Kana, 2001). Other factors responsible for regulating denitrification rates are temperature (Davidson and Seitzinger, 2006), O_2 consumption in sediments (Seitzinger, 1988 and 1990), external nitrogen inputs, mineralization of organic matter and other bacterial processes occurring under anoxic conditions such as sulphate reduction or methanogenesis (Seitzinger, 1988).

Anammox (anaerobic ammonia oxidation), the oxidation of ammonia coupled to nitrate reduction, yields N_2 . The mechanism is unknown but is thought to be common in wastewater and oxygen-depleted zones (Groffman, 2006).

The ecological significance of denitrification is that it (along with anammox) represents the only permanent sink in the nitrogen cycle by converting N into a form that cannot be used by most other organisms. In some habitats denitrification can ameliorate excess N loading (Seitzinger and Nixon, 1985) and is ultimately responsible for N-limitation of primary producers in a variety of habitats.

Dissimilatory Reduction of Nitrate to Ammonium (DNRA)

Dissimilatory reduction of nitrate to ammonium (DNRA) is the reduction of NO_3^- to NH_4^+ rather than nitrogen gas (Fennel et al., 2009). DNRA results in conservation of nitrogen in bioavailable form as opposed to denitrification which is a sink for nitrogen. The significance of DNRA in aquatic sediments is still not clear (Binnerup et al., 1992). An and Gardner (2002) measured DNRA using MIMS and HPLC in a shallow estuary in Texas and reported that DNRA consumed 15 to 75 % of added $^{15}NO_3^-$ compared to only 5 to 29% for denitrification. It is thought that high organic carbon content relative to nitrate concentrations favours DNRA over denitrification but there is little information about the competition between organisms response for DNRA compared to denitrification (Canavan et al., 2007). Christensen et al., (2000) studied denitrification and DNRA in sediments below marine fish farms in Denmark and reported DNRA activity 4 to 6 times greater than denitrification activity below the fish cages ($7 \mu\text{mol N/m}^2/\text{d}$) when nitrate concentrations were $13 \mu\text{M}$. High sulfide concentrations which inhibit denitrification may result in increased DNRA as available NO_3^- which is not consumed by denitrification is available to be consumed by DNRA. Sulfide may also act as an electron donor for DNRA (An and Gardner, 2002).

Ammonification/mineralization

Ammonification is the process where organic nitrogen is returned to the environment in the form of ammonia by decomposition of organic matter (also called N mineralization) mediated by heterotrophic bacteria or via excretion of organic matter from animals. Decomposition of organic matter is the only major source of reduced nitrogen other than nitrogen fixation. Bacteria, primarily heterotrophs, which remineralize organic matter as a carbon source leave behind ammonia which is then recycled by other organisms. Ammonification can occur in sediments or in the water column (Bianchi, 2007). Ammonifying bacteria are a diverse group of heterotrophic generalist organisms and may be anaerobic, aerobic or facultative with respect to O₂ respiration (Ward, 1996a). Ammonification occurs in oxic and anoxic conditions but ammonium generally does not accumulate because of nitrification and assimilation. Nitrification and ammonification may be tightly coupled in the photic zone of surface waters especially in the open ocean where material that is degraded is rapidly ammonified and nitrified. Nitrification and ammonification may become uncoupled in oxygen-depleted zones where lack of oxygen inhibits nitrification (Ward, 1996a). Ammonification is a very important process in the transformation and recycling of a limiting nutrient from complex organic compounds back to the dissolved inorganic nutrient pool.

Assimilation ***NH₄⁺ to N(NH₃)-R***

Inorganic nitrogen compounds such as NH₃ or NH₄⁺ can be oxidized to NO₂⁻ or NO₃⁻ or they can be taken up and used by organisms in the formation of proteins and other essential compounds (organic nitrogen). This is referred to as ammonia assimilation. Converting inorganic nitrogen to organic nitrogen makes nitrogen available to ecosystem food chains (Madigan et al., 2000). Since plants generally obtain most nitrogen from nitrate via reductive assimilation, direct ammonia assimilation provides a significant competitive advantage with respect to energy saving (Jaffe, 2000). Nitrate may also be simultaneously reduced and assimilated. This process is referred to as assimilatory nitrate reduction and is likely to be a dominant process when reduced nitrogen is limited such as during aerobic conditions (Jaffe, 2000).

Biological nitrogen fixation is the biologically-mediated process of reducing atmospheric N_2 gas to NH_3 , NH_4^+ or any organic N compound in the presence of nitrogenase enzyme (Madigan et al. 2000; Jaffe, 2000). Nitrogen is fixed by a phylogenetically and physiologically diverse array of highly specialized microorganisms including bacteria, cyanobacteria, actinomycetes and archaea (Ward, 2007). This inorganic nitrogen can then be used by terrestrial plants or marine algae. Atmospheric N_2 fixation can also occur during lightning strikes. However, this is thought to comprise less than 10% of total global N fixation. Industrial fixation occurs during fertilizer production but most ammonia is further processed to urea and ammonium nitrate. Industrial processes such as nitrogen fertilizer production, increased planting of leguminous crops and combustion of fossil fuels, almost doubles the rate of naturally occurring (i.e. biological and atmospheric) total global nitrogen fixation (Ayers et al., 1994). Nitrogen fixation was beyond the scope of this study as water contaminated by high levels of cipro (such as from WWTP effluent and perhaps feed-lot runoff) is expected to also have high loading of fixed nitrogen, and therefore nitrogen fixation is expected to be negligible. Inhibition of nitrogen fixation by cipro might then be significant statistically but environmentally irrelevant.

Summary of the Nitrogen Cycle

Nitrification is generally considered more sensitive to environmental stressors due to the low diversity of nitrifying obligate autotrophic ammonia-oxidizing and nitrite-oxidizing (some limited heterotrophic potential) bacteria. Given that nitrification is carried out by relatively small clades of closely related microbes, while other processes are carried out by large, diverse groups of microorganisms, nitrification may be the “weak link” in the nitrogen cycle and more vulnerable to the effects of environmental stressors like toxic chemicals. Denitrification and ammonification are often less sensitive than nitrification as result of the diversity of facultative heterotrophic organisms responsible for these processes.

1.2.2 Nitrogen Cycling In Aquatic Sediments

In aquatic sediments, nitrogen occurs mainly in organic form with a small fraction occurring as ammonia (NH_3). Typically NH_3 is formed by autolysis or mineralization by bacteria of organic matter. Ammonia is then dissolved in interstitial water or adsorbed by colloids within the sediments (Bianchi, 2007). In oxygenated environments, autotrophic bacteria convert ammonium into nitrate (nitrification). Under reducing conditions, denitrification is likely to occur. Nitrification and denitrification often occur in close proximity, often at the boundary between oxic and anoxic layers which usually occurs near the sediment-water interface.

A substantial part of the NO_3^- formed by nitrification afterwards diffuses to the anaerobic denitrification zone where it is reduced to N_2 (Nielsen et al., 1992). Nitrogen cycling processes in aquatic sediments are therefore dependent on dissolved oxygen concentrations, specifically the depth distribution of dissolved oxygen (Rysgaard et al., 1994).

Other factors contributing to exchange of nitrogen between sediments and surface water are: oxygen distribution, sulphide content and redox status of the sediments (An and Gardner, 2002) and the overlying water column and reduced microzones in aerobic zones within the sediments (Jenkins and Kemp, 1984); sorption/desorption processes such as cation exchange (Matson and Vitousek, 1987); microbial respiration (Smith et al., 2006); excretion by benthic organisms (Henriksen et al., 1981) and, depositional flux of nitrate, ammonium and organic matter to sediments (Cowan et al., 1996).

1.2.2.1 Direct Denitrification

Oxygen affects the flux of nitrogen across the sediment-water interface. Diffusive gradients may cause NO_3^- formed by nitrification in surface waters to flow downwards to sediments and be consumed by denitrification at the oxic-anoxic interface. The direction of the gradient determines the direction of nitrate diffusion (Jorgensen and Boudreau, 2001).

When nitrate concentrations in the surface water are high, the main source of nitrate for sediment denitrification is diffusion from above. Under conditions of low oxygen, the oxic zone is thin so diffusion of nitrate is rapid. Nitrate fluxes are high and subsequently, denitrification rates are also high. This is referred to as direct denitrification (Fennel et al., 2009). However, under high oxygen conditions, the oxic zone is relatively thicker so diffusion of nitrate from above is slow. Therefore, nitrate fluxes are low and denitrification rates become more closely tied to sediment nitrification (Jorgensen and Boudreau, 2001).

When nitrate concentrations in the surface water are low, the main source of nitrate for denitrification is nitrification occurring in the oxic layer. Under low oxygen conditions, nitrification rates decrease causing sediments to lose ammonium to the water column and denitrification rates also drop. Under high oxygen conditions, nitrification increases so denitrification rates are relatively higher (Jorgensen and Boudreau, 2001).

1.2.2.2 Coupled Nitrification-Denitrification

When organic material is deposited, nitrogen becomes buried in the sediments with the organic matter. Anaerobic degradation causes release of dissolved organic nitrogen and ammonium. NH_4^+ formed during anaerobic degradation of organic matter may either flow into overlying surface water to support nitrification or be retained in sediments. Sedimentary nitrogen cycling is strongly affected by interactions between oxygen and nitrogen metabolism. When the NH_4^+ arrives in the oxic zone, NH_4^+ is either lost to the surface water or nitrification occurs (Jorgensen and Boudreau, 2001). Coupling between nitrification and denitrification occurs when nitrification products (NO_2^- , NO_3^-) are transported across the redox boundary (Bianchi, 2007).

When organic matter deposition increases, ammonium from remineralization also increases. Coupled nitrification-denitrification can increase in response. However, when organic matter deposition is very high, oxygen consumption due to aerobic degradation of this material can greatly restrict the oxic layer. As the oxic layer becomes thinner, nitrification decreases and

coupled denitrification can also decrease as a result (Jorgensen and Boudreau, 2001). Rysgaard et al. (1994) reported that total denitrification would decrease under high O_2 conditions because direct denitrification would decrease but coupled denitrification would be stimulated except when nitrate concentrations in overlying water are low (direct denitrification decreases less than the increase in coupled denitrification). Coupled nitrification-denitrification is therefore also indirectly dependent on oxygen uptake during respiration and mineralization of organic matter (Seitzinger, 1990).

Risgaard-Petersen et al. (1994) measured the effect of light penetration, and therefore photosynthesis, on nitrification and denitrification in sediment from a Scandinavian fjord and reported increased oxygen penetration into sediments which stimulated nitrification resulting in an increased rate of nitrate release during illumination. This also caused an increase in coupled denitrification and reduced the rate of denitrification of nitrate supplied from the water column. However, coupled denitrification represented a small fraction (~14%) of total denitrification. Smith et al. (2006) measured direct and coupled-denitrification rates in the benthic sediments of two rivers within the upper Mississippi River basin that received variably high nitrate runoff from agricultural sources and concluded that coupled nitrification-denitrification was not a significant source of N_2 production. Overall, denitrification rates were positively correlated with nitrate concentration but not with sediment oxygen demand.

In general, heterotrophic bacteria have a much higher affinity for O_2 and are likely to out-compete nitrifying bacteria under low O_2 conditions (Blackburn, 1990).

1.2.3 Anthropogenic Influences on Nitrogen Cycling

Anthropogenic activities which affect global nitrogen cycling include agricultural runoff (increased denitrification and leaching of nitrates into surface and groundwater), wastewater treatment effluent, landfill leachate, draining of wetlands and fossil fuel combustion (release of solid nitrogen as NO_x , N_2O to the atmosphere). Galloway (1998) estimates that anthropogenic

activities including energy production, fertilizer production and cultivation of crops fixes approximately the same quantity of nitrogen (~150 Tg N/year) as biological fixation (90-130 Tg N/year).

Anthropogenic contaminants have been shown to affect nitrogen cycling processes, of which nitrification appears to be the most sensitive process. Atrazine, a widely-used synthetic herbicide, significantly altered N-cycling rates in stream sediments at a concentrations as low as 10 µg/L (Laursen and Carlton, 1999). At 10 µg/L, aerobic respiration (and, therefore, ammonification) was stimulated, reducing the thickness of the oxic zone. Nitrification and denitrification were also stimulated, possibly due to enhanced supply of ammonium to nitrifiers and coupling of nitrification and denitrification. Stimulation of aerobic respiration continued at atrazine concentrations up to 100 µg/L; however, nitrification and denitrification were inhibited at atrazine concentrations of 50 and 100 µg/L, respectively. Paraquat, another widely-used herbicide, completely inhibited both ammonium oxidation and nitrite oxidation for 40 days at concentrations as low as 1 mg/L. However, atrazine inhibited only ammonium oxidation at concentrations ranging from 5 to 10 mg/L for 40 days (Gadkari, 1988).

Trace metals have an effect on microorganisms especially nitrifying bacteria. Zinc concentrations ranging from 500 µg/L to 10 mg/L had an inhibitory effect on nitrate production and led to increased ammonium in sediments. Low levels of copper (1 µg/L) have a slight inhibitory effect on nitrification over a 24 hour period (Allanson and Baird, 1999). Free nickel and cadmium cations inhibit ammonium oxidation but not nitrite oxidation at concentrations of approximately 1 mmol/L (Hu et al., 2002).

The studies above, considered together, suggest that nitrification is generally more sensitive to a variety of chemical stressors than other nitrogen cycling processes. Similarly, cipro in freshwater sediments might be expected to have a greater impact on nitrification than on denitrification or ammonification, particularly if nitrification in freshwater sediments is dominated by bacteria. However, there is very little research on the impacts of antibacterial agents on N-cycling

processes in natural aquatic systems. It is important to determine the potential ecological impacts of contaminants such as cipro because aquatic ecosystems are complex and provide valuable ecological services. These services include amelioration of eutrophication by removal of excess nitrogen (denitrification), detoxification of ammonium through oxidation, and production of organic carbon that serves as the base of the food web through N-assimilation and nitrification.

1.3 Hypothesis and Rationale

1.3.1 Hypothesis

Given that ciprofloxacin is microbially active and was designed to have specifically detrimental physiological effects on pathogenic bacteria, it is reasonable to infer that cipro may also adversely affect environmental bacteria involved in nitrogen cycling processes. The purpose of these experiments was to assess what, if any, effect ciprofloxacin has on nitrogen cycling processes, specifically nitrification, denitrification and ammonification (mineralisation), in a simple microcosm consisting of lake sediment and surface water. Since the bacteria responsible for nitrification are less diverse and more specialized than bacteria carrying out other nitrogen cycling processes, the impact of ciprofloxacin is anticipated to be most significant on nitrification rates relative to denitrification and ammonification; microbial processes which are generally considered less sensitive to the effects of environmental stressors. Given that these processes are linked, an effect on one process would eventually be reflected in the other nitrogen cycling processes. By assessing the effects of various cipro concentrations on production of nitrogen species (including nitrate, ammonium and N_2) relative to reference microcosms, it may be possible to predict the effect of cipro on each of these processes.

1.3.2 Experimental Design and Rationale

Four processes were studied over the course of four experiments. The specific objective was to assess the impact of cipro on nitrification, denitrification, ammonification, and respiration.

Experiment 1: Effect of cipro on nitrate and ammonium fluxes

In experiment 1, sediment was spiked with various concentrations of cipro and the overlying water was amended with excess ammonium and then nitrate. Measurements of ammonium and nitrate concentrations in the overlying water provided an indication on the effect of cipro on nitrate and ammonium fluxes. The addition of excess ammonium or nitrate to surface water was intended to saturate ammonium oxidizers and denitrifiers, respectively. The purpose of this experiment was to analyse the concentrations of both ammonium and nitrate over time in the overlying surface water following a spike with each compound and calculate rates of flux of ammonium and nitrate.

Experiment 2: Effect of cipro on nitrification and DNRA using ^{15}N stable isotope methods

The objective of this experiment was to directly study the effect of cipro on nitrification and ammonification. In addition to measurements of nitrate and ammonium fluxes, rates of nitrification and ammonification were measured directly using the ^{15}N stable isotope microdiffusion method to study changes in isotopic composition of the nitrate and ammonium pool (Nielsen, 1992). This method, referred to as isotope dilution, provides a measure of nitrification where nitrate is released to overlying water (Jensen et al., 1996). In this method, the ^{15}N label in the nitrate pool becomes diluted as more nitrate is produced from ^{14}N ammonium. This method differs from the isotope pairing method which is used to measure denitrification. In the isotope pairing method, individual N isotopes "pair" to form the dinitrogen (N_2) molecule. Both isotope pairing and isotope dilution can be measured in the same experiment by addition of $^{15}\text{NO}_3^-$ (Experiments 3 and 4); the two methods, dilution and pairing, specifically measure nitrification and denitrification, respectively.

Experiment 3: Effect of cipro on denitrification under low nitrate conditions using MIMS

The objective of this experiment was to study the effect of cipro on denitrification and respiration in a nitrate limited environment. In addition to measurements of nitrate and ammonium fluxes, rates of denitrification and respiration were measured directly based on changes in $N_2:Ar$ and $O_2:Ar$ using membrane-inlet mass spectroscopy (MIMS).

N_2 production is normally difficult to quantify due to the high background concentrations of N_2 in the environment (Groffman et al., 2006). Accurate measurement of small changes in dissolved N_2 concentrations can be measured with a high level of precision using MIMS (Laursen and Seitzinger, 2002). This technique has become popular for studying denitrification because it provides rapid measurements on small samples with no pre-processing and provides high precision (0.03% for $N_2:Ar$ ratios) (Kana et al., 1994; Groffman et al., 2006).

This methodology allowed measurement of rates of denitrification, respiration and percent contribution by diffusion (% Diff). Membrane inlet mass spectrometry (MIMS) was used to determine the rate of denitrification for each treatment by calculating the rate of increase of N_2 relative to argon gas (Ar). MIMS was used to quantify changes in the ratios of dissolved $N_2:Ar$ and $O_2:Ar$ within the overlying water. By controlling the ratio of ^{15}N to ^{14}N in the microcosms, it was also possible to estimate the rate of denitrification of NO_3^- in the water column and also quantify coupled nitrification-denitrification.

The acetylene inhibition method of measuring denitrification rates (Stewart et al., 1967) was ruled out because acetylene selectively inhibits autotrophic nitrification without inhibiting heterotrophic nitrification (Knowles, 1990). As a result, the acetylene inhibition method has been shown to underestimate coupled denitrification, up to 25% in one study, which would defeat the objectives of this study (Steingruber et al., 2001).

Experiment 4: Effect of cipro on denitrification under non-limiting nitrate conditions using MIMS

The objective of this experiment was to study the effect of cipro on denitrification and respiration when nitrate is not limiting. Rates of denitrification and respiration were measured directly based on changes in $N_2:Ar$ and $O_2:Ar$ using MIMS, as described above.

2.0 MATERIALS AND METHODS

2.1 Experiment 1 – Net Flux of Ammonium and Nitrate under substrate-saturating conditions

Experiment 1 was conducted in two halves.

Potential Net Nitrification: The first half of the experiment consisted of spiking the overlying surface water in each of the microcosms with 70.6 $\mu\text{mol/L}$ (1 mg/L) ammonium. Samples of the overlying water were collected and analyzed for ammonium (ammonium flux into/out of sediments) and nitrate concentrations (nitrate flux into/out of sediments).

Potential Net Ammonification: The second half of the experiment consisted of spiking the overlying surface water in each of the microcosms with 70.6 $\mu\text{mol/L}$ (1 mg/L) nitrate. Samples of the overlying water were collected and analyzed for nitrate (nitrate flux into/out of sediments) and ammonium concentrations (ammonium flux into/out of sediments).

The overlying water was sampled at intervals over a period of 24 hours. All samples were analyzed for NH_4^+ and NO_3^- to assess both NO_3^- flux into/out of sediments and NH_4^+ flux into/out of sediments. Rates were calculated for each microcosm and averaged for each treatment. Rates of flux were calculated as micromoles of nitrogen per square metre per hour ($\mu\text{mol N/m}^2/\text{h}$).

Microcosm Set Up

Six microcosms were set up for the purpose of this experiment. Each microcosm consisted of a 500 mL glass jar with lid, approximately 20 g of sediment and approximately 450 mL of dechlorinated tap water. The microcosms were set up in the dark to prevent photodegradation of cipro from occurring during sediment amendment.

Three reference microcosms were not treated with cipro. Golet et al. (2003) reported cipro concentrations of 3.1 mg/kg in sewage sludge from a Swiss WWTP so this value was considered the theoretical upward limit of environmentally relevant concentrations for the cipro doses used in these experiments. The concentration of cipro in the three treated microcosms was approximately 2 mg cipro per kg of sediment. 5 mL of dissolved cipro solution was added to each of the three microcosms and allowed to equilibrate overnight. On June 7, 2007, dechlorinated tap water amended with 70.6 $\mu\text{mol/L}$ ammonium (1 mg N/L as ammonium chloride) was added to all of the microcosms. A 15 mL sample of this water was collected in a polyethylene centrifuge tube prior to addition into the microcosms to measure initial nitrate and ammonium concentrations. Samples of the overlying water (15 mL) from each microcosm were then collected and filtered (Whatman GF/F filter, nominal pore size 0.7 μm) at the following time intervals: 0.5, 2.5, 4.5, 8.5 and 21.5 hours. These samples were labelled and frozen at -20°C pending analysis.

The overlying water was then siphoned off carefully using a syringe so as to avoid sediment resuspension. Subsequently, dechlorinated tap water amended with 70.6 $\mu\text{mol/L}$ (1 mg N/L as sodium nitrate) was then added to all of the microcosms. A sample of this water was collected prior to addition into the microcosms. Samples from the overlying water from each microcosm were then collected using 50 mL polyethylene syringes and filtered (as above) at the following five time intervals over a 24 hour period: 0.5, 2.5, 4.5, 8 and 21 hours. These samples were labelled and frozen (-20°C) pending analysis.

The procedure for ammonium and nitrate analysis is described in Section 4.5 below.

The flux was calculated as the slope of a linear regression on dissolved nutrient concentration versus time (i.e. micromoles of nitrogen per square metre per hour ($\mu\text{mol N/m}^2/\text{h}$)). Rates were calculated for each microcosm and averaged for each treatment.

2.2 Experiment 2 – Nitrification using ^{15}N microdiffusion and nutrient fluxes

Nitrification and ammonification were measured directly using the ^{15}N stable isotope microdiffusion method described in Section 4.6.

Microcosm Set Up

Thirty microcosms were set up for the purpose of this experiment. Each microcosm consisted of a 500 mL glass jar with lid, approximately 20 g of sediment and approximately 450 mL of synthetic lake water (Ivorra et al., 1995) with 10% (atomic %) of the nitrate as $^{15}\text{NO}_3^-$. The total concentration of N as NO_3^- in the synthetic lake water was $70.6 \mu\text{mol/L}$ (equivalent to 1 mg N/L). The composition of synthetic lake water is described in Section 2.8. All 30 microcosms were set up in the dark to prevent photodegradation of cipro from occurring during sediment amendment.

This experiment consisted of five replicates of six treatments: Reference – 0 mg/kg cipro; Treatment 1 – 0.5 mg/kg cipro; Treatment 2 – 1.0 mg/kg cipro; Treatment 3 – 1.5 mg/kg cipro; Treatment 4 – 2.0 mg/kg cipro; Treatment 5 – 2.5 mg/kg cipro.

Twenty-five of the thirty microcosms were treated with 1 mL of ethanol and cipro solution and allowed to equilibrate for one hour (reference microcosms were not treated). The use of ethanol as a carrier for cipro is discussed in Section 2.9. The overlying water was decanted from each microcosm prior to treatment with cipro solution and fresh synthetic lake water was added approximately one hour after treatment. An aliquot of the synthetic lake water was collected prior to addition into the microcosms. Samples of the overlying water (5 mL for nitrate analysis, 10 mL for ammonium analysis and 100 mL for ^{15}N microdiffusion analysis) from each microcosm were then collected and filtered at the following two time points: 8 hours and 24 hours. In addition, 15 mL of sample were collected after 16 hours for nutrient analysis (5 mL for nitrate analysis, 10 mL for ammonium analysis). The decision to reduce the number of time

points analyzed was based on the linearity of changes in dissolved inorganic N species between 8 and 24 hours in the first experiment.

2.3 Experiment 3 – Denitrification under low nitrate conditions

Experiment 3 consisted of two parts: nutrient flux measurements and direct measurements of denitrification, percent contribution by diffusion (%Diff) and respiration based on changes in $\text{N}_2:\text{Ar}$ and $\text{O}_2:\text{Ar}$ using MIMS.

Microcosm Set Up

Sixty microcosms were set up for the purpose of this experiment. Each microcosm consisted of a 300 mL BOD bottle with stopper, approximately 20 g of sediment and approximately 300 mL of synthetic lake water (as described in Experiment 2). BOD bottles instead of jars were used to prevent loss of dissolved gases (N_2 , O_2 and Ar) from the microcosms. The difference in volume was accounted for in rate calculations. The total concentration of N as NO_3^- in the synthetic lake water was $7 \mu\text{mol/L}$ (equivalent to $0.1 \text{ mg NO}_3^-/\text{L}$). This concentration of nitrate approximates a lake in summer when nutrients have been depleted (Jensen et al., 1996). All microcosms were set up in the dark to prevent photodegradation of cipro from occurring during sediment amendment.

This experiment consisted of five replicates of six treatments: Reference (0 mg/kg cipro); Treatment 1 (0.5 mg/kg cipro); Treatment 2 (1.0 mg/kg cipro); Treatment 3 (1.5 mg/kg cipro); Treatment 4 (2.0 mg/kg cipro) and Treatment 5 (2.5 mg/kg cipro).

Two sets of BOD bottles for a total of sixty microcosms (one BOD bottle for each time interval) were required in order to collect samples for $\text{N}_2\text{:Ar}$ and $\text{O}_2\text{:Ar}$ analysis. Fifty of the sixty microcosms (excluding reference microcosms) were treated with 1 mL of ethanol and cipro solution and allowed to equilibrate for one hour. Approximately 300 mL of synthetic lake water was added to each of the 60 microcosms. An aliquot of the synthetic lake water was collected prior to addition into the microcosms.

Samples of the overlying water (15 mL for nitrate analysis, 10 mL for ammonium analysis and 5 mL for $\text{N}_2\text{:Ar}$ and $\text{O}_2\text{:Ar}$ analysis) from each microcosm were collected at the following time intervals: 8 hours and 24 hours. Samples for nitrate and ammonium analysis were filtered into centrifuge sample tubes. Water samples for $\text{N}_2\text{:Ar}$ and $\text{O}_2\text{:Ar}$ analysis were not filtered but collected in glass vials with Teflon septa by gravity siphoning using flexible plastic tubing from the neck of each BOD bottle. Approximately 500 μL of saturated ZnCl_2 preservative solution was added to the vial immediately prior to sample collection. Samples for nitrate and ammonium analysis were immediately frozen. Samples for $\text{N}_2\text{:Ar}$ and $\text{O}_2\text{:Ar}$ analysis using MIMS were stored submerged in deionized water at 4°C . The analytical method is discussed further in Section 2.7.

In addition to the microcosms set up as part of the experimental design, three microcosms were set up but were not treated with cipro in order to assess initial ^{15}N signature in the sediment. The three untreated microcosms used to assess initial ^{15}N signature in the sediment were shaken up to create a slurry of sediment and water. Once the slurry had settled, a sample of the overlying water was collected and analyzed using the method described in Section 2.7.

Three method blanks were also set up to assess the NH_4^+ content of the reagents used in the sample preparation process. A total of 122 water samples (including two method blanks) were collected for ammonium and nitrate analysis and 66 for $\text{N}_2\text{:Ar}$ and $\text{O}_2\text{:Ar}$ analysis (including two method blanks and three for ^{15}N signature).

2.4 Experiment 4 – Denitrification under non-limiting nitrate conditions

In Experiment 4, direct measurements of denitrification, percent contribution by diffusion (%Diff) and respiration were calculated based on changes in $\text{N}_2:\text{Ar}$ and $\text{O}_2:\text{Ar}$ using MIMS. Nutrient fluxes were not assessed.

Microcosm Set Up

The set up for Experiment 4 was repeated with a total concentration of NO_3^- in the synthetic lake water of $70.6 \mu\text{mol/L}$ (equivalent to 1 mg N/L). A 5 mL sample was collected for $\text{N}_2:\text{Ar}$ and $\text{O}_2:\text{Ar}$ analysis from each microcosm were collected at the following time intervals: 8 hours and 24 hours. Water samples for $\text{N}_2:\text{Ar}$ and $\text{O}_2:\text{Ar}$ analysis were not filtered but collected in glass vials with Teflon septa by gravity siphoning using flexible plastic tubing from the neck of each BOD bottle. Approximately $200 \mu\text{L}$ ZnCl_2 preservative solution (saturated) was added to the vial immediately prior to sample collection. Samples for $\text{N}_2:\text{Ar}$ analysis were stored submerged in deionized water at 4°C . The microcosms were discarded following sample collection. A total of 65 water samples were collected for $\text{N}_2:\text{Ar}$ and $\text{O}_2:\text{Ar}$ analysis.

2.5 Ammonium and Nitrate Analysis

Nitrate and ammonium concentrations were measured using methods adapted from the Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1998). Dissolved nitrogen species were quantified colorimetrically. Ammonium was measured using the phenate method (APHA method 4500- NH_3 F Phenate method) and nitrate by cadmium reduction to nitrite (APHA method 4500- NO_3^- E Cadmium reduction method). A summary of the method and deviations from the method are provided in Appendix 1.

Net fluxes of ammonium and nitrate were calculated by converting measured values of mg/L to micromoles N per m² per hour ($\mu\text{mol}/\text{m}^2/\text{h}$) by multiplying measured rates by total water volume in the microcosm and dividing by sediment surface area using the equation:

$$\text{Flux} = (\text{Final concentration} - \text{Initial Concentration}) \times \text{Volume} / \text{Area}$$

2.6 ¹⁵N Stable Isotope Microdiffusion

The stable N isotope dilution method was used to study nitrification and coupled denitrification occurring at the sediment-water boundary. The sample preparation method was adapted from Eyre et al. (2002) using methodology provided by Colorado Plateau Stable Isotope Laboratory (CPSIL) (Appendix 1).

Nitrification rates were determined using ¹⁵N microdiffusion technique adapted from Eyre et al. (2002). The methodological theory is that addition of magnesium oxide (MgO) makes the water sample alkaline and NH_4^+ is converted to NH_3 . The ammonium gas is captured and bound to an acidified GF/F filter (converting it back to ammonium) inside a diffusion packet. The filter is then rolled into a silver capsule which is combusted by an elemental analyser with an inline isotope ratio mass spectrometer (IRMS) and peaks measured for ratio of ¹⁴N:¹⁵N.

In order to measure nitrate concentrations, Devarda's alloy was added which acts as an electron donor. NO_3^- is reduced to NH_4^+ which is captured on the filter and measured in the same way.

2.6.1 Preparation of Diffusion Packets

Diffusion packets were prepared using a procedure provided by Colorado Plateau Stable Isotope Laboratory (CPSIL). Two filters were punched out from each GF/F filter using a 12 mm hole punch. Filters were placed in a loose foil packet in the muffle furnace at 500°C for 4 hours. After removal from the furnace, all work surfaces and implements were wiped down with ethanol or acetone (tweezers, scissors, glass vial, container for diffusion packets). Teflon tape

was cut into strips about 6 to 7 cm long. One filter was placed onto the left side of the strip of Teflon tape leaving about 1 cm on the left side. 20 μL 2.5M H_2SO_4 was pipetted onto the centre of the filter. The right half of the tape was folded over the filter. A glass vial was used to seal the teflon tape around the filter. Diffusion packets were stored in a sealed container immediately.

2.6.2 Sample Preparation

For each 100 mL sample added to a 250 mL Erlenmeyer flask, 100 mL of aqueous NaCl solution was added to decrease the solubility of NH_3 (g). Then 0.6 g MgO solid (98% ACS) was added to each flask along with one diffusion packet. Each flask was then covered with parafilm. The flasks were incubated at 30°C for 7 days. After 7 days, the diffusion packet was removed and replaced with a new diffusion packet. 150 mg Devarda's alloy was added to each flask and covered with parafilm. The flasks were returned to the incubator for another 7 days at 30°C.

Diffusion packets removed from the flasks were placed in a Nalgene desiccator for 7 days with approximately 50 mL of 5N H_2SO_4 . After 7 days, the filter was removed from the Teflon tape and the centre of the filter punched out using a 6 mm hole punch. The punched out portion was placed inside a silver capsule and rolled into a ball. Each ball was placed into a foil packet and labelled for shipping to Cornell Isotope Laboratory (COIL) for analysis. A total of 125 samples were submitted to COIL for analysis.

2.6.3 Sample Analysis

Percent composition of ^{15}N in solid samples submitted to COIL was measured using an elemental analyzer plumbed onto an isotope ratio mass spectrometer (EA-IRMS). COIL provided a description of the analysis method and EA-IRMS equipment (Appendix 1). Rates of nitrification and ammonification were calculated based on formulas adapted from Nielsen et al. (1992). 0.36 % ^{15}N was subtracted to account for naturally occurring background concentrations of the stable isotope (De Laeter et al., 2003).

Net nitrification rates were calculated as follows:

$$(\%^{15}\text{N}_8 / (\%^{15}\text{N}_{24} - 0.0036)) \times ((\text{Total } [\text{NO}_3^-]_8 + \text{Total } [\text{NO}_3^-]_{24}) / 2) \quad [\text{eq. 1}]$$

Where $\%^{15}\text{N}_{24}$ = Percent ^{15}N after 24 hours from $^{15}\text{NO}_3^-$

$\%^{15}\text{N}_8$ = Percent ^{15}N after 8 hours from $^{15}\text{NO}_3^-$

$[\text{NO}_3^-]_8$ = average nitrate concentration after 8 hours ($\mu\text{ mol N/L}$)

$[\text{NO}_3^-]_{24}$ = average nitrate concentration after 24 hours ($\mu\text{ mol N/L}$)

Net ammonification rates were calculated as follows:

Using the same calculation as net nitrification rate but inverting the formula because ammonia is produced not diluted:

$$((\%^{15}\text{N}_{24} - 0.0036) / \%^{15}\text{N}_8) \times ((\text{Total } [\text{NH}_3]_8 + \text{Total } [\text{NH}_3]_{24}) / 2) \quad [\text{eq. 2}]$$

Where $\%^{15}\text{N}_{24}$ = Percent ^{15}N after 24 hours from $^{15}\text{NH}_3$

$\%^{15}\text{N}_8$ = Percent ^{15}N after 8 hours from $^{15}\text{NH}_3$

$[\text{NH}_3]_8$ = average ammonia concentration after 8 hours ($\mu\text{ mol N/L}$)

$[\text{NH}_3]_{24}$ = average ammonia concentration after 24 hours ($\mu\text{ mol N/L}$)

Rates were converted to units of micromoles N per m² per hour ($\mu\text{mol}/\text{m}^2/\text{h}$) by multiplying measured rates by total water volume in the microcosm and dividing by sediment surface area and then dividing by 16 hours.

2.6.4 Inconclusive ¹⁵N Analyses

Two other approaches were attempted and discontinued. ¹⁵N analysis was attempted using a procedure adapted from Kieber et al. (1998) which uses chloroform extraction and FT-IR to analyze the concentration of an organic azide. This method was discarded due to poor resolution of the spectra which did not permit quantitative analysis of ¹⁴N and ¹⁵N.

¹⁵N analysis was also attempted using the diffusion method by Brooks et al. (1989). This method was discarded because excessive condensation in the test tubes caused the tin capsules to corrode and disintegrate so samples could not be analyzed.

2.7 N₂:Ar and O₂:Ar analysis using Membrane Isotope Mass Spec (MIMS)

The methodology used to analyze N₂:Ar and O₂:Ar using MIMS was adapted from Kana et al. (1994).

Water samples analyzed using MIMS are pumped directly from the sample container via a capillary tube into a glass inlet tube. The inlet tube passes through a water bath which regulates the temperature of the sample which then passes into a gas-permeable silicone tube where dissolved gases are sucked out of the line. As a result of reduced pressure in the tube, a vacuum is created and dissolved gases in the water sample diffuse out of the silicone tube and are swept under vacuum into the quadrupole mass spectrometer (QMS). The glass inlet tube has a U-bend into a dewar of liquid nitrogen. As gases are swept toward the mass spectrometer, water vapour and carbon dioxide are frozen and trapped preventing interference of CO with N₂ ($m/z = 28$ for each) and damage to filaments by water vapour. The QMS generates a magnetic field in sequence causing the molecules to oscillate. In the QMS, the N₂ molecules pick up a charge

which interacts with the QMS field. Smaller molecules travel further with an increased spiral radius. Therefore the distance along the QMS is critical to identifying the molecule. The distance along the path of flight determines the mass of the molecule (i.e. molecules with a smaller m/z travel further along the QMS). The standard used was tap water at a constant temperature of 20°C stirred slowly for at least 48 hours to achieve atmospheric equilibrium while preventing entrainment of gas bubbles. Concentrations of dissolved N_2 , O_2 and Ar were calculated using the solubility equations of Weiss (1970). However Ar is slightly easier to ionize than N_2 so a correction factor was required. The correction factor used was the average of Ar concentrations measured for each batch of samples. A standard was run after approximately 5 samples. Drift was corrected by integrating the values of the standards run before and after each batch of samples.

Denitrification was estimated directly based on changes in $N_2:Ar$ and $O_2:Ar$ using MIMS (Kana et al., 1994; Kana et al., 1998; Laursen and Seitzinger, 2002). N_2 was measured at $m/z = 28, 29$ and 30 which correspond to $^{14}N_2$, $^{14}N^{15}N$ and $^{15}N_2$. O_2 was measured at $m/z = 32$. Different isotopic compositions of nitrogen gas ($^{28}N_2$, ^{29}N , $^{30}N_2$) were monitored after adding $^{15}NO_3^-$ to the overlying water. Assuming that ^{14}N and ^{15}N randomly form N_2 gas, the production of each isotopic form would follow a binomial distribution. Therefore, where the proportion of ^{14}N is p (and ^{15}N is $1-p$), the production rates for each species ($^{28}N_2$, ^{29}N , $^{30}N_2$) would be p^2 , $2p(1-p)$ and $(1-p)^2$, respectively (An et al., 2001).

Measured ratios of isotopes included $^{28}N_2$, $^{29}N_2$, $^{30}N_2$, and calculated ratios included $^{30}N_2: ^{28}N_2$ and $O_2:Ar$. Using these data, ratios of $^{29}N_2: ^{28}N_2$, and $^{30}N_2: ^{28}N_2$ were calculated based on the measured values.

Denitrification rates were calculated as follows:

$$DNR = \text{Total } ^{14}N \text{ denitrified} + \text{Total } ^{15}N \text{ denitrified}$$

Where Total ^{14}N denitrified is calculated as:

$$^{14}\text{N-DNR} = (([^{28}\text{N}_2]_{24} - [^{28}\text{N}_2]_8)/16) \times 2 \times (\text{Volume/Surface Area}) + 0.5 \times (([^{29}\text{N}_2]_{24} - [^{29}\text{N}_2]_8)/16) \times 2 \times (\text{Volume/Surface Area})$$

Total ^{15}N denitrified is calculated as:

$$^{15}\text{N-DNR} = (([^{30}\text{N}_2]_{24} - [^{30}\text{N}_2]_8)/16) \times 2 \times (\text{Volume/Surface Area}) + 0.5 \times (([^{29}\text{N}_2]_{24} - [^{29}\text{N}_2]_8)/16) \times 2 \times (\text{Volume/Surface Area})$$

Using measurements of $^{28}\text{N}_2$, $^{29}\text{N}_2$ and $^{30}\text{N}_2$, relative contribution of NO_3^- diffusion supporting direct denitrification versus coupled nitrification-denitrification could be calculated (Nielsen et al. 1992, Jensen et al., 1996)

% contribution by diffusion was calculated as follows:

$$(\text{Total } ^{15}\text{N denitrified} / 0.1) / \text{Total denitrification}$$

Respiration rates were calculated as follows:

Actual O_2 concentrations were calculated by multiplying the ratio of O_2 : Ar by the correction factor for Ar. Then average rate of O_2 production in $\mu\text{mol O}_2/\text{m}^2/\text{hour}$ was calculated as:

$$(([\text{O}_2]_{24} - [\text{O}_2]_8)/16) \times (\text{Volume of microcosm/surface area of sediment})$$

where $[\text{O}_2]$ is micromoles O_2/L of water.

It is important to note that the isotope dilution technique used is based on the following two assumptions:

1. That the ^{15}N diffusing through the oxic nitrification zone is evenly mixed with ^{14}N produced by nitrification and there is no diffusional bias favouring delivery of one form (presumably ^{14}N) over another;
2. Random and homogenous pairing is occurring between the ^{15}N and ^{14}N atoms formed by denitrification

(Nielsen et al., 1996)

2.8 Materials

Lake sediment used in all of the experiments was obtained from Ms. Emily Awad at the Ministry of Environment. The sediment used consisted of a very fine silty clay material with high organic content. A summary of the originating lake, ELA reference Lake 240, is attached in Appendix 1. A detailed characterization of the sediment is also presented in Appendix 1.

Ciprofloxacin was obtained from Sigma-Aldrich and was $\geq 98\%$ purity.

Lake water was prepared according to Ivorra et al. (1995) (Table 2-1). However the following growth media ingredients were not added as the intention of these experiments was to assess the function of the naturally-occurring sediment bacteria rather than to cultivate additional bacterial growth (biotin, BaCl_2 , CoCl_2 , thiamine, HEPES and cyanocobalamin). The concentration of NaNO_3 in the lake water solution was 6 mg/L or 70.6 $\mu\text{mol/L}$ as NaNO_3 . For ^{15}N analysis, the ratio of ^{15}N : ^{14}N was 1:9 (i.e. 10% ^{15}N , 90% ^{14}N).

Table 2-1: Composition of synthetic lake water – Modified Woods Hole MBL medium

Compound	Concentration (mg/L)
$\text{CaCl}_2 \cdot \text{H}_2\text{O}$	36.8
NaNO_3	6
NaHCO_3	3.36
K_2HPO_4	0.87
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	37
Na_2EDTA	4.36
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	3.15
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.2
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.02
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.006
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.01

These experiments were done under oligo-mesotrophic conditions (nitrate concentrations of 70 $\mu\text{mol N/L}$ or less).

2.9 Ethanol as a carrier for cipro

Ethanol was used as a carrier to administer cipro in order to promote even distribution of cipro within the sediments. Ethanol was selected because of its rapid volatilization compared to other carriers. DMSO was not used because of the high organic content potentially causing cipro to bind and alter the predicted effects. Furthermore, 1 mL ethanol administered to the sediments and water was considered too dilute to adversely affect sediment bacteria populations and too small an amount to act as a nutrient.

Prior to starting experiment 2, two microcosms were set up to ensure no adverse effects of using ethanol as a carrier for cipro to promote even distribution within sediments. Oxygen content and bacteria population within the microcosm were measured. Dissolved oxygen (DO)

concentrations in the overlying water were measured every 3 hours over a period of 24 hours using a Winkler-type microelectrode (Unisense A/C, Aarhus, Denmark). The response of these electrodes is linear between 0 and 100% saturation. Electrodes were calibrated by sparging water in a calibration chamber with air (for 100% saturation) and N₂ (for 0% saturation).

In addition to assessing the effect of ethanol on DO, sediment microorganisms were cultured to assess the effect of ethanol on the sediment bacteria population. Approximately 1 mL of ethanol was added to the sediment in the second jar. An aliquot of sediment was removed from each jar and placed into two centrifuge tubes with 10 mL 0.1 M phosphate buffer. Both centrifuge tubes were shaken for 2 minutes and centrifuged for 20 minutes at 3,000 rpm. 0.1 mL of supernatant from each centrifuge tube was then applied to BBL tryptic soy agar using spread plate method and incubated at 30°C for 24 hours. This procedure was repeated after the sediment had been exposed to ethanol for 48 hours.

2.10 Statistics

Statistical analysis was performed using Systat 11. A summary of statistical analyses run on each data set is included in Appendix 3 with the results of the statistical analysis. 1-way ANOVA and post-hoc paired comparison tests were used to determine significance between cipro treatments. Prior to performing 1-way ANOVA, a one-sample KS test was performed and residuals were plotted to ensure the data were normally distributed. Alpha (α) of 0.05 was used to test the null hypothesis. Linear regression was used to assess dose-dependent effects on each process with increasing cipro concentration. Power analysis used to ensure an appropriate number of treatments and replicates was incorporated into the study design prior to the start of Experiment 2. Power analysis was also used after all experiments were completed to assess whether power could be increased with more treatments and/or replicates. Results of statistical analysis are incorporated into the relevant discussion sections below. Statistically different data groups are indicated on figures using the letters a and b.

3.0 RESULTS AND DISCUSSION

3.1 Experiment 1 (June 2007) – Effect of cipro on ammonium and nitrate fluxes under substrate-saturating conditions

In order to assess the effects of cipro on nitrogen cycling processes, net potential nitrification and ammonification were measured by analyzing nitrate and ammonium concentrations in overlying surface water of treated and reference microcosms over a 24 hour period.

3.1.1 Ammonium spike

In the first half of this experiment, the ammonium spike caused ammonium-oxidizers and nitrite-oxidizers to be saturated. Ammonium flux in the cipro-treated microcosms was significantly lower than the untreated microcosms ($F_{1,3} = 21.005$, $p = 0.020$) (Figure 3-1). Nitrate flux from sediments was also significantly lower in cipro-treated microcosms than from reference sediments ($F_{1,2} = 4.600$, $p = 0.165$) (Figure 3-2).

Figure 3-1: Ammonium flux following ammonium spike (Experiment 1)

Figure 3-2: Nitrate flux following ammonium spike (Experiment 1)

A comparison of Figures 3-1 and 3-2 indicates:

1. There was no significant difference in ammonium flux ($63.1 \mu\text{mol N/m}^2/\text{h}$) and nitrate flux ($52.0 \mu\text{mol N/m}^2/\text{h}$) in the reference dose. The reference rate of nitrate flux in Figure 3-2 ($52.0 \mu\text{mol N/m}^2/\text{h}$) may provide a measure of total nitrification because ammonium was not limited and the consumption of ammonium appeared to be driven strongly by ammonium oxidation.
2. The rate of ammonium flux in the cipro-treated microcosms ($6.2 \mu\text{mol N/m}^2/\text{h}$) was significantly lower relative to reference ($63.1 \mu\text{mol N/m}^2/\text{h}$) indicating that ammonium uptake increased (resulting in a lower flux) and/or mineralization (and possibly respiration) decreased due to the presence of cipro.
3. The addition of ammonium was intended to saturate ammonium oxidation so nitrate flux (measured as nitrate + nitrite) should approximate net nitrification. The rate of nitrate flux in the cipro-treated microcosms ($29.8 \mu\text{mol N/m}^2/\text{h}$) was slightly lower relative to reference ($52.0 \mu\text{mol N/m}^2/\text{h}$). Therefore, ammonium-oxidizers were being inhibited (i.e. nitrate production was decreased) and/or nitrate immobilization increased (by denitrification or microbial uptake) in the cipro-treated microcosms.
4. The reduced ammonium flux and nitrate flux in the treated microcosms suggests that cipro may have inhibited ammonium oxidizers and, perhaps, nitrogen immobilization (by sediment microbes).

3.1.2 Nitrate Spike

In the second half of this experiment, ammonifiers were saturated by the nitrate spike. The negative rates of ammonium flux (Figure 3-3) indicate that ammonium was being consumed (by nitrification or microbial uptake). There was no significant difference between the rate of ammonium flux in the treated ($-16.5 \mu\text{mol N/m}^2/\text{h}$) and untreated ($-19.4 \mu\text{mol N/m}^2/\text{h}$) microcosms ($F_{1,14} = 1.385$, $p = 0.305$) (Figure 3-3). However, there was a significant difference between the rate of nitrate flux in the treated ($-13.5 \mu\text{mol N/m}^2/\text{h}$) and untreated ($29.8 \mu\text{mol N/m}^2/\text{h}$) microcosms ($F_{1,2} = 4.503$, $p = 0.168$) (Figure 3-4). The positive nitrate flux (Figure 3-4) indicates that nitrate production (by nitrification or mineralization) was greater than nitrate consumption (denitrification or uptake) whereas the reverse appears to be occurring in the cipro-treated microcosms where the rate of nitrate flux was negative.

Figure 3-3: Ammonium flux following nitrate spike (Experiment 1)

Figure 3-4: Nitrate flux following nitrate spike (Experiment 1)

A comparison of Figures 3-3 and 3-4 indicates:

1. In the reference microcosms, the rate of nitrate flux out of sediments is positive ($29.8 \mu\text{mol N/m}^2/\text{h}$) compared to a negative rate of ammonium flux out of sediments ($-19.4 \mu\text{mol N/m}^2/\text{h}$). The difference in rates may provide an estimate of total denitrification, although this may be confounded by immobilization of nitrogen.
2. In the reference microcosms, flux of nitrate was positive (out of sediments) whereas in the cipro-treated microcosms, net flux of nitrate was negative (into sediments). Under reference conditions and despite nitrate supplied by diffusion, nitrification was greater than denitrification. The results suggest cipro reverses this relationship (i.e. nitrification < denitrification) by two possible mechanisms, namely 1) inhibition of nitrification and 2) stimulation of denitrification. Inhibition of nitrification is consistent with the ammonium spike results obtained in the first half of the experiment, although these two mechanisms are not mutually exclusive.

3.1.3 Summary of Experiment 1

In the first half of this experiment, the overlying surface water was spiked with ammonium. Therefore, ammonium was not limited and nitrification should theoretically have been maximized. Ammonium flux out of sediments into the overlying surface water in the cipro-treated microcosms was reduced (Figure 3-1) indicating that ammonium may be consumed more rapidly (or produced less rapidly) in the treated microcosms relative to the reference microcosms. Similarly, nitrate flux in the cipro-treated microcosms was slightly lower compared to the reference (Figure 3-2). This result might indicate that either nitrification by ammonium-oxidizers and nitrite-oxidizers was being inhibited and less nitrate was being produced and/or denitrification was being stimulated i.e. nitrification was unable to keep up with denitrification so nitrate was being consumed more rapidly than it was being replaced. As a result, nitrate flux was reduced.

In the second half of this experiment, the overlying surface water was spiked with nitrate. Therefore, nitrate was not limited and denitrification should theoretically have been maximized. There was no apparent effect of cipro on the rate of ammonium flux (Figure 3-3). Nitrate was not limited and nitrate flux into sediments in the treated microcosms was reduced relative to the reference microcosms indicating an inhibitory effect of cipro on nitrate flux (Figure 3-4). This result might indicate that nitrification was being inhibited or denitrification was being stimulated i.e. nitrification was unable to keep up with denitrification so nitrate was being consumed more rapidly than it was being replaced. As a result, the nitrate flux was reduced. These results are consistent with the results observed in the first half of the experiment.

Flux of nitrate in ammonium-saturated water saturated nitrifiers and provided an estimate of potential net nitrification. Flux of nitrate in nitrate-saturated water provided an estimate of potential net nitrate immobilization (denitrification and assimilation). Flux of ammonium minus flux of nitrate in ammonium-saturated water provided an estimate of potential net ammonium assimilation. Flux of ammonium in nitrate-saturated water provided an estimate of net potential ammonification.

Henriksen et al. (1981) measured the difference between ammonium accumulation in the presence and absence of a nitrification inhibitor (N-serve) in sediments of Danish waters and reported potential nitrification rates and nitrate fluxes. Potential nitrification rates ranged from 54.2 to 662.5 $\mu\text{mol N/m}^2/\text{h}$ in winter and 160.4 to 670.8 $\mu\text{mol N/m}^2/\text{h}$ in summer. Nitrate fluxes ranged from 4.2 to 25 $\mu\text{mol N/m}^2/\text{h}$ in winter and 0 to 29.2 $\mu\text{mol N/m}^2/\text{h}$ in summer. Hall (1986) published nitrification rate data in a mesotrophic lake sediment ranging from 1.6 to 5.6 $\mu\text{g N/g/d}$ (4.8×10^{-3} $\mu\text{mol N/g/h}$). Binnerup et al. (1992) published nitrate uptake rates and ammonium flux rates from sediments in a Scandinavian fjord in spring, summer and winter. In spring, nitrate uptake and ammonium flux ranged from 7.7 to 27.9 $\text{mmol N/m}^2/\text{d}$ and -3.8 to -1.9 $\text{mmol N/m}^2/\text{d}$, respectively. In summer, nitrate uptake and ammonium flux ranged from 7.8 to 14 $\text{mmol N/m}^2/\text{d}$ and 2.1 to 5.8 $\text{mmol N/m}^2/\text{d}$, respectively. In winter, nitrate uptake and ammonium flux ranged from 5.3 to 9.6 $\text{mmol N/m}^2/\text{d}$ and 2.5 to 6.7 $\text{mmol N/m}^2/\text{d}$, respectively.

The published rates vary from the rates measured in this study by one to three orders of magnitude which might be expected given the variations in methodology (use of general inhibitor) and study design (temperature and initial conditions).

By comparing all four rates (Figure 3-5), we can conclude that:

- Cipro appears to have an effect on either nitrification or denitrification or both
- Cipro does not appear to have a significant effect on ammonification
- Rates of ammonification were negative, indicating nitrate was being lost through denitrification (rather than ammonification)

Figure 3-5: Summary of flux changes in Experiment 1

Based on data obtained from this experiment, the combined results may indicate that two possible mechanisms may be at work: 1) inhibition of nitrification and 2) stimulation of denitrification. It is important to note that an inhibitory effect on nitrification does not necessarily preclude stimulation of denitrification and vice versa and, therefore, both processes were studied explicitly. There was no apparent effect on ammonification.

There is little published information on the effects of cipro, or other antibiotics, on nitrification. Zhou et al. (2008) assessed the effects of enrofloxacin and ciprofloxacin (5 mg/kg to 50 mg/kg) on nitrification and ammonification and reported that both FQs inhibited nitrification early in the study period (2 to 4 days) but there was no clear concentration-response relative to nitrification rates. Neither FQ had an apparent effect on ammonification which is consistent with the results

of this experiment. Ciprofloxacin is a metabolite of enrofloxacin which is used extensively in veterinary medicine (Sukul and Spiteller, 2007). Although the concentrations studied by Zhou et al. are considerably higher than the concentrations studied in this experiment, one might expect to see a continuation of the trend of inhibition of nitrification and/or stimulation of denitrification at high cipro concentrations.

In order to measure the effects of cipro on nitrification and ammonification directly, ^{15}N microdiffusion methods were used to study both processes explicitly. Two separate experiments were conducted to assess the effect of cipro on denitrification under nitrate limiting and non-limiting conditions.

3.2 Experiment 2 (May 2008) – Effect of cipro on nitrification and DNRA

To better determine the explanation for the results of Experiment 1, the objective of Experiment 2 was to study changes in the isotopic composition of the nitrate and ammonium pool. Experiment 2 consisted of two parts: nutrient flux measurements and direct measurements of nitrification and ammonification using ^{15}N stable isotope dilution.

3.2.1 Nutrient Fluxes

Nitrate fluxes were positive except for a large negative nitrate flux (i.e. flux into sediments at 0.5 mg/kg cipro) ($F_{5,20} = 7.162$, $p = 0.001$) (Figure 3-6). This data might indicate that either nitrate was lost more rapidly in this treatment due to increased denitrification or that nitrification was being inhibited. In general, ammonium flux decreased with increasing cipro concentration ($F_{5,22} = 2.057$, $p = 0.110$) (Figure 3-7).

Figure 3-6: Nitrate Flux (Experiment 2)

Figure 3-7: Ammonium Flux (Experiment 2)

3.2.2 Nitrification and DNRA Rates

Changes in the isotopic composition of the nitrate and ammonium pool were measured directly. As a result of nitrification, $^{15}\text{NO}_3^-$ in the overlying water became more dilute as new $^{14}\text{NO}_3^-$ was produced from ^{14}N in the sediments. The ammonium pool became enriched in $^{15}\text{NH}_4^+$ over time as some $^{15}\text{NO}_3^-$ was reduced by DNRA. Rates of nitrification and DNRA measured directly are presented in Figures 3-8 and 3-9. There was a slight treatment effect of cipro on production of new nitrate based on isotopic dilution of the $^{15}\text{NO}_3^-$ pool (Figure 3-8). 1-way ANOVA analysis indicated there was no significant treatment effect of cipro on nitrification ($F_{5,23} = 1.818$, $p = 0.149$). However, linear regression analysis indicated a slight, dose-dependent effect of cipro ($F_{1,27} = 5.836$, $p = 0.023$, $r^2 = 0.612$) on nitrification i.e. nitrification decreased with increasing cipro concentration. 1-way ANOVA indicated there was no significant treatment effect on ammonification (with and without Treatment 3) (1-way ANOVA $p = 0.147$; omit treatment 3, $p = 0.364$). Linear regression indicated no dose-dependent response to cipro ($F_{1,28} = 1.767$, $p = 0.195$, $r^2 = 0.275$).

In general, production of $^{15}\text{NH}_4^+$ (from the $^{15}\text{NO}_3^-$ pool) was generally lower at higher cipro concentrations (Figure 3-9). This slight effect might indicate an increase in ammonium uptake. In addition, the magnitude of ammonification measured by ^{15}N microdiffusion was considerably smaller than the rates calculated by measuring gross ammonium flux. Given the relatively short time frame of this experiment (24 hours), the production of $^{15}\text{NH}_4^+$ probably represents mainly dissimilatory nitrate reduction to ammonium (DNRA). However, some NO_3^- may also be remineralized following nitrate uptake and incorporation into cells and no distinction was made in this study between assimilatory and dissimilatory ammonification of nitrate. Assuming most of the $^{15}\text{NO}_3^-$ conversion to $^{15}\text{NH}_4^+$ was by DNRA, this pathway appears to be a minor contributor to ammonium production in sediment.

1-way ANOVA

$$F_{5,23} = 1.818, p = 0.149$$

$$F_{4,19} = 1.096, p = 0.387 \text{ (omit 1.0 mg/kg)}$$

Linear regression

$$F_{1,27} = 5.836, p = 0.023, r^2 = 0.612$$

Figure 3-8: Average Nitrification Rates

1-way ANOVA

$$F_{5,24} = 1.821, p = 0.147$$

$$F_{4,19} = 1.146, p = 0.364 \text{ (omit 1.0 mg/kg)}$$

Linear regression

$$F_{1,28} = 1.767, p = 0.195, r^2 = 0.275$$

Figure 3-9: Average DNRA Rates

Nitrification rates have generally been estimated using ^{15}N tracer techniques in bottle incubations (Ward, 1996a). Binnerup et al. (1992) reported uncoupled nitrification rates measured in $^{15}\text{NO}_3^-$ dilution in a Scandinavian fjord ranging from 4 to 8.0 mmol N/m²/d in spring, 2.7 to 3.7 mmol N/m²/d in summer and -1.2 to 0.6 mmol N/m²/d in winter. Caffrey et al. (2003) used the acetylene inhibition method to study potential nitrification in estuarine sediments of a small estuary in central California and reported spring nitrification rates ranging from 0 to 1.7 mmol N/m²/d and, in winter ranging from 2.5 to 5.5 mmol N/m²/d. The published rates are approximately one order of magnitude higher than the rates measured in this experiment.

3.2.3 Summary of Experiment 2

Nitrification

The results of both experiments indicate that nitrification is slightly inhibited by cipro. In the previous experiment, nitrate flux was lower in the treated microcosms suggesting either nitrification was inhibited or denitrification was stimulated or both. In this experiment, nitrification was measured indirectly and directly. Both sets of results indicated that there was a slight decrease in nitrification with increasing cipro concentration. Linear regression analysis confirmed a slight dose-dependent response of cipro on nitrification.

In the past, nitrification and DNRA has proven difficult to quantify so published data on these rates in sediments is limited especially in freshwater sediments (Jensen et al., 1996; An and Gardner, 2002). Jensen et al. (1996) measured nitrification and denitrification rates in marine sediments in Germany and reported nitrification rates ranging from 24 to 408 $\mu\text{mol}/\text{m}^2/\text{d}$ for sandy sediments and nitrification rates ranging from 120 to 672 $\mu\text{mol}/\text{m}^2/\text{d}$ for muddy sediments which the authors attributed to increased organic loading and mineralization.

DNRA

There was no apparent effect on ammonium flux in the first experiment. The results of the second experiment indicated a decrease in ammonium flux with increasing cipro concentration; however, 1-way ANOVA indicated this was not a significant effect. In this experiment, DNRA was also measured directly. Both sets of results indicated that there was no significant effect of cipro on ammonification. Furthermore, 1-way ANOVA indicated no significant treatment effect of cipro on DNRA and linear regression analysis indicated no dose-dependent effect of cipro on DNRA.

In general, nitrification showed a tendency toward inhibition by cipro when measured explicitly by this microdiffusion method. However, there was no indication that DNRA was affected by the presence of cipro. The results of this experiment were generally consistent with the results of the previous experiment.

3.3 Experiment 3 – Effect of cipro on denitrification under low nitrate conditions

Based on the results of Experiment 1, nitrification may have been inhibited or denitrification was stimulated or both. In order to assess the results of Experiment 1, denitrification was measured directly in this experiment.

3.3.1 Nutrient Flux Measurements

Nitrate fluxes were negative except for a positive nitrate flux (i.e. flux out sediments) at 1.5 mg/kg cipro (Figure 3-10) where consumption of nitrate by sediments or denitrification exceeded production of nitrate by nitrification or mineralization. More specifically, at higher cipro concentrations, the magnitude of nitrate fluxes was considerably less compared to the reference microcosms and Treatment 1 (0.5 mg/kg). The nitrate fluxes for this experiment were also considerably lower compared to Experiment 2. The magnitude of nitrate flux may have been lower overall due to the reduced concentration of nitrate in overlying surface water. The nitrate concentration in the overlying surface water at the start of the experiment was approximately 7 $\mu\text{mol/L}$ (equivalent to 0.1 mg/L NO_3^-) and therefore, the conditions for this experiment were relatively nitrate-limited compared to the other experiments. These data might also indicate that either nitrate was lost more rapidly in Treatment 1 due to increased denitrification or that nitrification was being inhibited. 1-way ANOVA indicated a slight treatment effect of cipro on nitrate flux ($F_{5,22} = 2.914$, $p = 0.036$).

In general, ammonium flux was unaffected by cipro concentration ($F_{5,23} = 0.524$, $p = 0.756$) (Figure 3-11). Note that the overall scale for nitrate flux rates was considerably smaller relative to the magnitude of ammonium flux rates, possibly as a result of nitrate-limited conditions. Results from the previous experiment indicated a slight effect on ammonification at higher cipro concentrations which might indicate an increased uptake of ammonium as a result of increased DNRA. However, there was no corresponding denitrification data for the previous experiment.

Figure 3-10: Nitrate flux under nitrate-limiting conditions

Figure 3-11: Ammonium flux under nitrate-limiting conditions

3.3.2 Denitrification, Percent Contribution by Diffusion and Respiration

Denitrification

Denitrification decreased at higher cipro concentrations relative to reference and was, in general, positive (Figure 3-12). A decrease in denitrification was observed where nitrate flux reversed direction in Treatment 4, 1.5 mg/kg cipro (Figure 3-10). There was no significant effect on ammonium flux ($F_{5,23} = 0.524$, $p = 0.756$) (Figure 3-11). The change in direction of nitrate flux (Figure 3-10) could be due to an increase in nitrification although this was not demonstrated by the results of Experiment 2 or by a reduction in denitrification. However, denitrification did not increase with cipro concentration as might be expected from the nitrate flux results suggesting there is some other process responsible for decreased nitrate flux into sediments, potentially a decrease in nitrate uptake by autotrophic and heterotrophic microorganisms.

1-way ANOVA

$$F_{5,20} = 18.107, p \leq 0.05$$

$$F_{4,19} = 19.584, p \leq 0.05 \text{ (omit 2.0 mg/kg)}$$

Linear Regression

$$F_{1,24} = 14.567, p = 0.001, r^2 = 0.378$$

$$F_{1,22} = 32.091, p \leq 0.05 \text{ (omit 2.0 mg/kg)},$$

$$r^2 = 0.593$$

Figure 3-12: Denitrification rates under nitrate-limiting conditions

Statistical analysis using 1-way ANOVA indicated a significant treatment effect on denitrification ($F_{5, 20} = 18.107$, $p \leq 0.05$). There was also a significant treatment effect on denitrification when Treatment 4 (2.0 mg/kg cipro) was omitted from the 1-way ANOVA analysis ($F_{4, 19} = 19.584$, $p \leq 0.05$). Linear regression analysis indicated a strong dose-dependent effect of cipro on denitrification with Treatment 4 (2.0 mg/kg) ($F_{1, 24} = 14.567$, $p = 0.001$, $r^2 = 0.378$) and without ($F_{1, 22} = 32.091$, $p \leq 0.05$, $r^2 = 0.593$).

Costanzo et al. (2005) evaluated the effects of short-term exposure to antibiotics including cipro on sediment denitrification rates in sewage effluent and downstream from a sewage discharge in Brisbane, Australia. Cipro concentrations were 0, 0.1, 10 and 1,000 $\mu\text{g/L}$. The results of the study indicated no significant difference on denitrification between treatments and control except at cipro concentrations above 1 mg/L. However, this may be explained by the study methodology (using the acetylene blockage technique) which may have underestimated denitrification. The results of their study may also be explained by the effect of seawater cations (Ca^{2+} and Mg^{2+}) inhibiting the action of cipro. The authors also referred to anecdotal information from the aquarium industry which relies heavily on nitrogen processing bacteria in biological filters in which inhibition has been reported following antibiotic additions aimed at treating infections (Costanzo et al., 2005).

Percent contribution by diffusion

Figure 3-13: % Contribution by Diffusion under nitrate-limiting conditions

Percent contribution by diffusion (%Diff) is a measure of the percentage of nitrogen contributed to denitrification from nitrification occurring in the overlying surface water versus nitrate obtained from other processes, primarily coupled nitrification-denitrification processes occurring in sediments (Nielsen et al., 1996). At a relatively lower nitrate concentration, tight coupling was observed between nitrification and denitrification in sediments with nitrate diffusion supplying only 6-14% of N_2 denitrified, (Figure 3-13). Statistical analysis using 1-way ANOVA indicated there was a significant treatment effect on %Diff ($F_{5,20} = 3.279, p = 0.025$). However, linear regression analysis indicated there was no dose-dependent effect of cipro on %Diff ($F_{1,24} = 0.777, p = 0.387, r^2 = 0.031$).

It remains unclear from this experiment, then, if denitrification was directly inhibited by higher concentrations of cipro, or if denitrification was indirectly inhibited by substrate limitation due to effects of cipro on nitrification.

Note that standard deviation for %Diff is high because these percentages are calculated using measured values of $^{28}\text{N}_2$, $^{29}\text{N}_2$, and $^{30}\text{N}_2$. The individual standard deviations for each measurement therefore affect the standard deviation values for %Diff.

Respiration

As mentioned previously, although not a part of the nitrogen cycle, respiration has a direct effect on nitrogen cycling in sediments as a result of O_2 distribution and remineralization of organic N. Cipro concentration did not appear to have a significant effect on respiration rates ($F_{5,20} = 1.356$, $p = 0.279$) (Figure 3-14). Linear regression ($F_{1,26} = 2.315$, $p = 0.140$, $r^2 = 0.082$) indicated there was no significant treatment effect on respiration. Overall, this data indicates there may be increase in ammonium uptake rather than a decrease in nitrate mineralization.

1-way ANOVA

$$F_{5,20} = 1.356, p = 0.279$$

Linear regression

$$F_{1,26} = 2.315, p = 0.140, r^2 = 0.082$$

Figure 3-14: Respiration rates under nitrate-limiting conditions

3.3.3 Summary of Experiment 3

Under limiting conditions, there appeared to be a dose-dependent response of denitrification to cipro. However, %Diff data indicated these results may be due to tightly coupled nitrification-denitrification. Therefore it is not possible to conclude whether or not denitrification is directly affected by cipro concentrations. There was no effect on respiration at these concentrations of cipro.

Nielsen and Glud (1996) measured denitrification using the isotope pairing method both in-situ and in the lab. In-situ denitrification rates were 350 and 470 $\mu\text{mol N/m}^2/\text{d}$ in June and November, respectively. Measured rates in the lab study were 290 and 500 $\mu\text{mol N/m}^2/\text{d}$ which were not significantly different. Jensen et al. (1996) measured nitrification and denitrification rates in marine sediments in Germany and reported seasonally variable rates depending on nitrate concentrations in the overlying water. When nitrate concentrations were less than 2 $\mu\text{mol/L}$ (summer and early winter), denitrification rates ranged from 0 to 192 $\mu\text{mol/m}^2/\text{d}$ and, when nitrate concentrations were approximately 70 $\mu\text{mol/L}$ (late winter and spring), denitrification rates ranged from 264 to 1,488 $\mu\text{mol/m}^2/\text{d}$, depending on sediment type. Direct denitrification accounted for 60 to 90% of total denitrification for most of the year except when nitrate was depleted during the summer months. The highest rates of coupled nitrification-denitrification of approximately 50% of total denitrification were reported in the spring. Summer and fall rates of coupled nitrification-denitrification were between 1 and 5% of total denitrification. Denitrification rates and %Diff measured in this experiment were similar to the published rates.

Risgaard-Petersen et al. (1994) reported that coupled denitrification in a Scandinavian fjord represented a small fraction (~14%) of total denitrification which is consistent with the results of this experiment. Smith et al. (2006) measured direct and coupled-denitrification rates in benthic sediments of two rivers using IRMS and MIMS and reported good correlation between the two techniques. Furthermore, the authors reported that coupled nitrification-denitrification was not a significant source of N_2 production.

Binnerup et al. (1992) published O_2 uptake rates in a Scandinavian fjord in spring, summer and winter. The rates ranged from 56 to 65 mmol $O_2/m^2/d$ in spring, 62 to 87 mmol $O_2/m^2/d$ in summer and 38 to 57 mmol $O_2/m^2/d$ in winter. The published rates are considerably higher than the rates measured in these experiments, likely because of abundant in-fauna burrowing in the sediments of the shallow fjord (Binnerup et al., 1992).

3.4 Experiment 4 (September 2008) – Effect of cipro on denitrification under non-limiting nitrate conditions

In order to assess the results of experiment 3, denitrification was studied under non-limiting conditions.

3.4.1 Denitrification

In general, this data demonstrated a trend of increasing denitrification rate with increasing cipro concentration (Figure 3-15). Linear regression analysis indicated a strong dose-dependent response ($F_{1,25} = 23.048$, $p \leq 0.05$, $r^2 = 0.480$) of denitrification to cipro concentration i.e. increasing denitrification with increasing cipro concentration. This was supported by 1-way ANOVA analysis which indicated a significant treatment effect on denitrification ($F_{5,21} = 55.419$, $p \leq 0.05$). This trend is a reversal of earlier data obtained in the previous experiment under nitrate-limiting conditions.

1-way ANOVA

$$F_{5,21} = 55.419, p \leq 0.05$$

Linear Regression

$$F_{1,25} = 23.048, p \leq 0.05, r^2 = 0.480$$

Figure 3-15: Denitrification rates under non-limiting conditions

3.4.2 Percent contribution by diffusion

The reversed trend observed in the denitrification data (discussed above) was also apparent in the results for percent contribution by diffusion (%Diff). In this experiment, %Diff ranged from 62 to 100% in the reference and lower cipro doses but dropped in the higher cipro doses, ranging from 13 to 50% (Figure 3-16). %Diff appeared to decrease at the same time denitrification increased (Figure 3-16, 1.5 mg/kg). Denitrification and nitrification are uncoupled at lower cipro concentrations (reference, 0.5 mg/kg and 1.0 mg/kg) (i.e. greater contribution of nitrate from the overlying water relative to nitrate contributed by sediment nitrification) but appear to be tightly coupled at higher cipro concentrations (1.5 mg/kg to 2.5 mg/kg) (i.e. greater contribution to denitrification of nitrate from sediment nitrification processes). 1-way ANOVA analysis indicated a significant treatment effect ($F_{5,21} = 3.279, p \leq 0.05$) on %Diff and linear regression analysis indicated a strong dose-dependent response ($F_{1,25} = 17.543, p \leq 0.05, r^2 = 0.412$) to cipro of %Diff.

1-way ANOVA

$$F_{5,21} = 3.279, p \leq 0.05$$

Linear regression

$$F_{1,25} = 17.543, p \leq 0.05, r^2 = 0.412$$

Figure 3-16: % Contribution by Diffusion under non-limiting conditions

3.4.3 Respiration

Rates of O_2 uptake were slightly larger in magnitude than the rates measured in experiment 3, likely because denitrification was higher due to increased nitrate concentrations. The results of this experiment indicated no effect on respiration at these cipro concentrations (Figure 3-17).

1-way ANOVA analysis and linear regression analysis did not indicate a significant treatment effect of cipro on respiration ($F_{5,24} = 1.353, p = 0.277$) or a strong dose-dependent response on respiration ($F_{1,28} = 0.096, p = 0.759, r^2 = 0.003$). The results of this experiment confirmed the results of the previous experiment which indicated that cipro does not have an effect on respiration at these concentrations.

1-way ANOVA

$F_{5,24} = 1.353, p = 0.277$

Linear regression

$F_{1,28} = 0.096, p = 0.759, r^2 = 0.003$

Figure 3-17: Respiration rates under non-limiting conditions

3.4.4 Summary of Experiment 4 –

In this experiment, under non-limiting conditions, denitrification appears to have been stimulated by increasing cipro concentrations which confirms the results of the first experiment when nitrate was also not limiting. In the first experiment, nitrate fluxes appeared to indicate that denitrification may have been stimulated (nitrate was consumed at a higher rate). When nitrate concentrations in the overlying water were relatively high (1 mg N-NO₃⁻/L), denitrification again increased with increasing cipro concentrations.

When nitrate concentrations in the overlying surface water were lower in experiment 3 (i.e. nitrate availability in the overlying surface water was limited), the denitrification rate appeared to decrease with increasing cipro concentration with the exception of Treatment 4 (Figure 3-12). Under nitrate-limiting conditions, at higher cipro concentrations (Treatments 3, 4 and 5), there was tight coupling between nitrification and denitrification as a result of low nitrate availability (Figure 3-13). This resulted in lower rates of denitrification indicating that

denitrification may have been limited as a result of decreased supply of nitrate. This is supported by the directly measured nitrification rates i.e. decrease in nitrification rate with cipro concentration (Figure 3-8).

In experiment 4, the amount of nitrate contributed to denitrification processes as a result of diffusion from overlying surface water was proportionately greater (Figure 3-16) compared to the contribution of nitrate to denitrification when nitrate was limiting (Figure 3-13).

When nitrate concentrations in the overlying surface water were higher (relative to the previous experimental conditions), these higher levels of nitrate may have been supporting higher rates of denitrification. Denitrification increased with increasing cipro concentration, and may even have been stimulated by cipro at higher concentrations. The results of these two experiments indicate that denitrifiers may be limited by nitrate availability rather than being inhibited directly by cipro.

3.5 Results of DO and CFU counts for ethanol carrier

DO concentrations decreased less than 20% in the jar without ethanol (from 230.645 to 184.799 $\mu\text{mol O}_2/\text{L}$) and decreased less than 16% (from 230.645 to 193.855 $\mu\text{mol O}_2/\text{L}$) in the jar containing ethanol (Table A3-1). The difference between the two microcosms was not considered to be significant ($z = 0.103$, $p = 0.918$). Cell counts for each plate were not considered significantly different after 48 hours ($F_{1,2} = 0.009$, $p = 0.934$) (Table A3-2). As such, ethanol was considered to be an acceptable carrier for administering cipro to sediments.

3.6 Overall experimental statistical analysis

Results of 2-way ANOVA indicated that there was a significant experiment effect between experiments 3 and 4 when Treatment 4 (experiment 3, 2.0 mg/kg cipro) was omitted ($F_{1,44} = 5.676$, $p = 0.022$). This was to be expected given the initial concentrations of nitrate in overlying surface water varied by one order of magnitude (0.1 mg/L and 6 mg/L, respectively). However, there was no significant experimental effect of concentration between the two experiments ($F_{5,1} = 1.057$, $p = 0.397$). Results of power analysis indicated an acceptable number of replicates were used in the study design.

3.7 Ecological implications

Based on these results, it is possible to build a simple predictive model for net nitrate change as a function of cipro concentration.

3.7.1 Net Change in Nitrification

Using the results of the linear regression analysis on nitrification data, it is possible to predict shifts in nitrification as a result of cipro concentrations measured in the environment.

Based on the regression equation obtained from the nitrification data in experiment 2 (omitting Treatment 2):

$$\text{Nitrification} = 738.11 - 25.58 \times \text{cipro concentration}$$

Therefore, for a concentration of 1 mg/kg cipro in the environment, one would expect a 3.5% drop in nitrification rate. For a concentration of 2.5 mg/kg cipro in the environment, one would expect an 8.7 % decrease in nitrification. Given the size of error bars for this data (some in excess of double the measured rate value), these decreases are not likely to be substantial or ecologically significant.

3.7.2 Net Change in Denitrification

Changes in denitrification are more difficult to predict because denitrification is dependent on nitrate concentrations in overlying water and/or in sediment.

Based on the results of these experiments, under limiting conditions, denitrification might decrease in the presence of cipro. If denitrification is tied to nitrification (low nitrate conditions), any effect of cipro on nitrification would subsequently be reflected in denitrification. Since nitrification was inhibited by cipro, under limiting conditions, denitrification would decrease as the supply of nitrate for coupled nitrification-denitrification decreases.

Using the regression equation obtained from the denitrification data in experiment 3 (omitting Treatment 4, 2.0 mg/kg):

$$\text{Denitrification} = 237.718 - 58.501 \times \text{cipro concentration}$$

In general, denitrification could be expected to decrease with increasing cipro concentration. For a concentration of 1 mg/kg cipro in the environment, one would expect a 25% drop in denitrification rate. For a concentration of 2.5 mg/kg cipro in the environment, one would expect a 62 % decrease in denitrification.

Under non-limiting conditions, denitrification becomes uncoupled from nitrification and might be expected to increase as a result of the additional supply of nitrate from overlying water. If denitrification is controlled by diffusion of nitrate from overlying water, the effects of cipro on denitrification should be independent of the effects of nitrification in sediments. This was demonstrated at the lower cipro concentrations in Experiment 4 when denitrification and nitrification became uncoupled.

Based on the regression equation obtained from the denitrification data in experiment 4:

$$\text{Denitrification} = 80.611 \times \text{cipro concentration} - 4.188$$

In general, denitrification could be expected to increase slightly with increasing cipro concentration. Denitrification could potentially be stimulated by cipro because of reduced competition for nitrate by other bacteria. This may also be a slight hormetic effect where small doses of a contaminant cause increased metabolic activity. Again, as substantial increases in denitrification are only likely to be realized where cipro concentrations are on the high end of the scale of environmental relevance, the impacts of cipro are expected to be negligible to marginally beneficial in culturally-eutrophied systems.

3.7.3 Respiration Effects on Nitrification and Denitrification

Nitrification and denitrification are dependent on respiration which determines the depth and distribution of the oxic zone. In this study, where rates were measured in the dark, aerobic respiration rates determined depth and distribution of oxygen in sediments rather than photosynthetic activity. However, the results of these experiments indicate respiration was not affected by cipro at these concentrations. If cipro inhibits respiration, the oxic zone should become broader. Nitrification might be expected to increase as a result. However, the effect on respiration could be mitigated by direct impacts of cipro on nitrification. Since these experiments demonstrated an effect of cipro on nitrification, it is not inconceivable that an effect on respiration was not observed because nitrification was inhibited and the nitrifiers would normally compete for O_2 with heterotrophs thereby obscuring the effect of cipro on nitrification.

If cipro inhibits respiration, denitrification could either increase or decrease. Denitrification would increase if denitrification was controlled predominantly by coupled nitrification-denitrification because the oxic zone would increase in thickness effectively increasing nitrification by increasing the volume of sediments where nitrification occurs. If, however,

nitrate conditions were limiting and cipro inhibits respiration, denitrification might increase if the supply of nitrate was predominantly from overlying water because the oxic zone would become thinner thereby decreasing the distance required for diffusion of nitrate from the overlying water. But, under the same nitrate-limiting conditions, if respiration was inhibited, denitrification would decrease if the source of nitrate for denitrification was diffusion of nitrate from overlying water because the zone of denitrification would be further from the sediment surface (i.e. deeper).

However, the results of these experiments indicated cipro does not have an effect on respiration at these concentrations. Therefore, since respiration was not affected, denitrification was not affected by change in oxygen distribution. Maul et al. (2006) assessed the effect of cipro on respiration on leaf-associated microbial community and found no effects at 1.0 to 10 $\mu\text{g/L}$. Naslund et al. (2008) studied the effect of cipro on mineralization of sediment-associated ^{14}C -labelled pyrene and sediment community bacterial structure in natural marine sediment. The authors reported that pyrene degradation was negatively affected by cipro and showed a dose-dependent response to concentration. An EC_{50} for inhibition of pyrene mineralization of 560 $\mu\text{g/L}$ cipro corresponded to a dry sediment weight of 0.4 $\mu\text{g/kg}$ cipro using reported $\log K_d$ values of 2.9-4.3. Furthermore, bacterial community structure changed in addition to a decrease in diversity at cipro concentrations above 1 mg/L . The lowest effect level occurred at 200 $\mu\text{g/L}$ which corresponds to approximately 0.1 $\mu\text{g/kg}$ cipro (Naslund et al., 2008).

3.7.4 Ammonification and DNRA

In general, there was no significant effect on ammonium flux although a slight decrease in ammonification measured directly was observed in Experiment 2. This decrease in ammonium flux may have been due to increased ammonium uptake as a result of increased denitrification or, more likely given the short time-frame of this experiment, due to DNRA processes.

If effects of cipro on ammonification were observed, these effects would also be observed in respiration data because the process of oxidizing organic compounds consumes O_2 and releases NH_4^+ (van Beelen and Doelman, 1996). However, no effect was observed in either process which is not unexpected given that ammonification and respiration are not very sensitive processes relative to nitrification.

4.0 CONCLUSION

In summary, these experiments were designed to assess the effects of cipro on four processes: nitrification, denitrification, ammonification (DNRA) and remineralization (using respiration as a proxy) in a simple microcosm of lake sediment and surface water. Nitrate and ammonium fluxes were first measured indirectly to provide an indication of the effect of cipro on potential net nitrification and potential net ammonification. Nitrification and ammonification were then studied directly using ^{15}N isotope dilution methods. Finally, denitrification and respiration were studied directly under nitrate-limiting and non-limiting conditions by measuring changes in $\text{N}_2:\text{Ar}$ and $\text{O}_2:\text{Ar}$ using MIMS.

Of these four processes, ammonification and respiration were not significantly affected by these concentrations of cipro. Nitrification appeared to be slightly inhibited by cipro at concentrations ranging from 0.5 to 2.5 mg/kg in sediment. Based on these data, it is not possible to conclusively state whether denitrification is specifically affected by cipro or by limitation of nitrate. In nitrate-limiting conditions, denitrification was tightly coupled to nitrification and therefore inhibited by low nitrate concentrations. Denitrification appeared to be stimulated by cipro when nitrate concentrations were not limiting.

The methods used in these experiments demonstrated that cipro does have an effect on nitrogen cycling, although the impacts on nitrification and denitrification are likely to be realized only at the highest concentrations measured in the environment, possibly only in spatially localized areas or hot spots. At the lower end of the environmentally relevant concentration range, observed impacts are not likely to be ecologically important, especially when averaged over an entire lake ecosystem.

Previous studies have focused on toxicity effects and growth inhibition of cipro and other FQs (Robinson et al., 2005; Backhaus et al., 2000; Froehner et al., 2000; Wollenberger et al., 2000; Backhaus and Grimme, 1999; Holten-Lutzhof et al., 1999) but few have assessed microbial function such as nitrification (Halling-Sorensen, 2001; Kümmerer et al., 2000; Al-Ahmad et al., 1999), denitrification (Costanzo et al., 2005) and respiration (Naslund et al., 2008; Maul et al., 2006). This study has demonstrated that cipro affects nitrification and, indirectly, denitrification as a result of nitrification inhibition. As predicted, slight shifts in the function of nitrogen cycling bacteria were observed in the presence of cipro. Gomez et al. (1996) reported no effects of five antibiotics on nitrifying sludge (no FQs were studied) indicating that heterotrophic organisms may be less sensitive to antibiotics than other autotrophs. So, although nitrifying bacteria may be inhibited by cipro, the implications for ecosystem function may not be considerable. The significance of these shifts over the long-term and the implications for development of microbial resistance to antibiotics have yet to be determined. Future research directions might include the use of molecular biology techniques such as DNA amplification using PCR to assess shifts in community composition or assessment of possible synergistic effects of cipro and other antibiotics or pharmaceuticals on nitrogen cycling.

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APPENDIX 1

Materials and Methods

A1.1 Ammonium Analysis

Ammonium concentrations were measured using the colorimetric phenol-hypochlorite method. Alkaline phenol and hypochlorite react with ammonium to form indophenol blue which is proportional to ammonium concentration. The blue colour is intensified by sodium nitroprusside. For each 10 mL sample of overlying water collected in a sample tube, two 5 mL replicates were extracted for analysis. The following reagents were added to each 5 mL subsample: 200 μ L phenol solution, 200 μ L sodium nitroprusside (0.5% w/v) and 500 μ L oxidizing solution (alkaline citrate and sodium hypochlorite). A new bottle of sodium hypochlorite (household bleach) was purchased each week and oxidizing solution was prepared fresh for each batch of samples analyzed. The samples were covered and stored in the dark for at least 1 hour to allow colour development to occur. Colorimetric analysis was performed on a Perkin Elmer Lambda 20 UV/Vis spectrophotometer using a wavelength of 640 nm. A standard curve was created by diluting a stock solution of 7.1 mmol N/L (100 mg/L) ammonium bromide solution to the following concentrations: 0, 7.1 μ mol/L, 17.8 μ mol/L, 35.5 μ mol/L and 71 μ mol/L (0, 0.1, 0.25, 0.5 and 1.0 mg/L).

Deviations from the method included use of ammonium bromide stock solution instead of ammonium chloride, reduction of sample volume and corresponding volumes of colour reagents.

A1.2 Nitrate Analysis

Nitrate + nitrite concentrations were measured using a modified copper-cadmium reduction column to reduce nitrate to nitrite which is then quantified by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly coloured reddish purple azo dye which is measured colorimetrically using a Perkin Elmer Lambda 20 UV/Vis spectrophotometer (American Public Health Association, 1998). Nitrate was measured as nitrate plus nitrite. Quantification of nitrite concentrations was not considered relevant for the purposes of this study, particularly as it was not a component of the synthetic lake water and concentrations of nitrite were not expected to be significant.

The cadmium reduction column construction consisted of a 4 mm inside diameter 10 cm length stainless steel tube with approximately 55 cm long 2 mm inside diameter flexible plastic tubing. Cadmium granules were rinsed in a solution of 50 % 10 N hydrochloric acid, then washed three times in copper sulphate solution and rinsed in deionized water. The column was then packed with cadmium granules. The column was rinsed with NH_4Br -EDTA buffer solution and primed with a 25% solution of 7.1 mmol N/L (100 mg/L) sodium nitrate. Solution was pumped through the column using a Rainin Rabbit[®] peristaltic pump. The pumping rate was approximately 5 mL per minute.

According to the column dimensions and length of plastic tubing, the volume of the system was approximately 3.7 mL. For each sample reduced through the column, approximately 5 mL was purged prior to sample collection. The column porosity was not accounted for in this calculation so 5 mL was considered a sufficient quantity to ensure that the volume of sample in the column had been completely replaced.

For each 5 mL sample of overlying water collected in a sample tube, 15 mL of NH_4Br -EDTA buffer solution was added to the sample. The entire volume was then pumped from a graduated cylinder through the column. Two 5 mL samples were collected in a test tube and 200 μL colour reagent was added immediately. The samples were covered and stored in the dark for a maximum of 2 hours to allow colour development to occur. Colorimetric analysis was performed on a Perkin Elmer Lambda 20 UV/Vis spectrophotometer using a wavelength of 543 nm. A standard curve was created by diluting a stock solution of 100 mg/L sodium nitrate solution to the following concentrations: 0, 7.1 $\mu\text{mol/L}$, 17.8 $\mu\text{mol/L}$, 35.5 $\mu\text{mol/L}$ and 71 $\mu\text{mol/L}$ (0, 0.1, 0.25, 0.5 and 1.0 mg/L).

Deviations from the method included use of sodium nitrate stock solution instead of potassium nitrate, reduction of sample size and corresponding colour reagent and buffer volumes.

CPSIL Method for NH_4^+ , NO_3^- Diffusions

1. Materials for Construction of a Diffusion Packet

- 1.1. Glass Microfiber Filter paper (combusted 400°C for 3 hours)
- 1.2. Furnace
- 1.3. Kimwipes
- 1.4. Acetone/alcohol
- 1.5. Aluminum foil
- 1.6. 1.2 cm diameter paper punch
- 1.7. Forceps
- 1.8. Appropriate lab attire, i.e. Nitrile gloves, lab coat and goggles.
- 1.9. Teflon tape – one inch width
- 1.10. 20 mL crimp- or snap-top glass vial with an opening of ~1.5 to 2cm
- 1.11. 2.5 M KHSO_4 [17.03g KHSO_4 in 40 mL water]
- 1.12. 20 μL pipette and tips (Eppendorf adjustable pipette 10-100 μL)
- 1.13. Small Nalgene bottles
- 1.14. Paper towels

2. Construction of a diffusion packet

- 2.1. Pre-combust an adequate number of filter papers in the furnace at 400°C for 4 hours.
- 2.2. Wipe down work space and surrounding area with acetone or alcohol. Roll out the outer layer of aluminum foil, cut and discard. Roll out a 12" sheet of aluminum foil, cut, and place on the countertop.
- 2.3. Wipe down the paper punch, forceps, and all utensils with acetone or alcohol.
- 2.4. Wearing gloves remove the filter paper(s) from the furnace and carefully cut the filter disks using paper punch.
- 2.5. Roll out the outer layer of Teflon tape, cut, and discard. Roll out another piece that is 8 cm long and fold, creating 2 layers of Teflon tape 4 cm long.
- 2.6. Place 1.2 cm filter disk onto the Teflon tape allowing enough room to fold the end over to create the packet
- 2.7. Using a micropipette, add 20 μL of 2.5 M KHSO_4 to the filter paper. Fold the Teflon tape over the filter paper to create the packet.
- 2.8. Seal the Teflon tape by pressing down on the layers on top of the foil and three layers of paper towels with the opening of the glass vial. The Teflon will become transparent when the proper seal is made. Place the diffusion packet into a Nalgene bottle and seal the top to minimize exposure of the packet to the air.

3. Materials for NH_4^+ , NO_3^- Diffusion

- 3.1. Appropriate lab attire, i.e. Nitrile gloves, lab coat and goggles.
- 3.2. Nalgene bottles (appropriate number and volume)
- 3.3. Analytical balance
- 3.4. NaCl - J.T. Baker A.C.S. Reagent grade [VWR:3642-19; CAS#: 7647-14-5]
- 3.5. Diffusion packets
- 3.6. Magnesium Oxide (MgO) – J.T. Baker [VWR:2484-011; CAS#:1309-48-4]

- 3.7. Micropipette and pipette tips (Eppendorf adjustable pipette 10-100 μ L)
- 3.8. Shaker table w/temperature setting
- 3.9. Desiccator with desiccant [EM t.h.e. desiccant, 8-mesh, silica gel*cobalt dichloride]
- 3.10. 250 mL beaker filled to 150 mL w/ conc. H₂SO₄
- 3.11. Devarda's alloy – Fluka, puriss. p.a. powder [Aldrich:31385; CAS#:8049-11-4]

4. NH₄⁺, NO₃⁻ Diffusion Procedure for Natural Abundance, Freshwater Samples

- 4.1. Ideally each filter should have 40 μ g N. To calculate the volume of sample required to obtain 40 μ g N, divide 40 by the concentration of N from NH₄⁺ or NO₃⁻.
- 4.2. The filters should not have more than 160 μ g N. If the N-NH₄⁺ and N-NO₃⁻ concentrations are very different (more than 4 times different) then 2 separate diffusions may be necessary. For example: If the volume required to obtain 40 μ g N from NH₄⁺ will give >160 μ g N from NO₃⁻, then a separate diffusion should be carried out to target 40 μ g N-NO₃⁻. If there is not enough material to obtain 40 μ g N from the second ion, use all of the material.
- 4.3. Wear gloves.
- 4.4. Add 50 g/L of NaCl to each sample bottle.
- 4.5. Transfer the sample to the diffusion Nalgene bottle.
- 4.6. Bring the volume in each Nalgene to a consistent level. For example, if you add 20 ml of sample to a 125 ml Nalgene, bring the volume up to 100 ml with NANOpure water. Be consistent so that all of the samples have the same amount of headspace.
- 4.7. Add one diffusion packet to each sample bottle.
- 4.8. Add MgO to each sample (3.0 g/L), quickly seal the top, and gently shake to stir the MgO. [Be consistent with the error regarding the mass of MgO when weighing.]
- 4.9. Incubate the samples at 30°C for seven days on a shaker table at a speed of 50 rpm.
- 4.10. After seven days, remove the samples from the shaker table. Prepare a clean work space by wiping down with acetone/alcohol. Clean all utensils with acetone/alcohol.
- 4.11. Remove each trap from the sample using forceps. Each trap should be placed in a labeled aluminum foil packet, loosely sealed and placed in the dessicator for 3-4 days, or until dry. Dessicator should contain an open top beaker of concentrated sulfuric acid and silica desiccating agent.
- 4.12. Calculate the mass of Devarda's alloy necessary to obtain a concentration of 75 mg/100ml of sample. [Be consistent with the error when weighing the alloy.]
- 4.13. Add a new diffusion packet to each sample.
- 4.14. Immediately add Devard's alloy to each sample and close the bottle tightly.
- 4.15. Incubate the samples at 30°C for seven days on a shaker table at a speed of 50 rpm.
- 4.16. Repeat steps 4.10 through 4.11.

A1.4 COIL Methodology

COIL provided the following description of the analysis method and EA-IRMS equipment.

"The Cornell Isotope Laboratory utilizes a Finnigan Delta Plus isotope ratio mass spectrometer (Bremen, Germany) interfaced to a Carlo Erba NC2500 elemental analyzer (Italy) for the analysis of N based organic materials. This process involves combustion at a high temperature (1000°C) in the presence of chromium III oxide and copper oxide. Once combustion is complete the resultant analyte gas is further reduced to N₂ in the presence of copper. Water is removed from the sample using a magnesium perchlorate trap. The sample is then sent through a gas Chromatography column and sent into the IRMS. In the IRMS the analyte N₂ gas is ionized at upwards of 3 kilovolts and separated by mass into heavy ($^{29}\text{N}_2 - ^{15}\text{N} + ^{14}\text{N}$) and light ($^{28}\text{N}_2 - ^{14}\text{N} + ^{14}\text{N}$) isotope ratios. The masses are detected in a collector system at the end of the mass spectrometer flight tube and calculated into $^{15}\text{N}/^{14}\text{N}$ ratios. A delta ^{15}N signature is then derived based on the Atmospheric Air reference scale.

In addition to simply deriving isotope signatures, the precision of the instrument is determined by analyzing internal standard materials. These are run for accuracy and precision purposes but also to check instrument linearity. In the case of enriched sample types it is an effective way to determine carryover from 1 sample to the next. We used several internal and chemical standards. These are biannually calibrated against known IAEA reference materials. Precision on enriched sample types is not guaranteed but we are able to show just how much carryover we are getting based on these internal standards." (Pers. corr.)

Lake 240 Summary

Mean Depth:	6.1m ^a	Shoreline length:	3440 m ^a
Maximum Depth:	13.1m ^a	Residence Time:	1.5yr ^b
Surface Area:	44.1 x 10 ⁴ m ^{2a}	pH:	6.48-7.31 ^c
Lake Volume:	26.7 x 10 ⁵ m ^{3a}	DOC:	550-640μmol/L ^c

^aBrunskill and Schindler, 1971

^b1970-1994 annual mean; Beaty and Lyng unpublished data; cited in Sellers *et al.*, 2001.

^cSellers *et al.*, 2001; n=12, 1995

Lake 240 (49°39'35.06"N, 93°43'43.24"W) is a drainage lake located in the boreal forest of the Precambrian Shield of northwestern Ontario at the Experimental Lakes Area (ELA) near Kenora (Brunskill and Schindler, 1971). It is part of a 723-ha watershed characterized by wetlands, six lakes, and forests dominated by immature jackpine and paper birch (St. Louis *et al.*, 1994). Lake 240 has only one outflow into Dryberry Lake which drains into Lake of the Woods (Sellers *et al.*, 2001, Brunskill and Schindler, 1971). It receives water directly from Lake 239 and Lake 470, which are connected to it by short streams (Sellers *et al.*, 2001). The Experimental Lakes field station is located at the junction of lakes 239 and 240. A 6.6 km road leads into the field station and the necessary electrical, water, and sewage systems have been in place since it's opening in 1968 (Johnson and Vallentyne, 1971).

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A1.4 Sediment characterization results

The following grain size and analysis of sediment used in the microcosms was provided by Ms. Emily Awad of the Ministry of Environment.

LIMS_PARMNAME	DESCRIPTION	RESULT_B	DESCRIPTION
Aluminum	ALUMINIUM, UNFILTERED TOTAL	18000	MICROGRAM PER GRAM DRY
Barium	BARIUM, UNFILTERED TOTAL	74	MICROGRAM PER GRAM DRY
BDE-100	BDE-100	0.41	NANOGRAM PER GRAM DRY
BDE-119	BDE-119	0.002	NANOGRAM PER GRAM DRY
BDE-126	BDE-126		NANOGRAM PER GRAM DRY
BDE-138	BDE-138	0.006	NANOGRAM PER GRAM DRY
BDE-153	BDE-153	0.028	NANOGRAM PER GRAM DRY
BDE-154	BDE-154	0.049	NANOGRAM PER GRAM DRY
BDE-17	BDE-17	0.022	NANOGRAM PER GRAM DRY
BDE-183	BDE-183	0.0094	NANOGRAM PER GRAM DRY
BDE-209	BDE-209	2.1	NANOGRAM PER GRAM DRY
BDE-28	BDE-28	0.054	NANOGRAM PER GRAM DRY
BDE-47	BDE-47	1.8	NANOGRAM PER GRAM DRY
BDE-49	BDE-49	0.064	NANOGRAM PER GRAM DRY
BDE-66	BDE-66	0.029	NANOGRAM PER GRAM DRY
BDE-71	BDE-71	0.005	NANOGRAM PER GRAM DRY
BDE-77	BDE-77	0.0004	NANOGRAM PER GRAM DRY
BDE-85	BDE-85	0.03	NANOGRAM PER GRAM DRY
BDE-99	BDE-99	1.5	NANOGRAM PER GRAM DRY
Beryllium	BERYLLIUM, UNFILTERED TOTAL	0.8	MICROGRAM PER GRAM DRY
Calcium	CALCIUM, UNFILTERED TOTAL	3000	MICROGRAM PER GRAM DRY
Cadmium	CADMIUM, UNFILTERED TOTAL	1.1	MICROGRAM PER GRAM DRY
Cobalt	COBALT, UNFILTERED TOTAL	8.7	MICROGRAM PER GRAM DRY
Chromium	CHROMIUM, UNFILTERED TOTAL	22	MICROGRAM PER GRAM DRY
Copper	COPPER, UNFILTERED TOTAL	20	MICROGRAM PER GRAM DRY
DDT & Metabolites	DDT & METABOLITES	5	NANOGRAM PER GRAM DRY
Iron	IRON, UNFILTERED TOTAL	19000	MICROGRAM PER GRAM DRY
Mercury	MERCURY, UNFILTERED TOTAL	0.14	MICROGRAM PER GRAM DRY
Magnesium	MAGNESIUM, UNFILTERED TOTAL	2200	MICROGRAM PER GRAM DRY
Manganese	MANGANESE, UNFILTERED TOTAL	260	MICROGRAM PER GRAM DRY
Molybdenum	MOLYBDENUM, UNFILTERED TOTAL	2.9	MICROGRAM PER GRAM DRY
Nickel	NICKEL, UNFILTERED TOTAL	15	MICROGRAM PER GRAM DRY
Aldrin	ALDRIN	1	NANOGRAM PER GRAM DRY
a-BHC (hexachlorocyclohexane)	HEXACHLOROCYCLOHEX, ALPHA-BHC	1	NANOGRAM PER GRAM DRY
b-BHC (hexachlorocyclohexane)	HEXACHLOROCYCLOHEX, BETA-BHC	1	NANOGRAM PER GRAM DRY
g-BHC (hexachlorocyclohexane)	HEXACHLOROCYCLOHEX, GAMMA-BHC	1	NANOGRAM PER GRAM DRY
a-Chlordane	CHLORDANE, ALPHA	2	NANOGRAM PER GRAM DRY
g-Chlordane	CHLORDANE, GAMMA	2	NANOGRAM PER GRAM DRY
Dieldrin	DIELDRIN	2	NANOGRAM PER GRAM DRY
Methoxychlor	DMDT METHOXYCHLOR	5	NANOGRAM PER GRAM DRY
Endosulphan I	ENDOSULFAN I	2	NANOGRAM PER GRAM DRY
Endosulphan II	ENDOSULFAN II	4	NANOGRAM PER GRAM DRY
Endrin	ENDRIN	4	NANOGRAM PER GRAM DRY
Endosulphan sulphate	ENDOSULFAN, SULPHATE	4	NANOGRAM PER GRAM DRY
Heptachlor epoxide	HEPTACHLOREPOXIDE	1	NANOGRAM PER GRAM DRY
Heptachlor	HEPTACHLOR	1	NANOGRAM PER GRAM DRY
Mirex	MIREX	5	NANOGRAM PER GRAM DRY
Oxychlordane	OXYCHLORDANE	2	NANOGRAM PER GRAM DRY
op-DDT	OP-DDT	5	NANOGRAM PER GRAM DRY
PCB; total	PCB TOTAL	38	NANOGRAM PER GRAM DRY
pp-DDD	PP-DDD	5	NANOGRAM PER GRAM DRY
pp-DDE	PP-DDE	5	NANOGRAM PER GRAM DRY
pp-DDT	PP-DDT	5	NANOGRAM PER GRAM DRY
Toxaphene	TOXAPHENE	50	NANOGRAM PER GRAM DRY
2378-tetrachlorodioxin	2378-T4-CDD	0.41	PICOGRAM PER GRAM DRY
2378-tetrachlorofuran	2378-T4-CDF	2.3	PICOGRAM PER GRAM DRY
12378-pentachlorodioxin	12378-P5-CDD	1.1	PICOGRAM PER GRAM DRY
12378-pentachlorofuran	12378-P5-CDF	0.66	PICOGRAM PER GRAM DRY

LIMS_PARMNAME	DESCRIPTION	RESULT_B	DESCRIPTION
23478-pentachlorofuran	23478-P5-CDF	1.2	PICOGRAM PER GRAM DRY
123478-hexachlorodioxin	123478-H6-CDD	0.92	PICOGRAM PER GRAM DRY
123678-hexachlorodioxin	123678-H6-CDD	1.6	PICOGRAM PER GRAM DRY
123789-hexachlorodioxin	123789-H6-CDD	2.7	PICOGRAM PER GRAM DRY
234678-hexachlorofuran	234678-H6-CDF	0.78	PICOGRAM PER GRAM DRY
123478-hexachlorofuran	123478-H6-CDF	2.2	PICOGRAM PER GRAM DRY
123678-hexachlorofuran	123678-H6-CDF	1	PICOGRAM PER GRAM DRY
123789-hexachlorofuran	123789-H6-CDF	0.22	PICOGRAM PER GRAM DRY
1234678-heptachlorodioxin	1234678-H7-CDD	24	PICOGRAM PER GRAM DRY
1234678-heptachlorofuran	1234678-H7-CDF	8	PICOGRAM PER GRAM DRY
1234789-heptachlorofuran	1234789-H7-CDF	0.67	PICOGRAM PER GRAM DRY
Octachlorodioxin	OCTCHLORODIBENZPIOXIN	150	PICOGRAM PER GRAM DRY
Octachlorofuran	OCTCHLORODIBENZO FURAN	12	PICOGRAM PER GRAM DRY
Lead	LEAD, UNFILTERED TOTAL	36	MICROGRAM PER GRAM DRY
3,3',4,4'-tetrachlorobiphenyl	3,3',4,4'-TETRACHLOROBIPHENYL	22	PICOGRAM PER GRAM DRY
3,4,4',5-tetrachlorobiphenyl	3,4,4',5-TETRACHLOROBIPHENYL	0.7	PICOGRAM PER GRAM DRY
2,3,3',4,4'-pentachlorobiphenyl	2,3,3',4,4'-PENTACHLOROBIPHENY	77	PICOGRAM PER GRAM DRY
2,3,4,4',5-pentachlorobiphenyl	2,2',3,4,5'-PENTACHLOROBIPHENY	4.1	PICOGRAM PER GRAM DRY
2,3',4,4',5-pentachlorobiphenyl	2,3',4,4',5-PENTACHLOROBIPHENY	180	PICOGRAM PER GRAM DRY
2',3,4,4',5-pentachlorobiphenyl	2',3,4,4',5-PENTA(CL)BIPHENYL	16	PICOGRAM PER GRAM DRY
3,3',4,4',5-pentachlorobiphenyl	3,3',4,4',5-PENTACHLOROBIPHENY	2	PICOGRAM PER GRAM DRY
2,3,3',4,4',5-hexachlorobiphenyl	2,3,3',4,4',5-HEXACHLOROBIPHENY	15	PICOGRAM PER GRAM DRY
2,3,3',4,4',5'-hexachlorobiphenyl	2,3,3',4,4',5'-HEXACHLOROBIPHENY	4.6	PICOGRAM PER GRAM DRY
23',44',55'-hexachlorobiphenyl	23',44',55'-HEXA(CL)BIPHENYL	6.8	PICOGRAM PER GRAM DRY
3,3',4,4',55'-hexachlorobiphenyl	3,3',4,4',55'-HEXACHLOROBIPHENY	1	PICOGRAM PER GRAM DRY
233'44'55'-heptachlorobiphenyl	233'44'55'-HEPTA(CL)BIPHENYL	2.3	PICOGRAM PER GRAM DRY
%Sand,very coarse(1000-2000um)	PARTICLES, 1000-2000 MICRONS	0	PERCENT
% <1000 um, >42.2 um	PARTICLES, 42.2-1000 MICRONS	10	PERCENT
% <42.2 um	PARTICLES, <42.2 MICRO	90	PERCENT
% <62 um, >42.2 um	PARTICLES, < 62 MICRONS	8.1	PERCENT
% <88 um, >62 um	PARTICLES, < 88 MICRONS	2.2	PERCENT
% <1000 um, >704 um	PARTICLES, 704-1000 MICRONS	0.2	PERCENT
% <10.5 um, >7.46 um	PARTICLES, < 10.55 MICRONS	12.3	PERCENT
% <125 um, >88 um	PARTICLES, < 125 MICRONS	0.5	PERCENT
% <14.9 um, >10.5 um	PARTICLES, < 14.92 MICRONS	13.2	PERCENT
% <176 um, >125 um	PARTICLES, < 176 MICRONS	0.5	PERCENT
% <1.01 um, >0.66 um	PARTICLES, < 1.01 MICRONS	1.7	PERCENT
% <1.69 um, >1.01 um	PARTICLES, < 1.69 MICRONS	2.8	PERCENT
% <21.1 um, >14.9 um	PARTICLES, < 21.1 MICRONS	12.5	PERCENT
% <250 um, >176 um	PARTICLES, < 250 MICRONS	1	PERCENT
% <29.8 um, >21.1 um	PARTICLES, < 29.85 MICRONS	10.1	PERCENT
% <2.63 um, >1.69 um	PARTICLES, < 2.63 MICRONS	4.2	PERCENT
% <352 um, >250 um	PARTICLES, < 352 MICRONS	1	PERCENT
% <3.73 um, >2.63 um	PARTICLES, < 3.73 MICRONS	4.9	PERCENT
% <42.2 um, >29.8 um	PARTICLES, < 42.21 MICRONS	9.6	PERCENT
% <500 um, >352 um	PARTICLES, < 500 MICRONS	0.5	PERCENT
% <5.27 um, >3.73 um	PARTICLES, < 5.27 MICRONS	7	PERCENT
% <704 um, >500 um	PARTICLES, < 704 MICRONS	0.2	PERCENT
% <7.46 um, >5.27 um	PARTICLES, < 7.46 MICRONS	10.3	PERCENT
% <0.21 um, >0.10 um	PARTICLES, < .21 MICRONS	0.1	PERCENT
% <0.34 um, >0.21 um	PARTICLES, < .34 MICRONS	0.1	PERCENT
% <0.43 um, >0.34 um	PARTICLES, < .43 MICRONS	0.1	PERCENT
% <0.66 um, >0.43 um	PARTICLES, < .66 MICRONS	1.1	PERCENT
Strontium	STRONTIUM, UNFILTERED TOTAL	21	MICROGRAM PER GRAM DRY
% <62 um, >2.63 um, sum	% <62 UM, >2.63 UM, SUM	88	PERCENT
% <1000 um, >62 um, sum	% <1000 UM, >62 UM, SUM	2	PERCENT
% <2.63 um, >0.10 um, sum	% <2.63 UM, >0.10 UM, SUM	9.8	PERCENT
Titanium	TITANIUM, UNFILTERED TOTAL	210	MICROGRAM PER GRAM DRY
Carbon; total organic	CARBON, TOTAL ORGANIC	120	MILLIGRAM PER GRAM DRY
Vanadium	VANADIUM, UNFILTERED TOTAL	32	MICROGRAM PER GRAM DRY
Zinc	ZINC, UNFILTERED TOTAL	84	MICROGRAM PER GRAM DRY

APPENDIX 2

Data Tables

Results of DO and CFU for assessment of ethanol carrier

Table A2-1 - Results of DO measurements with and without ethanol carrier

Time	Hours elapsed	Dissolved oxygen ($\mu\text{mol O}_2/\text{L}$)	
		Reference	Ethanol carrier
5.05 pm	0	230.645	230.645
7.55 pm	3	235.173	236.305
11.30 pm	6.5	235.173	233.475
2.05 am	9	210.835	224.985
8.10 am	15	212.250	209.420
11.20 am	18	200.930	215.363
2.35 pm	21	206.590	206.590
5.10 pm	24	184.799	193.855

Table A2-2 - Results of CFU counts with and without ethanol carrier

Hours elapsed	Reference	Ethanol carrier
2	109	117
48	122	102

Ammonium and nitrate concentrations following ammonia spike for Experiment 1

Table A2-3 - Ammonium concentration (mg/L)

Microcosm	Replicate	Time (hours)				
		0.5	2.5	4.5	8.5	21.5
1	A	1.0811	1.1554	0.9208	1.1324	1.0856
	B	1.0724	1.1824	0.9786	1.0827	1.1613
	Mean	1.0767	1.1689	0.9497	1.1076	1.1234
	Std Dev	0.0061	0.0191	0.0409	0.0352	0.0535
2	A	1.0863	1.1992	0.9959	1.1703	1.2351
	B	1.1231	1.2301	1.0045	1.1359	1.2119
	Mean	1.1047	1.2146	1.0002	1.1531	1.2235
	Std Dev	0.0260	0.0218	0.0061	0.0243	0.0165
3	A	1.0940	1.1869	1.0313	1.1355	1.1532
	B	1.1127	1.1399	0.9986	1.0911	1.1387
	Mean	1.1034	1.1634	1.0059	1.1133	1.1459
	Std Dev	0.0132	0.0333	0.0102	0.0314	0.0102
4	A	1.0605	1.1671	1.1102	1.0958	1.1216
	B	1.0401	1.1350	1.1128	1.1122	1.1001
	Mean	1.0503	1.1511	1.1115	1.1040	1.1109
	Std Dev	0.0145	0.0227	0.0019	0.0116	0.0125
5	A	1.0453	1.0830	1.1486	1.1197	1.1540
	B	1.1357	1.1766	1.1309	1.1372	1.1363
	Mean	1.0905	1.1298	1.1397	1.1285	1.1452
	Std Dev	0.0640	0.0662	0.0125	0.0124	0.0125
6	A	1.0820	1.1652	1.1669	1.1839	1.1816
	B	1.1277	1.1857	1.1606	1.1895	1.1952
	Mean	1.1049	1.1754	1.1638	1.1867	1.1884
	Std Dev	0.0324	0.0145	0.0045	0.0040	0.0096

Microcosms 1, 2, 3 = Reference microcosms

Microcosms 4, 5, 6 = Cipro treated sediments (2 mg/kg)

A and B refer to replicate analysis run on same sample

Concentration of NH_4 in NH_4 solution (prior to addition to microcosms)

A	1.0031
B	1.0472
Mean	1.0251
Std Dev	0.0312

Table A2-4 - Nitrate concentration (mg/L)

Microcosm	Replicate	Time (hours)				
		0.5	2.5	4.5	8.5	21.5
1	A	0.9210	1.0110	0.8517	0.8988	0.9744
	B	N/A	N/A	N/A	N/A	N/A
	Mean	-	-	-	-	-
	Std Dev	-	-	-	-	-
2	A	0.9837	1.0132	0.8410	1.0537	0.9102
	B	N/A	N/A	N/A	N/A	N/A
	Mean	-	-	-	-	-
	Std Dev	-	-	-	-	-
3	A	0.9903	1.0118	0.8556	0.9039	0.9876
	B	N/A	N/A	N/A	N/A	N/A
	Mean	-	-	-	-	-
	Std Dev	-	-	-	-	-
4	A	0.7429	0.9792	0.8520	0.8086	0.8226
	B	N/A	N/A	N/A	N/A	N/A
	Mean	-	-	-	-	-
	Std Dev	-	-	-	-	-
5	A	0.9163	1.0263	0.8255	0.8893	0.9317
	B	N/A	N/A	N/A	N/A	N/A
	Mean	-	-	-	-	-
	Std Dev	-	-	-	-	-
6	A	0.9465	1.0261	0.8731	0.8780	0.9168
	B	N/A	N/A	N/A	N/A	N/A
	Mean	-	-	-	-	-
	Std Dev	-	-	-	-	-

Microcosms 1, 2, 3 = Reference microcosms

Microcosms 4, 5, 6 = Cipro treated sediments (2 mg/kg)

Insufficient sample volume precluded running a replicate sample for NO_3 analysisConcentration of NO_3 in NH_4 solution (prior to addition to microcosms)

A	1.0488
B	-
Mean	-
Std Dev	-

Ammonium and nitrate concentrations following nitrate spike for Experiment 1

Table A2-5 - Ammonium concentration (mg/L)

Microcosm	Replicate	Time (hours)				
		0.5	2.5	4.5	8.5	21.5
1	A	0.1332	0.1374	0.1300	0.1460	0.1043
	B	0.1329	0.1300	0.1362	0.1425	0.0943
	Mean	0.1330	0.1337	0.1331	0.1442	0.0993
	Std Dev	0.0002	0.0052	0.0044	0.0025	0.0071
2	A	0.1465	0.1385	0.1380	0.1423	0.0898
	B	0.1416	0.1505	0.1474	0.1342	0.0892
	Mean	0.1441	0.1445	0.1427	0.1383	0.0895
	Std Dev	0.0035	0.0085	0.0067	0.0057	0.0004
3	A	0.1332	0.1362	0.1301	0.1308	0.0959
	B	0.1261	0.1315	0.1325	0.1241	0.0931
	Mean	0.1297	0.1339	0.1313	0.1274	0.0945
	Std Dev	0.0050	0.0033	0.0017	0.0048	0.0020
4	A	0.1214	0.1208	0.1193	0.1262	0.0870
	B	0.1204	0.1171	0.1216	0.1186	0.0867
	Mean	0.1209	0.1190	0.1205	0.1224	0.0868
	Std Dev	0.0007	0.0026	0.0017	0.0053	0.0002
5	A	0.1348	0.1329	0.1329	0.1265	0.0991
	B	0.1338	0.1357	0.1322	0.1337	0.0868
	Mean	0.1343	0.1343	0.1326	0.1301	0.0929
	Std Dev	0.0007	0.0020	0.0005	0.0051	0.0087
6	A	0.1378	0.1399	0.1388	0.1280	0.1057
	B	0.1286	0.1406	0.1418	0.1334	0.1028
	Mean	0.1332	0.1403	0.1409	0.1307	0.1043
	Std Dev	0.0065	0.0005	0.0022	0.0038	0.0021

Microcosms 1, 2, 3 = Reference microcosms

Microcosms 4, 5, 6 = Cipro treated sediments (2 mg/kg)

A and B refer to replicate analysis run on same sample

Concentration of NH₄ in NO₃ solution (prior to addition to microcosms)

A	0.1110
B	0.1106
Mean	0.1108
Std Dev	0.0003

Table A2-6 - Nitrate concentration (mg/L)

Microcosm	Replicate	Time (hours)				
		0.5	2.5	4.5	8.5	21.5
1	A	1.8666	1.9443	1.7223	1.7191	1.7417
	B	N/A	N/A	N/A	N/A	N/A
	Mean	-	-	-	-	-
	Std Dev	-	-	-	-	-
2	A	1.8455	1.9076	1.6972	1.7445	1.7217
	B	N/A	N/A	N/A	N/A	N/A
	Mean	-	-	-	-	-
	Std Dev	-	-	-	-	-
3	A	1.8387	1.9092	1.6976	1.7007	1.8082
	B	N/A	N/A	N/A	N/A	N/A
	Mean	-	-	-	-	-
	Std Dev	-	-	-	-	-
4	A	1.9006	1.8977	1.8528	1.7617	1.8584
	B	N/A	N/A	N/A	N/A	N/A
	Mean	-	-	-	-	-
	Std Dev	-	-	-	-	-
5	A	1.8663	1.9353	1.8771	1.9278	1.8608
	B	N/A	N/A	N/A	N/A	N/A
	Mean	-	-	-	-	-
	Std Dev	-	-	-	-	-
6	A	1.9019	1.8508	1.8462	1.7956	1.8035
	B	N/A	N/A	N/A	N/A	N/A
	Mean	-	-	-	-	-
	Std Dev	-	-	-	-	-

Microcosms 1, 2, 3 = Reference microcosms

Microcosms 4, 5, 6 = Cipro treated sediments (2 mg/kg)

Insufficient sample volume precluded running a replicate sample for NO₃ analysisConcentration of NO₃ in NO₃ solution (prior to addition to microcosms)

A	0.7461
B	-
Mean	-
Std Dev	-

Ammonium and Nitrate Fluxes for Experiment 1

Table A2-7 - Ammonium fluxes following ammonium spike

Microcosm	Rate (mg N/L/h)	Rate (micromoles N/m ² /h)	Average Rate (micromoles N/m ² /h)	Standard deviation
1	0.008	58.20523633	63.05567269	16.5484492
2	0.0112	81.48733087		
3	0.0068	49.47445088		
4	0.0001	-0.727565454	6.18430636	2.572332332
5	0.0006	4.365392725		
6	0.0011	8.003219996		

Table A2-8 - Nitrate fluxes following ammonium spike

Microcosm	Rate (mg N/L/h)	Rate (micromoles N/m ² /h)	Average Rate (micromoles N/m ² /h)	Standard deviation
1	0.0069	50.20201634	52.02092997	2.572332332
2	0.0003	2.182696362		
3	0.0074	53.83984361		
4	-0.001	-7.275654542	29.83018362	14.40506106
5	0.0055	40.01609998		
6	0.0027	19.64426726		

Table A2-9 - Ammonium fluxes following nitrate spike

Microcosm	Rate (mg N/L/h)	Rate (micromoles N/m ² /h)	Average Rate (micromoles N/m ² /h)	Standard deviation
1	-0.0024	-17.4615709	-19.40174544	4.007118067
2	-0.0033	-24.00965999		
3	-0.0023	-16.73400545		
4	-0.0022	-16.00643999	-16.49148363	1.514548268
5	-0.0025	-18.18913635		
6	-0.0021	-15.27887454		

Table A2-10 - Nitrate fluxes following nitrate spike

Microcosm	Rate (mg N/L/h)	Rate (micromoles N/m ² /h)	Average Rate (micromoles N/m ² /h)	Standard deviation
1	0.0013	9.458350904	21.58444181	24.87947633
2	0.0007	5.092958179		
3	0.0069	50.20201634		
4	0.0021	15.27887454	-13.4599609	1.543399399
5	-0.002	-14.55130908		
6	-0.0017	-12.36861272		

Microcosms 1, 2, 3 = Reference microcosms

Microcosms 4, 5, 6 = Cipro treated sediments (2 mg/kg)

Conversion factors	
1 mg N	7.14286E-05 mol N
Volume of surface water	0.45 L
Sediment surface area	0.004417865 m ²

Italics - outlier; data discarded

Ammonium and nitrate concentrations for Experiment 2

Table A2-11 - Ammonium concentration (mg/L)

Treatment	Replicate	Time		
		8.5	15.5	26
Reference	1	0.1538	0.1776	0.2853
	2	0.2115	0.1606	0.3622
	3	0.1611	0.1964	0.363
	4	0.1638	0.2032	0.4747
	5	0.1807	0.28	0.4834
1	1	9.25	15.5	26
	2	0.2182	0.2154	0.3954
	3	0.209	0.4377	0.5012
	4	0.4277	0.4302	0.4947
	5	0.3079	0.4931	0.5728
2	1	0.2548	0.3524	0.5155
	2	10.25	16.5	26
	3	0.2762	0.4239	0.5204
	4	0.2908	0.4854	0.5413
	5	0.2589	0.4229	0.5451
3	1	0.4105	0.422	0.5049
	2	0.2414	0.498	0.5307
	3	11.25	16.5	26
	4	0.306	0.3614	0.3984
	5	0.3233	0.4088	0.405
4	1	0.3488	0.3662	0.398
	2	0.3522	0.2046	0.2714
	3	0.3235	0.4204	0.5579
	4	12	16.5	26
	5	0.3437	0.4309	0.5171
5	1	0.3286	0.3796	0.4197
	2	0.3124	0.4264	0.4868
	3	0.3976	0.5252	0.5566
	4	0.3508	0.5041	0.5098
	5	13	17	26
6	1	0.4231	0.3405	0.3866
	2	0.467	0.4676	0.5741
	3	0.4581	0.5476	0.5935
	4	0.5396	0.4206	0.5887
	5	0.4574	0.4828	0.5276

Concentration of ammonium in lake water = -0.012 mg/L

Treatment	Cipro (mg/kg)
Reference	0
1	0.5
2	1
3	1.5
4	2
5	2.5

Italics - outlier discarded; reps not averaged

Table A2-12 - Nitrate concentration (mg/L)

Treatment	Replicate	Time		
		8.5	15.5	26
Reference	1	0.8083	0.7332	0.8194
	2	0.9096	0.817	0.9449
	3	0.9309	0.8982	0.9678
	4	0.9293	0.8346	0.9798
	5	0.9567	0.9038	0.9083
1	1	9.25	15.5	26
	2	0.8576	0.9844	0.7479
	3	0.8804	0.8626	0.745
	4	Sample lost	0.8975	0.936
	5	0.981	0.9308	0.8858
2	1	1.1214	0.8973	0.9783
	2	10.25	16.5	26
	3	0.8831	0.8541	0.9349
	4	0.8947	0.8245	0.8938
	5	0.8765	0.7288	1.0323
3	1	0.9117	0.7839	0.9437
	2	0.8534	0.8562	0.9607
	3	11.25	16.5	26
	4	0.8947	0.8448	0.8679
	5	0.8597	0.8816	0.8368
4	1	0.8421	0.9328	0.8997
	2	0.8367	0.9427	0.9211
	3	0.8005	0.9469	0.8409
	4	12	16.5	26
	5	0.9203	0.882	0.6569
5	1	0.8907	0.9384	0.8916
	2	0.7989	0.8827	0.9174
	3	0.8672	0.8272	0.8598
	4	0.8585	0.8115	0.8709
	5	13	17	26
6	1	0.7947	0.7455	0.9066
	2	0.7829	0.8451	0.8581
	3	0.7746	0.8281	0.8266
	4	0.8542	0.7492	0.81
	5	0.8154	0.7407	0.6974

Concentration of nitrate in lake water = 1.0160 mg/L

Ammonium and Nitrate Fluxes for Experiment 2

Table A2-13 - Ammonium Fluxes for Experiment 2

Treatment	Replicate	Rate (mg N/L/h)	Rate (micro mol N/m ² /h)	Average Rate (micromoles N/m ² /h)	Standard deviation
Reference	1	0.0077	432.27	728.69	268.75
	2	0.0094	527.71		
	3	0.0119	668.06		
	4	0.0184	1032.96		
	5	0.0175	982.44		
1	1	0.0113	634.37	700.62	281.58
	2	0.0163	915.07		
	3	0.0042	235.79		
	4	0.015	842.09		
	5	0.0156	875.77		
2	1	0.0151	847.70	799.42	260.72
	2	0.0151	847.70		
	3	0.0177	993.67		
	4	0.0062	348.06		
	5	0.0171	959.98		
3	1	0.006	336.84	418.24	314.93
	2	0.0048	269.47		
	3	0.0033	185.26		
	4	-0.004	-224.56		
	5	0.0157	881.39		
4	1	0.0119	668.06	554.66	126.56
	2	0.0062	348.06		
	3	0.0115	645.60		
	4	0.0101	567.01		
	5	0.0097	544.55		
5	1	-0.0015	-84.21	419.64	112.13
	2	0.0088	494.03		
	3	0.0095	533.32		
	4	0.0063	353.68		
	5	0.0053	297.54		

Treatment	Cipro (mg/kg)
Reference	0
1	0.5
2	1
3	1.5
4	2
5	2.5

Italics - outlier; data discarded

Table A2-14 - Nitrate Fluxes for Experiment 2

Treatment	Replicate	Rate (mg N/L/h)	Rate (micro mol N/m ² /h)	Average Rate (micromoles N/m ² /h)	Standard deviation
Reference	1	0.0012	67.37	143.16	58.07
	2	0.0028	157.19		
	3	0.0025	140.35		
	4	0.0037	207.72		
	5	-0.0025	-140.35		
1	1	-0.0082	-460.34	-407.01	75.39
	2	-0.0084	-471.57		
	3	0.0037	207.72		
	4	-0.0055	-308.77		
	5	-0.0069	-387.36		
2	1	0.0037	207.72	295.29	242.40
	2	0.0005	28.07		
	3	0.0117	656.83		
	4	0.0032	179.65		
	5	0.0072	404.20		
3	1	-0.0013	-72.98	64.00	158.91
	2	-0.0019	-106.66		
	3	0.003	168.42		
	4	0.0048	269.47		
	5	0.0011	61.75		
4	1	-0.0196	-1100.33	123.37	213.43
	2	-0.0007	-39.30		
	3	0.0077	432.27		
	4	0.00009	5.05		
	5	0.0017	95.44		
5	1	0.0102	572.62	237.19	275.84
	2	0.0051	286.31		
	3	0.0033	185.26		
	4	-0.0017	-95.44		
	5	-0.0084	-471.57		

15N Nitrification Data for Experiment 2

Table A2-15 - 15N03 Data and Nitrification Rates for Experiment 2

		8 hours	At%15N			24 hours	At%15N			
Treatment	Replicate	Total NO ₃ mg N/L	%	Total ¹⁵ NO ₃ (μmol N/L)	Total ¹⁴ NO ₃ (μmol N/L)	Total NO ₃ mg N/L	%	Total ¹⁵ NO ₃ (μmol N/L)	Total ¹⁴ NO ₃ (μmol N/L)	Net nitrification (μmol N/m2/h)
		8.5				26				
Reference	1	0.8083	5.5594	3.210	54.526	0.8194	5.0100	2.932	55.596	691.34
	2	0.9096	5.5234	3.589	61.383	0.9449	4.8801	3.294	64.199	805.06
	3	0.9309	5.4423	3.619	62.874	0.9678	5.1492	3.560	65.569	766.51
	4	0.9293	5.4443	3.614	62.765	0.9798	5.0979	3.568	66.418	779.34
	5	0.9567	4.9154	3.359	64.977	0.9083	4.9904	3.238	61.641	703.33
		9.25				26				
1	1	0.8576	5.3467	3.275	57.982	0.7479	4.9200	2.628	50.793	668.77
	2	0.8804	5.0224	3.158	59.727	0.745	4.9329	2.625	50.589	634.20
	3	Sample lost	5.1749	-	-	0.936	4.7833	-	-	-
	4	0.981	5.2770	3.698	66.374	0.8858	4.5930	2.906	60.365	826.75
	5	1.1214	4.4958	3.601	76.499	0.9783	4.9752	3.477	66.402	726.64
		10.25				26				
2	1	0.8831	5.1391	3.242	59.837	0.9349	4.6860	3.129	63.649	767.25
	2	0.8947	5.4001	3.451	60.456	0.8938	4.9368	3.152	60.691	749.68
	3	0.8765	5.3547	3.352	59.255	1.0323	4.9897	3.679	70.057	784.31
	4	0.9117	4.7826	3.114	62.007	0.9437	4.6918	3.163	64.245	727.74
	5	0.8534	5.3788	3.279	57.678	0.9607	4.9277	3.381	65.240	758.91
		11.25				26				
3	1	0.8947	5.3714	3.433	60.474	0.8679	5.0713	3.144	58.849	713.91
	2	0.8597	4.7294	2.904	58.503	0.8368	4.7758	2.855	56.917	645.48
	3	0.8421	5.3374	3.210	56.940	0.8997	5.0040	3.216	61.048	711.18
	4	0.8367	5.4466	3.255	56.509	0.9211	5.1230	3.371	62.422	714.10
	5	0.8005	5.3151	3.039	54.139	0.8409	4.9111	2.950	57.114	681.01
		12				26				
4	1	0.9203	5.2459	3.448	62.287	0.6569	4.8981	2.298	44.623	647.71
	2	0.8907	5.1265	3.262	60.360	0.8916	4.7862	3.048	60.638	733.35
	3	0.7989	5.1051	2.913	54.151	0.9174	4.8510	3.179	62.350	693.09
	4	0.8672	4.5001	2.788	59.155	0.8598	4.8228	2.962	58.452	618.65
	5	0.8585	5.2232	3.203	58.118	0.8709	4.8652	3.026	59.181	712.30
		13				26				
5	1	0.7947	5.4062	3.069	53.695	0.9066	4.9174	3.184	61.573	716.96
	2	0.7829	5.0991	2.851	53.070	0.8581	4.8152	2.951	58.341	667.22
	3	0.7746	5.3155	2.941	52.388	0.8266	4.2681	2.520	56.523	773.68
	4	0.8542	5.3447	3.261	57.753	0.81	4.8576	2.810	55.047	702.58
	5	0.8154	4.7989	2.795	55.448	0.6974	4.9427	2.462	47.352	562.79

Conversion factors		
Molar mass N	14	g/mol
Concentration of NaNO ₃	6	mg/L
Molarity of NO ₃ in lake water	428.5714286	μmol/L
Volume	0.45	L
Surface Area	0.002827433	m ²

Treatment	Cipro (mg/kg)
Reference	0
1	0.5
2	1
3	1.5
4	2
5	2.5

Italics - outlier; data discarded

15N Ammonification Data for Experiment 2

Table A2-16 - 15NH4 Data and Ammonification Rates for Experiment 2

Treatment	Replicate	8 hours	At%15N	Total ¹⁵ NH4 (μmol N/L)	Total ¹⁴ NH4 (μmol N/L)	24 hours		Total ¹⁵ NH4 (μmol N/L)	Total ¹⁴ NH4 (μmol N/L)	Net ammonification (μmol N/m2/h)
		Total NH4 mg N/L	%			Total NH4 mg N/L	%			
1	1	0.1538	0.3785	4.16E-02	10.94	0.2853	0.3844	7.83E-02	20.30	0.606
	2	0.2115	0.3788	5.72E-02	15.05	0.3622	0.3822	9.89E-02	25.77	0.506
	3	0.1611	0.3781	4.35E-02	11.46	0.363	0.3873	1.00E-01	25.83	0.821
	4	0.1638	0.3775	4.42E-02	11.66	0.4747	0.3911	1.33E-01	33.77	1.199
	5	0.1807	0.3838	4.95E-02	12.86	0.4834	0.3919	1.35E-01	34.39	1.118
		9.25				26				
2	1	0.2182	0.3883	6.05E-02	15.53	0.3954	0.4013	1.13E-01	28.13	0.971
	2	0.209	0.3914	5.84E-02	14.87	0.5012	0.4041	1.45E-01	35.66	1.360
	3	0.4277	0.4014	1.23E-01	30.43	0.4947	0.3871	1.37E-01	35.20	0.392
	4	0.3079	0.3819	8.40E-02	21.91	0.5728	0.3991	1.63E-01	40.75	0.955
	5	0.2548	0.3912	7.12E-02	18.13	0.5155	0.4040	1.49E-01	36.67	1.144
		10.25				26				
3	1	0.2762	0.3893	7.68E-02	19.65	0.5204	0.4031	1.50E-01	37.02	1.048
	2	0.2908	0.3980	8.27E-02	20.69	0.5413	0.4183	1.62E-01	38.50	1.370
	3	0.2589	0.3892	7.20E-02	18.42	0.5451	0.3981	1.55E-01	38.78	1.035
	4	0.4105	0.3866	1.13E-01	29.21	0.5049	0.3908	1.41E-01	35.92	0.493
	5	0.2414	0.3851	6.64E-02	17.18	0.5307	0.4302	1.63E-01	37.74	2.012
		11.25				26				
4	1	0.306	0.3841	8.40E-02	21.77	0.3984	0.3882	1.10E-01	28.35	0.482
	2	0.3233	0.3842	8.87E-02	23.00	0.405	0.3992	1.15E-01	28.81	0.645
	3	0.3488	0.3812	9.50E-02	24.82	0.398	0.3935	1.12E-01	28.32	0.506
	4	0.3522	0.3811	9.59E-02	25.06	0.2714	0.3927	7.61E-02	19.31	0.336
	5	0.3235	0.3927	9.08E-02	23.02	0.5579	0.4089	1.63E-01	39.69	1.078
		12				26				
5	1	0.3437	0.3859	9.47E-02	24.46	0.5171	0.4019	1.48E-01	36.79	0.821
	2	0.3286	0.3883	9.11E-02	23.38	0.4197	0.3982	1.19E-01	29.86	0.633
	3	0.3124	0.3893	8.69E-02	22.23	0.4868	0.3978	1.38E-01	34.63	0.761
	4	0.3976	0.3913	1.11E-01	28.29	0.5566	0.4021	1.60E-01	39.60	0.757
	5	0.3508	0.3889	9.75E-02	24.96	0.5098	0.3972	1.45E-01	36.27	0.698
		13				26				
6	1	0.4231	0.3906	1.18E-01	30.10	0.3866	0.4023	1.11E-01	27.50	0.499
	2	0.467	0.4111	1.37E-01	33.22	0.5741	0.4399	1.80E-01	40.83	1.200
	3	0.4581	0.4072	1.33E-01	32.59	0.5935	0.4114	1.74E-01	42.22	0.821
	4	0.5396	0.3886	1.50E-01	38.39	0.5887	0.3981	1.67E-01	41.88	0.537
	5	0.4574	0.3959	1.29E-01	32.54	0.5276	0.4063	1.53E-01	37.53	0.678

Conversion factors

Molar mass N	14
Concentration of NaNO ₃	6
Molarity of NO ₃ in lake water	0.000428571
	428.5714286
Volume	0.45
Surface Area	0.002827433

Treatment	Cipro (mg/kg)
Reference	0
1	0.5
2	1
3	1.5
4	2
5	2.5

Italics - outlier; data discarded

Nitrification and Ammonification Rates for Experiment 2

Table A2-17 - Nitrification and Ammonification Rates for Experiment 2

Treatment	Replicate	Net nitrification ($\mu\text{mol N/m}^2/\text{h}$)	Average ($\mu\text{mol N/m}^2/\text{h}$)	Standard deviation	Net ammonification ($\mu\text{mol N/m}^2/\text{h}$)	Average ($\mu\text{mol N/m}^2/\text{h}$)	Standard Deviation
Reference	1	691.340	749.12	49.45	0.606	0.85	0.31
	2	805.058			0.506		
	3	766.511			0.821		
	4	779.341			1.199		
	5	703.335			1.118		
1	1	668.774	714.09	84.23	0.971	0.96	0.36
	2	634.202			1.360		
	3	Sample lost			0.392		
	4	826.751			0.955		
	5	726.642			1.144		
2	1	767.249	757.58	20.99	1.048	1.19	0.56
	2	749.680			1.370		
	3	784.306			1.035		
	4	727.742			0.493		
	5	758.915			2.012		
3	1	713.910	693.13	30.06	0.482	0.61	0.28
	2	645.481			0.645		
	3	711.176			0.506		
	4	714.097			0.336		
	5	681.008			1.078		
4	1	647.713	681.02	47.07	0.821	0.73	0.07
	2	733.350			0.633		
	3	693.092			0.761		
	4	618.653			0.757		
	5	712.298			0.698		
5	1	716.965	684.65	78.18	0.499	0.75	0.28
	2	667.221			1.200		
	3	773.684			0.821		
	4	702.580			0.537		
	5	562.787			0.678		

Treatment	Cipro (mg/kg)
Reference	0
1	0.5
2	1
3	1.5
4	2
5	2.5

Ammonium and nitrate concentrations for Experiment 3

Table A2-18 - Ammonium concentration (mg/L)

Treatment	Replicate	Time	
		10.75	23.75
Reference	1	0.524	0.6249
	2	1.1037	0.4895
	3	0.7257	0.5132
	4	1.057	0.7095
	5	0.4775	0.4223
1	1	11.75	25.75
	2	0.5172	0.5144
	3	0.7796	0.4452
	4	1.3192	0.5704
	5	1.2228	0.5332
2	1	1.7702	0.5204
	2	12.75	26.75
	3	0.5526	0.546
	4	1.0465	0.5122
	5	0.7223	0.4493
3	1	0.6677	0.5672
	2	1.2087	0.455
	3	13.75	27.75
	4	1.0648	0.3985
	5	1.0082	0.5706
4	1	1.2588	0.4094
	2	0.8874	0.5338
	3	0.4891	0.329
	4	14.75	28.75
	5	0.9819	0.4878
5	1	1.0309	0.9657
	2	0.9922	0.3515
	3	1.0799	0.554
	4	1.1997	0.4011
	5	16	29.5
	1	1.0947	0.5434
	2	1.0294	0.384
	3	0.8386	0.5878
	4	0.4765	0.4033
	5	1.236	0.3496

Concentration of ammonium in stock solution = -0.095 mg/L

Treatment	Cipro (mg/kg)
Reference	0
1	0.5
2	1
3	1.5
4	2
5	2.5

Table A2-19 - Nitrate concentration (mg/L)

Treatment	Replicate	Time	
		10.75	23.75
Reference	1	0.1734	0.1952
	2	0.0772	0.0805
	3	0.202	0.0124
	4	0.0628	0.0049
	5	0.1499	-0.017
1	1	11.75	25.75
	2	0.1919	-0.006
	3	0.1128	0.0034
	4	0.0874	0.024
	5	0.0769	0.0315
2	1	0.0801	-0.009
	2	12.75	26.75
	3	0.1336	0.1147
	4	0.0921	0.0894
	5	0.1005	0.0845
3	1	0.1189	0.0838
	2	0.0637	0.092
	3	13.75	27.75
	4	0.1406	0.2665
	5	0.0675	0.1384
4	1	0.0746	0.0959
	2	0.0788	0.1112
	3	0.1275	0.0916
	4	14.75	28.75
	5	0.0826	0.1944
5	1	0.0959	0.0728
	2	0.2008	0.0703
	3	0.0582	0.0805
	4	0.085	0.0745
	5	16	29.5
	1	0.0412	0.078
	2	0.0553	0.0936
	3	0.1948	0.0742
	4	0.1387	0.0798
	5	0.0219	0.0664

Concentration of nitrate in lake water = 0.1213 mg/L

Ammonia and Nitrate Fluxes for Experiment 3

Table A2-20 - Ammonia Fluxes for Experiment 3

Treatment	Replicate	Rate (mg N/L/h)	Rate (micro mol N/m ² /h)	Average Rate (micromoles N/m ² /h)	Standard deviation
Reference	1	0.0078	656.83	-1458.50	1778.88
	2	-0.0472	-3974.66		
	3	-0.0163	-1372.61		
	4	-0.0267	-2248.38		
	5	-0.0042	-353.68		
1	1	-0.002	-168.42	-3671.51	2773.03
	2	-0.0239	-2012.59		
	3	-0.0535	-4505.18		
	4	-0.0493	-4151.50		
	5	-0.0893	-7519.86		
2	1	-0.0005	-42.10	-2007.54	1856.56
	2	-0.0382	-3216.78		
	3	-0.0195	-1642.07		
	4	-0.0072	-606.30		
	5	-0.0538	-4530.44		
3	1	-0.0476	-4008.35	-2969.21	1621.79
	2	-0.0313	-2635.74		
	3	-0.0607	-5111.48		
	4	-0.0253	-2130.49		
	5	-0.0114	-959.98		
4	1	-0.0353	-2972.58	-2344.04	1919.20
	2	-0.0047	-395.78		
	3	-0.0458	-385.68		
	4	-0.0376	-3166.26		
	5	-0.057	-4799.91		
5	1	-0.0408	-3435.73	-3002.89	2011.74
	2	-0.0478	-4025.19		
	3	-0.0186	-1566.29		
	4	-0.0054	-454.73		
	5	-0.0657	-5532.53		

Treatment	Cipro (mg/kg)
Reference	0
1	0.5
2	1
3	1.5
4	2
5	2.5

Italics - outlier; data discarded

Table A2-21 - Nitrate Fluxes for Experiment 3

Treatment	Replicate	Rate (mg N/L/h)	Rate (micro mol N/m ² /h)	Average Rate (micromoles N/m ² /h)	Standard deviation
Reference	1	0.0017	143.16	-503.57	626.53
	2	0.0003	25.26		
	3	-0.0146	-1229.45		
	4	-0.0045	-378.94		
	5	-0.0128	-1077.87		
1	1	-0.0141	-1187.35	-606.30	357.02
	2	-0.0078	-656.83		
	3	-0.0045	-378.94		
	4	-0.0032	-269.47		
	5	-0.0064	-538.94		
2	1	-0.0014	-117.89	-53.89	142.24
	2	-0.0002	-16.84		
	3	-0.0011	-92.63		
	4	-0.0025	-210.52		
	5	0.002	168.42		
3	1	0.009	757.88	257.68	363.40
	2	0.0051	429.47		
	3	0.0015	126.31		
	4	0.0023	193.68		
	5	-0.0026	-218.94		
4	1	0.008	673.67	-37.05	525.23
	2	-0.0017	-143.16		
	3	-0.0093	-783.14		
	4	0.0016	134.73		
	5	-0.0008	-67.37		
5	1	0.0027	227.36	-75.79	462.27
	2	0.0028	235.79		
	3	-0.0089	-749.46		
	4	-0.0044	-370.52		
	5	0.0033	277.89		

MIMS Data for Experiment 3

Table A2-22 - MIMS Data for Experiment 3

			Instrument Measured Values							
Time	Treatment	Replicate	N ₂ -28	N ₂ -29	N ₂ -30	O ₂	Ar	29/28	N ₂ /Ar	
8 hours	Standard		6.83E-11	4.07E-13	1.01E-13	3.03E-11	2.68E-12	5.97E-03	25.49	
	Reference	1	6.29E-11	4.02E-13	9.29E-14	1.97E-11	2.45E-12	6.39E-03	25.67	
		2	6.37E-11	4.04E-13	8.69E-14	2.04E-11	2.47E-12	6.34E-03	25.76	
		3	6.32E-11	3.92E-13	7.84E-14	2.09E-11	2.46E-12	6.21E-03	25.71	
		4	6.40E-11	4.06E-13	7.55E-14	1.92E-11	2.47E-12	6.35E-03	25.95	
		5	6.24E-11	4.04E-13	7.25E-14	1.82E-11	2.42E-12	6.47E-03	25.83	
	1	1	6.31E-11	3.99E-13	6.64E-14	1.97E-11	2.43E-12	6.33E-03	25.92	
		2	6.26E-11	3.94E-13	6.17E-14	1.80E-11	2.42E-12	6.30E-03	25.87	
		3	6.23E-11	3.92E-13	5.82E-14	1.80E-11	2.41E-12	6.29E-03	25.88	
		4	6.21E-11	3.87E-13	5.42E-14	1.86E-11	2.40E-12	6.23E-03	25.91	
		5	6.22E-11	3.95E-13	5.43E-14	1.75E-11	2.39E-12	6.36E-03	25.98	
	2	1	6.19E-11	3.83E-13	4.96E-14	1.90E-11	2.39E-12	6.19E-03	25.95	
		2	6.16E-11	3.84E-13	4.77E-14	1.84E-11	2.37E-12	6.23E-03	26.01	
	Standard		6.64E-11	3.87E-13	4.31E-14	2.86E-11	2.54E-12	5.83E-03	26.11	
	Standard		6.61E-11	3.84E-13	4.24E-14	2.86E-11	2.53E-12	5.80E-03	26.14	
	2	3	6.19E-11	3.80E-13	4.46E-14	1.94E-11	2.36E-12	6.13E-03	26.28	
		5	6.33E-11	3.95E-13	4.42E-14	1.80E-11	2.40E-12	6.24E-03	26.37	
	Standard		6.74E-11	3.89E-13	3.82E-14	2.89E-11	2.55E-12	5.77E-03	26.41	
	Standard		6.75E-11	3.88E-13	3.71E-14	2.90E-11	2.54E-12	5.75E-03	26.53	
	3	1	6.38E-11	3.88E-13	3.91E-14	1.90E-11	2.38E-12	6.08E-03	26.84	
		2	6.43E-11	3.92E-13	3.75E-14	2.02E-11	2.39E-12	6.11E-03	26.89	
		3	6.38E-11	3.86E-13	3.47E-14	2.00E-11	2.38E-12	6.04E-03	26.86	
		4	6.32E-11	3.88E-13	3.42E-14	1.88E-11	2.36E-12	6.13E-03	26.82	
		5	6.34E-11	3.85E-13	3.15E-14	1.92E-11	2.36E-12	6.07E-03	26.81	
	Standard		6.78E-11	3.87E-13	2.71E-14	2.88E-11	2.53E-12	5.71E-03	26.79	
	4	1	6.25E-11	3.80E-13	3.08E-14	1.66E-11	2.32E-12	6.09E-03	26.91	
		2	7.05E-11	4.32E-13	3.12E-14	2.03E-11	2.48E-12	6.13E-03	28.46	
		3	6.41E-11	3.90E-13	2.83E-14	1.85E-11	2.37E-12	6.08E-03	27.00	
		4	6.34E-11	3.85E-13	2.68E-14	1.84E-11	2.35E-12	6.07E-03	27.02	
		5	6.32E-11	3.84E-13	2.58E-14	1.87E-11	2.33E-12	6.07E-03	27.13	
	Standard		6.73E-11	3.79E-13	2.05E-14	2.84E-11	2.49E-12	5.64E-03	27.05	
	5	1	6.21E-11	3.64E-13	2.26E-14	1.91E-11	2.28E-12	5.87E-03	27.22	
		2	6.38E-11	3.86E-13	2.40E-14	1.83E-11	2.34E-12	6.05E-03	27.29	
		3	6.42E-11	3.91E-13	2.32E-14	1.78E-11	2.35E-12	6.10E-03	27.33	
		4	6.36E-11	3.85E-13	2.14E-14	1.80E-11	2.33E-12	6.05E-03	27.34	
		5	6.34E-11	3.82E-13	2.06E-14	1.82E-11	2.32E-12	6.03E-03	27.35	
	2	4	6.44E-11	3.87E-13	1.92E-14	1.91E-11	2.35E-12	6.01E-03	27.36	
	Standard		6.76E-11	3.80E-13	1.40E-14	2.82E-11	2.48E-12	5.62E-03	27.32	
	Standard		6.76E-11	3.78E-13	1.41E-14	2.84E-11	2.47E-12	5.60E-03	27.38	
24 hours	Reference	1	6.42E-11	4.16E-13	2.89E-14	1.42E-11	2.33E-12	6.47E-03	27.59	
		2	6.50E-11	4.28E-13	2.90E-14	1.41E-11	2.36E-12	6.58E-03	27.51	
		3	6.50E-11	4.24E-13	2.61E-14	1.43E-11	2.36E-12	6.52E-03	27.54	
		4	6.43E-11	4.23E-13	2.57E-14	1.39E-11	2.33E-12	6.58E-03	27.57	
		5	6.43E-11	4.18E-13	2.39E-14	1.41E-11	2.33E-12	6.51E-03	27.58	
	Standard		6.67E-11	3.73E-13	8.66E-15	2.77E-11	2.43E-12	5.59E-03	27.49	
	1	1	6.38E-11	4.19E-13	2.61E-14	1.35E-11	2.31E-12	6.56E-03	27.64	
		2	6.42E-11	4.12E-13	2.27E-14	1.45E-11	2.31E-12	6.42E-03	27.74	
		3	6.39E-11	4.11E-13	2.19E-14	1.45E-11	2.31E-12	6.43E-03	27.70	
		4	6.39E-11	4.13E-13	2.13E-14	1.40E-11	2.31E-12	6.46E-03	27.72	
		5	6.37E-11	4.06E-13	1.85E-14	1.51E-11	2.30E-12	6.37E-03	27.72	
	Standard		6.65E-11	3.68E-13	4.92E-15	2.75E-11	2.40E-12	5.53E-03	27.69	
	2	1	6.37E-11	4.06E-13	2.03E-14	1.51E-11	2.28E-12	6.37E-03	27.99	
		2	6.61E-11	4.28E-13	2.08E-14	1.38E-11	2.37E-12	6.48E-03	27.84	
		3	6.20E-11	3.98E-13	1.81E-14	1.32E-11	2.24E-12	6.43E-03	27.72	
		4	6.48E-11	4.20E-13	1.87E-14	1.33E-11	2.33E-12	6.48E-03	27.86	
		5	6.45E-11	4.16E-13	1.81E-14	1.37E-11	2.32E-12	6.46E-03	27.83	
	Standard		6.68E-11	3.69E-13	2.46E-15	2.74E-11	2.40E-12	5.52E-03	27.84	
	3	1	6.36E-11	3.94E-13	1.40E-14	1.55E-11	2.26E-12	6.18E-03	28.10	
		2	8.43E-11	5.09E-13	1.41E-14	2.87E-11	3.10E-12	6.04E-03	27.17	
		3	6.47E-11	4.10E-13	1.76E-14	1.45E-11	2.30E-12	6.34E-03	28.16	
		4	6.41E-11	3.79E-13	9.50E-15	1.94E-11	2.28E-12	5.92E-03	28.06	
		5	6.51E-11	4.18E-13	1.77E-14	1.35E-11	2.31E-12	6.42E-03	28.13	
	Standard		6.76E-11	3.71E-13	1.58E-15	2.78E-11	2.40E-12	5.49E-03	28.12	
	4	1	6.45E-11	4.08E-13	1.75E-14	1.52E-11	2.27E-12	6.33E-03	28.37	
		2	6.58E-11	4.21E-13	1.79E-14	1.46E-11	2.32E-12	6.40E-03	28.37	
		3	6.58E-11	4.17E-13	1.57E-14	1.57E-11	2.33E-12	6.33E-03	28.27	
		4	6.61E-11	4.22E-13	1.62E-14	1.38E-11	2.33E-12	6.39E-03	28.37	
		5	6.57E-11	4.23E-13	1.60E-14	1.38E-11	2.31E-12	6.44E-03	28.39	
	Standard		6.78E-11	3.72E-13	-5.15E-16	2.75E-11	2.40E-12	5.49E-03	28.29	
	Replaced liquid nitrogen									
	Standard		5.66E-11	3.06E-13	-6.05E-15	2.35E-11	2.05E-12	5.40E-03	27.54	
	5	1	5.51E-11	3.31E-13	2.65E-15	1.50E-11	1.98E-12	6.01E-03	27.84	
		2	5.66E-11	3.57E-13	7.60E-15	1.22E-11	2.04E-12	6.31E-03	27.79	
		3	5.73E-11	3.59E-13	6.93E-15	1.33E-11	2.06E-12	6.26E-03	27.78	
		4	5.75E-11	3.58E-13	5.87E-15	1.36E-11	2.06E-12	6.23E-03	27.88	
		5	5.81E-11	3.67E-13	6.94E-15	1.23E-11	2.09E-12	6.32E-03	27.79	
	Standard		6.21E-11	3.36E-13	-6.55E-15	2.54E-11	2.22E-12	5.41E-03	27.98	
	0 hours	0-1	-	5.88E-11	3.22E-13	-5.51E-15	1.93E-11	2.09E-12	5.47E-03	28.21
0-2		-	6.01E-11	3.31E-13	-5.15E-15	1.89E-11	2.13E-12	5.51E-03	28.29	
0-3		-	7.57E-11	4.32E-13	-2.71E-15	2.20E-11	2.39E-12	5.70E-03	31.66	
Lkwtr		-	5.98E-11	3.18E-13	-7.62E-15	2.28E-11	2.10E-12	5.32E-03	28.48	
0Blank		-	5.99E-11	3.17E-13	-7.67E-15	2.29E-11	2.10E-12	5.30E-03	28.55	
24Blank		-	6.38E-11	3.42E-13	-6.96E-15	2.39E-11	2.20E-12	5.35E-03	28.94	
Standard			6.63E-11	3.57E-13	-6.38E-15	2.67E-11	2.32E-12	5.38E-03	28.61	

Treatment	Cipro (mg/kg)
Reference	0
1	0.5
2	1
3	1.5
4	2
5	2.5

Denitrification and Respiration Rates and % Contribution by Diffusion for Experiment 3

Table A2-23 - Dentrification, %Diff and Respiration Rates for Experiment 3

Treatment	Replicate	Total denitrification			% Contribution by Diffusion			Respiration		
		Denitrification (micromoles N/m ² /h)	Average rate (micromoles N/m ² /h)	Standard deviation	% contribution by diffusion	Average (%)	Standard deviation	Respiration (micromoles O ₂ /m ² /h)	Average rate (micromoles N/m ² /h)	Standard deviation
Reference	1	234.288	210.19	23.12	9.978	11.16	1.44	-276.951	-288.43	60.61
	2	197.352			12.248			-335.667		
	3	226.821			10.673			-360.998		
	4	177.177			13.085			-258.768		
	5	215.298			9.819			-209.781		
1	1	195.489	215.05	14.53	10.778	8.85	1.10	-330.925	-202.66	88.13
	2	231.454			8.365			-166.726		
	3	222.743			8.481			-166.760		
	4	220.634			8.618			-246.698		
	5	204.920			8.013			-102.185		
2	1	315.891	232.67	57.86	5.507	7.03	1.17	-192.876	-281.42	69.00
	2	269.142			6.379			-289.693		
	3	177.247			8.643			-347.058		
	4	200.323			7.228			-346.716		
	5	200.766			7.376			-230.765		
3	1	115.919	119.32	10.89	9.978	9.45	1.15	-160.601	-274.38	100.32
	2	-			-			-		
	3	126.035			9.955			-312.411		
	4	105.549			7.725			-		
	5	129.780			10.148			-350.121		
4	1	270.584	252.53	25.53	4.532	6.23	2.40	-54.175	-202.85	102.52
	2	-			-			-269.143		
	3	234.486			7.930			-137.202		
	4	-			-			-265.063		
	5	-			-			-288.665		
5	1	129.981	83.48	31.28	6.429	13.19	5.84	-108.627	-197.64	78.10
	2	92.973			10.984			-267.386		
	3	67.742			14.562			-159.498		
	4	80.705			11.738			-161.125		
	5	45.987			22.242			-291.559		

Treatment	Cipro (mg/kg)
Reference	0
1	0.5
2	1
3	1.5
4	2
5	2.5

"-" = outlier; data discarded

MIMS Data for Experiment 4

Table A2-24 - MIMS Data for Experiment 4

Time	Treatment	Replicate	Instrument Measured Values						
			N ₂ -28	N ₂ -29	N ₂ -30	O ₂	Ar	29/28	N ₂ /Ar
8 hours	Standard		6.77E-11	3.62E-13	-5.28E-15	2.74E-11	2.34E-12	5.3496E-03	28.98
	Standard		6.81E-11	3.63E-13	-4.98E-15	2.75E-11	2.35E-12	5.3336E-03	29.03
	Standard		6.87E-11	3.67E-13	-5.31E-15	2.77E-11	2.36E-12	5.3451E-03	29.12
	Standard		6.91E-11	3.70E-13	-5.02E-15	2.78E-11	2.37E-12	5.3537E-03	29.16
	Reference	1	6.46E-11	3.49E-13	-6.70E-15	2.06E-11	2.17E-12	5.4057E-03	29.73
		2	6.53E-11	3.49E-13	-7.23E-15	2.08E-11	2.21E-12	5.3521E-03	29.60
		3	6.54E-11	3.48E-13	-7.97E-15	2.20E-11	2.20E-12	5.3231E-03	29.66
		4	6.55E-11	3.51E-13	-8.05E-15	2.09E-11	2.20E-12	5.3546E-03	29.71
		5	6.51E-11	3.49E-13	-8.62E-15	1.98E-11	2.19E-12	5.3678E-03	29.72
	Standard		7.02E-11	3.75E-13	-6.90E-15	2.81E-11	2.39E-12	5.3400E-03	29.37
	1	1	6.56E-11	3.51E-13	-7.78E-15	2.05E-11	2.20E-12	5.3519E-03	29.76
		2	6.58E-11	3.51E-13	-8.41E-15	2.10E-11	2.22E-12	5.3244E-03	29.72
		3	6.62E-11	3.53E-13	-8.41E-15	2.10E-11	2.22E-12	5.3248E-03	29.80
		4	6.62E-11	3.52E-13	-8.80E-15	2.11E-11	2.22E-12	5.3164E-03	29.85
		5	6.64E-11	3.54E-13	-9.12E-15	2.05E-11	2.22E-12	5.3243E-03	29.90
	Standard		7.06E-11	3.76E-13	-7.64E-15	2.82E-11	2.39E-12	5.3322E-03	29.60
	2	1	6.67E-11	3.53E-13	-8.62E-15	2.08E-11	2.21E-12	5.2886E-03	30.23
		2	6.69E-11	3.56E-13	-9.51E-15	2.08E-11	2.22E-12	5.3257E-03	30.12
		3	6.73E-11	3.57E-13	-9.54E-15	2.20E-11	2.24E-12	5.3071E-03	30.08
		4	6.65E-11	3.51E-13	-9.99E-15	2.10E-11	2.20E-12	5.2764E-03	30.15
		5	6.70E-11	3.55E-13	-1.02E-14	2.09E-11	2.22E-12	5.3048E-03	30.16
	Standard		7.12E-11	3.79E-13	-9.03E-15	2.83E-11	2.39E-12	5.3197E-03	29.83
	Standard		7.13E-11	3.80E-13	-9.29E-15	2.84E-11	2.39E-12	5.3212E-03	29.88
	Standard		7.15E-11	3.79E-13	-8.81E-15	2.84E-11	2.39E-12	5.3014E-03	29.94
	3	1	6.64E-11	3.52E-13	-1.03E-14	2.04E-11	2.19E-12	5.3001E-03	30.37
		2	6.73E-11	3.57E-13	-1.12E-14	2.07E-11	2.22E-12	5.3066E-03	30.37
		3	6.73E-11	3.55E-13	-1.13E-14	2.08E-11	2.21E-12	5.2802E-03	30.47
		4	6.71E-11	3.56E-13	-1.19E-14	2.02E-11	2.20E-12	5.3014E-03	30.45
		5	6.70E-11	3.54E-13	-1.23E-14	2.06E-11	2.20E-12	5.2777E-03	30.46
	Standard		7.17E-11	3.79E-13	-1.06E-14	2.84E-11	2.38E-12	5.2861E-03	30.09
	4	1	6.61E-11	3.48E-13	-1.20E-14	2.03E-11	2.16E-12	5.2595E-03	30.55
		2	6.69E-11	3.53E-13	-1.27E-14	2.06E-11	2.19E-12	5.2683E-03	30.58
		3	6.71E-11	3.53E-13	-1.26E-14	2.12E-11	2.20E-12	5.2568E-03	30.57
		4	6.69E-11	3.52E-13	-1.27E-14	2.10E-11	2.19E-12	5.2627E-03	30.55
		5	6.71E-11	3.55E-13	-1.31E-14	2.06E-11	2.19E-12	5.2885E-03	30.63
	Standard		7.16E-11	3.79E-13	-1.15E-14	2.83E-11	2.37E-12	5.2871E-03	30.23
	5	1	6.59E-11	3.46E-13	-1.31E-14	2.05E-11	2.15E-12	5.2478E-03	30.68
		2	6.66E-11	3.48E-13	-1.34E-14	2.07E-11	2.17E-12	5.2235E-03	30.71
		3	6.73E-11	3.56E-13	-1.39E-14	2.01E-11	2.19E-12	5.2892E-03	30.79
		4	6.70E-11	3.54E-13	-1.39E-14	1.97E-11	2.19E-12	5.2918E-03	30.63
		5	6.65E-11	3.50E-13	-1.43E-14	2.04E-11	2.17E-12	5.2645E-03	30.73
	Standard		7.22E-11	3.82E-13	-1.22E-14	2.85E-11	2.38E-12	5.2851E-03	30.37
	Reference	1	6.67E-11	3.87E-13	-1.22E-14	1.68E-11	2.17E-12	5.8062E-03	30.81
		2	6.83E-11	3.83E-13	-1.30E-14	1.74E-11	2.21E-12	5.6117E-03	30.96
		3	6.84E-11	4.02E-13	-1.31E-14	1.46E-11	2.21E-12	5.8729E-03	30.98
		4	6.73E-11	3.82E-13	-1.44E-14	1.64E-11	2.18E-12	5.6752E-03	30.87
		5	6.69E-11	3.77E-13	-1.44E-14	1.66E-11	2.18E-12	5.6387E-03	30.73
	Standard		7.16E-11	3.79E-13	-1.37E-14	2.81E-11	2.36E-12	5.2851E-03	30.39
	1	1	6.67E-11	3.90E-13	-1.32E-14	1.55E-11	2.15E-12	5.8364E-03	31.04
		2	6.77E-11	3.98E-13	-1.34E-14	1.50E-11	2.18E-12	5.8805E-03	31.04
		3	6.72E-11	3.79E-13	-1.47E-14	1.68E-11	2.17E-12	5.6288E-03	30.96
		4	6.70E-11	3.66E-13	-1.53E-14	1.81E-11	2.16E-12	5.4650E-03	30.97
		5	6.72E-11	3.73E-13	-1.50E-14	1.73E-11	2.17E-12	5.5508E-03	30.97
	Standard		7.12E-11	3.76E-13	-1.41E-14	2.80E-11	2.33E-12	5.2765E-03	30.50
	2	1	6.65E-11	3.85E-13	-1.38E-14	1.56E-11	2.14E-12	5.7866E-03	31.14
		2	6.69E-11	3.82E-13	-1.45E-14	1.58E-11	2.15E-12	5.7041E-03	31.18
		3	6.62E-11	3.67E-13	-1.57E-14	1.60E-11	2.13E-12	5.5492E-03	31.06
		4	6.64E-11	3.79E-13	-1.56E-14	1.51E-11	2.14E-12	5.6973E-03	31.09
		5	6.64E-11	3.82E-13	-1.58E-14	1.40E-11	2.14E-12	5.7456E-03	31.02
	Standard		7.06E-11	3.71E-13	-1.48E-14	2.77E-11	2.31E-12	5.2635E-03	30.49
	3	1	6.50E-11	3.73E-13	-1.53E-14	1.50E-11	2.07E-12	5.7366E-03	31.32
		2	6.70E-11	3.83E-13	-1.59E-14	1.52E-11	2.14E-12	5.7198E-03	31.28
		3	6.71E-11	3.83E-13	-1.60E-14	1.55E-11	2.14E-12	5.7008E-03	31.32
		4	6.69E-11	3.88E-13	-1.61E-14	1.42E-11	2.14E-12	5.8025E-03	31.32
		5	6.67E-11	3.87E-13	-1.64E-14	1.41E-11	2.13E-12	5.8035E-03	31.29
	Standard		7.03E-11	3.71E-13	-1.55E-14	2.76E-11	2.30E-12	5.2717E-03	30.63
	4	1	6.57E-11	3.78E-13	-1.59E-14	1.47E-11	2.10E-12	5.7640E-03	31.33
		2	6.55E-11	3.62E-13	-1.68E-14	1.52E-11	2.09E-12	5.5287E-03	31.29
		3	6.62E-11	3.76E-13	-1.67E-14	1.49E-11	2.11E-12	5.6842E-03	31.33
		4	6.74E-11	3.89E-13	-1.65E-14	1.52E-11	2.14E-12	5.7690E-03	31.48
		5	6.61E-11	3.76E-13	-1.69E-14	1.51E-11	2.11E-12	5.6950E-03	31.31
	Standard		7.00E-11	3.67E-13	-1.61E-14	2.75E-11	2.28E-12	5.2406E-03	30.73
	5	1	6.45E-11	3.75E-13	-1.56E-14	1.51E-11	2.05E-12	5.8121E-03	31.42
		2	6.65E-11	3.82E-13	-1.65E-14	1.58E-11	2.10E-12	5.7328E-03	31.65
		3	6.90E-11	3.97E-13	-1.65E-14	1.56E-11	2.14E-12	5.7556E-03	32.19
		4	6.50E-11	3.74E-13	-1.76E-14	1.42E-11	2.07E-12	5.7525E-03	31.46
		5	6.52E-11	3.78E-13	-1.78E-14	1.44E-11	2.07E-12	5.7980E-03	31.47
	Standard		6.80E-11	3.54E-13	-1.79E-14	2.66E-11	2.21E-12	5.2168E-03	30.78
0 hours	0-1	-	6.11E-11	3.11E-13	-1.98E-14	2.07E-11	1.95E-12	5.0884E-03	31.37
	0-2	-	6.15E-11	3.13E-13	-1.99E-14	2.13E-11	1.96E-12	5.0926E-03	31.41
	0-3	-	6.21E-11	3.16E-13	-2.02E-14	2.14E-11	1.97E-12	5.0954E-03	31.45
	0Blank	-	6.42E-11	3.30E-13	-1.98E-14	2.32E-11	2.06E-12	5.1405E-03	31.21
	24Blank	-	6.34E-11	3.26E-13	-2.00E-14	2.35E-11	2.04E-12	5.1434E-03	31.18
	Standard		6.77E-11	3.52E-13	-1.87E-14	2.67E-11	2.19E-12	5.1968E-03	30.97

Treatment	Cipro (mg/kg)
Reference	0
1	0.5
2	1
3	1.5
4	2
5	2.5

Denitrification and Respiration Rates and % Contribution by Diffusion for Experiment 4

Table A2-25 - Denitrification, %Diff and Respiration Rates for Experiment 4

Treatment	Replicate	Total denitrification			% Contribution by Diffusion			Respiration		
		Denitrification (micromoles N/m ² /h)	Average rate (micromoles N/m ² /h)	Standard deviation	% contribution by diffusion	Average (%)	Standard deviation	Respiration (micromoles O ₂ /m ² /h)	Average rate (micromoles N/m ² /h)	Standard deviation
Reference	1	-	54.24	25.77	-	61.76	33.18	-264.944	-307.45	117.60
	2	74.420			32.246			-241.426		
	3	74.916			49.644			-509.567		
	4	46.519			55.962			-304.957		
	5	21.096			109.204			-216.363		
1	1	41.027	32.04	17.30	81.557	92.74	27.52	-332.693	-282.18	89.52
	2	57.857			63.697			-406.821		
	3	24.841			99.592			-272.973		
	4	21.155			82.462			-191.596		
	5	15.321			136.414			-206.834		
2	1	-	9.53	4.48		100.00	101.89	-328.910	-366.75	49.56
	2	14.376			194.559			-317.072		
	3	5.553			388.501			-365.423		
	4	8.649			345.692			-379.742		
	5	-						-442.609		
3	1	131.316	117.94	9.63	23.177	25.70	2.80	-302.507	-343.10	41.31
	2	123.090			23.212			-326.525		
	3	111.421			25.334			-315.265		
	4	117.017			26.919			-369.570		
	5	106.880			29.847			-401.643		
4	1	55.917	57.63	22.04	54.540	50.73	11.33	-352.548	-350.20	24.15
	2	40.146			48.338			-319.903		
	3	55.130			50.565			-380.283		
	4	94.866			34.410			-364.998		
	5	42.087			65.789			-333.291		
5	1	246.359	293.01	58.34	15.820	13.49	2.45	-294.840	-287.79	30.55
	2	290.470			12.995			-266.158		
	3	393.111			9.749			-255.971		
	4	275.075			13.310			-287.266		
	5	260.016			15.582			-334.721		

Treatment	Cipro (mg/kg)
Reference	0
1	0.5
2	1
3	1.5
4	2
5	2.5

"-" = outlier; data discarded

APPENDIX 3

Results of Statistical Analysis

Experiment	Analysis	File Name	Description
Dissolved oxygen			
Jun-07	-	O2_output	Test of significance for ethanol carrier
CFU			
Jun-07	1 way ANOVA	1 way ANOVA CFU	1 way ANOVA for CFU data
Ammonia and Nitrate Fluxes			
Jun-07	1 way ANOVA	Expt 1A 1 way ANOVA	1 way ANOVA for June 2007 data (Part 1 of experiment)
Jun-07	1 way ANOVA	Expt 1B 1 way ANOVA	1 way ANOVA for June 2007 data (Part 2 of experiment)
May-08	1 way ANOVA	1 way ANOVA all	1 way ANOVA for July 2008 experiment data
Jul-08	1 way ANOVA	1 way ANOVA all	1 way ANOVA for August 2008 experiment data
Nitrification			
May-08	1 way ANOVA	1 way ANOVA all	1 way ANOVA for May 2008 data
May-08	1 way ANOVA	1 way ANOVA omit T3	1 way ANOVA for May 2008 without treatment 3
May-08	Linear regression	Linear regression all	Linear regression for May 2008 data
May-08	Linear regression	Linear regression omit T3	Linear regression for May 2008 data without treatment 3
Ammonification			
May-08	1 way ANOVA	1 way ANOVA all	1 way ANOVA for May 2008 data
May-08	1 way ANOVA	1 way ANOVA omit T3	1 way ANOVA for May 2008 without treatment 3
May-08	Linear regression	Linear regression all	Linear regression for May 2008 data
May-08	Linear regression	Linear regression omit T3	Linear regression for May 2008 data without treatment 3
Denitrification			
Jul-08	1 way ANOVA	1 way ANOVA E4_July08	1 way ANOVA for July 2008 experiment data
Jul-08	1 way ANOVA	1 way ANOVA E4_July08 omit T5 for DNR	1 way ANOVA for July 2008 experiment data without Treatment 5 DNR data
Jul-08	Linear regression	Linear regression E4_July08	Linear regression for July 2008 data
Jul-08	Linear regression	Linear regression E4 omit T5 for DNR	Linear regression for July 2008 data without Treatment 5 DNR data
Aug-08	1 way ANOVA	1 way ANOVA E5_Aug08	1 way ANOVA for August 2008 experiment data
Both July and August	-	denitrification all	All denitrification data (DNR, % contribution by DNR, respiration)
Both July and August	-	denitrification all omit T5 for DNR	All denitrification data except DNR for treatment 5
Jul-08	-	denitrification E4_July08	July 08 experiment data
Jul-08	-	denitrification E4 omit T5 for DNR	July 08 experiment data excluding DNR for treatment 5
Aug-08	-	denitrification E5_Aug08	August 08 experiment data
Aug-08	Linear regression	Linear regression E5_Aug08	Linear regression for August 2008 experiment data
Both July and August	2 way ANOVA	2 way ANOVA E4 and E5	2 way ANOVA for both July and August 08 experiments
Both July and August	2 way ANOVA	2 way ANOVA E4 and E5 omit T5 E4 for DNR	2 way ANOVA for both July and August 08 experiments without July Treatment 5 DNR data

*Note that E4 in the stats corresponds to E3 in the text and E5 in the stats corresponds to E4 in the text.

SYSTAT Rectangular file C:\Documents and Settings\Owner\Local Settings\Temporary Internet Files\Content.IE5\2S5B7AL3\DO_data[1].syd,
 created Sat Jul 25, 2009 at 19:07:31, contains variables:

MICROCOSM O2_CONC VAR00003

16 cases and 3 variables processed and saved.

SYSTAT Rectangular file C:\Documents and Settings\Owner\Desktop\Desktop Clean-up\Students\Graduate Students\Monisa Nandi\Monisa_O2.syd,
 created Sat Jul 25, 2009 at 19:09:05, contains variables:

MICROCOSM O2_CONC O2_CONC2

Test for equality of correlation coefficients between MICROCOSM & O2_CONC
 and between MICROCOSM & O2_CONC2 with 8 and 8 cases respectively
 Ho: correlation of set1 = correlation of set2 against Alternative = 'not equal'

Population	N	Mean	S
MICROCOSM	8	4.500	2.449
O2_CONC	8	214.549	17.979
MICROCOSM	8	4.500	2.449
O2_CONC2	8	218.830	14.983

Correlation coefficient of set1 = -0.917
 Correlation coefficient of set2 = -0.926
 Difference between correlation coefficients = 0.010
 z = 0.103
 p-value = 0.918

File: C:\Documents and Settings\Monisa\My Documents\Systat files\O2_Output.syo
 File: C:\Documents and Settings\Monisa\My Documents\Systat files\O2_Output.syo
 File: C:\Documents and Settings\Monisa\My Documents\Systat files\O2_Output.syo

SYSTAT Rectangular file C:\Documents and Settings\Monisa\My Documents\Systat files\O2_Data.syd,
 created Sun Jul 26, 2009 at 01:31:30, contains variables:

TIME O2_CONC1 O2_CONC2

Test for equality of correlation coefficients between TIME & O2_CONC1
 and between TIME & O2_CONC2 with 8 and 8 cases respectively
 Ho: correlation of set1 = correlation of set2 against Alternative = 'not equal'

Population	N	Mean	S
TIME	8	4.500	2.449
O2_CONC1	8	214.549	17.979
TIME	8	4.500	2.449
O2_CONC2	8	218.830	14.983

Correlation coefficient of set1	=	-0.917
Correlation coefficient of set2	=	-0.926
Difference between correlation coefficients	=	0.010
z	=	0.103
p-value	=	0.918

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

TREATMENT (2 levels)

1, 2

Dep Var: CFU N: 4 Multiple R: 0.066 Squared multiple R: 0.004

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	1.000	1	1.000	0.009	0.934
Error	232.000	2	116.000		

Least Squares Means

*** WARNING ***

Case 2 is an outlier (Studentized Residual = 2.500)
 Case 4 is an outlier (Studentized Residual = -2.500)

Durbin-Watson D Statistic 1.845

First Order Autocorrelation -0.172

COL/

ROW TREATMENT

1 1

2 2

Using least squares means.

Post Hoc test of CFU

Using model MSE of 116.000 with 2 df.

Matrix of pairwise mean differences:

	1	2
1	0.000	
2	-1.000	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2
1	1.000	
2	0.935	1.000

4 cases and 3 variables processed and saved.

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\CFU_data.syd,
 created Fri Jul 24, 2009 at 17:28:18, contains variables:

TREATMENT CFU VAR00003

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Flux measurements\Expt_1A.syd,
created Tue Jul 14, 2009 at 15:54:06, contains variables:

TREATMENT CIPRO_CONC AMM_FLUX NITR_FLUX

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

TREATMENT (2 levels)

1, 2

1 case(s) deleted due to missing data.

Dep Var: AMM_FLUX N: 5 Multiple R: 0.935 Squared multiple R: 0.875

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	3881.223	1	3881.223	21.005	0.020
Error	554.319	3	184.773		

Least Squares Means

*** WARNING ***

Case	2 is an outlier	(Studentized Residual =	4.773)
Case	3 is an outlier	(Studentized Residual =	-1.412)

Durbin-Watson D Statistic	3.100
First Order Autocorrelation	-0.574

Residuals have been saved.

COL/

ROW TREATMENT

1 1

2 2

Using least squares means.

Post Hoc test of AMM_FLUX

Using model MSE of 184.773 with 3 df.

Matrix of pairwise mean differences:

	1	2
1	0.000	
2	-56.871	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2
1	1.000	
2	0.020	1.000

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

TREATMENT (2 levels)

1, 2

2 case(s) deleted due to missing data.

Dep Var: NITR_FLUX N: 4 Multiple R: 0.835 Squared multiple R: 0.697

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	492.429	1	492.429	4.600	0.165
Error	214.123	2	107.061		

Least Squares Means

*** WARNING ***

Case	5 is an outlier	(Studentized Residual =	5.600)
Case	6 is an outlier	(Studentized Residual =	-5.600)

Durbin-Watson D Statistic	2.327
First Order Autocorrelation	-0.413

Residuals have been saved.

COL/

ROW TREATMENT

1 1

2 2

Using least squares means.

Post Hoc test of NITR_FLUX

Using model MSE of 107.061 with 2 df.

Matrix of pairwise mean differences:

	1	2
1	0.000	
2	-22.191	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2
1	1.000	
2	0.165	1.000

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

TREATMENT (2 levels)

1, 2

Dep Var: AMM_FLUX N: 6 Multiple R: 0.507 Squared multiple R: 0.257

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	12.704	1	12.704	1.385	0.305
Error	36.702	4	9.175		

Least Squares Means

*** WARNING ***

Case 2 is an outlier (Studentized Residual = -4.437)

Durbin-Watson D Statistic 3.101

First Order Autocorrelation -0.622

COL/

ROW TREATMENT

1 1

2 2

Using least squares means.

Post Hoc test of AMM_FLUX

Using model MSE of 9.175 with 4 df.

Matrix of pairwise mean differences:

	1	2
1	0.000	
2	2.910	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2
1	1.000	
2	0.305	1.000

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

TREATMENT (2 levels)

1, 2

1 case(s) deleted due to missing data.

Dep Var: NITR_FLUX N: 5 Multiple R: 0.737 Squared multiple R: 0.543

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	1473.732	1	1473.732	3.564	0.155
Error	1240.359	3	413.453		

Least Squares Means

*** WARNING ***

Case 3 is an outlier (Studentized Residual = 14.363)

Durbin-Watson D Statistic 2.371

First Order Autocorrelation -0.245

COL/

ROW TREATMENT

1 1

2 2

Using least squares means.

Post Hoc test of NITR_FLUX

Using model MSE of 413.453 with 3 df.

Matrix of pairwise mean differences:

	1	2
1	0.000	
2	-35.044	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2
1	1.000	
2	0.155	1.000

RATE_AMMONIF	
N of cases	28
Minimum	185.260
Maximum	1032.960
Mean	616.730
Standard Dev	263.471

Kolmogorov-Smirnov one sample test using normal(602.70, 277.55) distribution

Variable	N-of-Cases	MaxDif	Probability (2-tail)
RATE_AMMONIF	28.000	0.163	0.447

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

TREATMENT (6 levels)

1, 2, 3, 4, 5, 6

2 case(s) deleted due to missing data.

Dep Var: RATE_AMMONIF N: 28 Multiple R: 0.564 Squared multiple R: 0.319

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	596981.628	5	119396.326	2.057	0.110
Error	1277274.533	22	58057.933		

Least Squares Means

Durbin-Watson D Statistic 2.289

First Order Autocorrelation -0.185

COL/

ROW TREATMENT

1	1
2	2
3	3
4	4
5	5
6	6

Using least squares means.

Post Hoc test of RATE_AMMONI

Using model MSE of 58057.933 with 22 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	-28.070	0.000			
3	70.734	98.804	0.000		
4	-310.448	-282.378	-381.182	0.000	
5	-174.032	-145.962	-244.766	136.416	0.000
6	-309.051	-280.981	-379.785	1.397	-135.019

6	
6	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	1.000	1.000			
3	0.997	0.986	1.000		
4	0.417	0.517	0.214	1.000	
5	0.858	0.926	0.603	0.956	1.000
6	0.421	0.523	0.217	1.000	0.957

6	
6	1.000

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

TREATMENT (6 levels)

1, 2, 3, 4, 5, 6

4 case(s) deleted due to missing data.

Dep Var: RATE_NITR N: 26 Multiple R: 0.801 Squared multiple R: 0.642

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	1303649.157	5	260729.831	7.162	0.001
Error	728124.601	20	36406.230		

Least Squares Means

Durbin-Watson D Statistic 2.347

First Order Autocorrelation -0.254

COL/

ROW TREATMENT

1	1
2	2
3	3
4	4
5	5
6	6

Using least squares means.

Post Hoc test of RATE_NITR

Using model MSE of 36406.230 with 20 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	-550.163	0.000			
3	152.141	702.304	0.000		
4	-79.153	471.010	-231.294	0.000	
5	-19.788	530.375	-171.929	59.365	0.000
6	94.035	644.198	-58.106	173.188	113.822

6	
6	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.007	1.000			
3	0.837	0.000	1.000		
4	0.988	0.016	0.421	1.000	
5	1.000	0.009	0.758	0.997	1.000
6	0.980	0.001	0.997	0.753	0.955

6	
6	1.000

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Flux measurements\Expt 4\Flux_rates.syd, created Tue Jul 14, 2009 at 17:22:13, contains variables:

TREATMENT CIPRO_CONC AMM_FLUX NITR_FLUX

WARNING

The file

C:\Documents and Settings\mnandi\My Documents\School\Systat files\Flux measurements\Expt 4\Flux_rates.syd was read for processing, and its contents have been replaced by saving the processed data into it.

30 cases and 4 variables processed and saved.

	AMM_FLUX
N of cases	29
Minimum	-7519.860
Maximum	-42.100
Mean	-2687.079
Standard Dev	1924.012

Kolmogorov-Smirnov one sample test using normal(-2687.08, 1924.01) distribution

Variable	N-of-Cases	MaxDif	Probability (2-tail)
AMM_FLUX	29.000	0.102	0.925

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

TREATMENT (6 levels)

1, 2, 3, 4, 5, 6

1 case(s) deleted due to missing data.

Dep Var: AMM_FLUX N: 29 Multiple R: 0.320 Squared multiple R: 0.102

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	1.05980E+07	5	2119606.766	0.524	0.756
Error	9.30530E+07	23	4045782.818		

Least Squares Means

Durbin-Watson D Statistic 1.940
First Order Autocorrelation -0.026

COL/
ROW TREATMENT

1 1
2 2

3 3
 4 4
 5 5
 6 6

Using least squares means.

Post Hoc test of AMM_FLUX

Using model MSE of 4045782.818 with 23 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	-1684.177	0.000			
3	-20.206	1663.972	0.000		
4	-981.876	702.302	-961.670	0.000	
5	-356.710	1327.468	-336.504	625.166	0.000
6	-1015.562	668.616	-995.356	-33.686	-658.852

6	0.000
---	-------

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.809	1.000			
3	1.000	0.778	1.000		
4	0.976	0.993	0.972	1.000	
5	1.000	0.898	1.000	0.996	1.000
6	0.973	0.995	0.968	1.000	0.995

6	1.000
---	-------

NITR_FLUX	
N of cases	28
Minimum	-1229.450
Maximum	757.880
Mean	-180.147
Standard Dev	514.619

Kolmogorov-Smirnov one sample test using normal(-180.15, 514.62) distribution

Variable	N-of-Cases	MaxDif	Probability (2-tail)
NITR_FLUX	28.000	0.100	0.942

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

TREATMENT (6 levels)

1, 2, 3, 4, 5, 6

2 case(s) deleted due to missing data.

Dep Var: NITR_FLUX N: 28 Multiple R: 0.631 Squared multiple R: 0.398

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
TREATMENT	2848791.967	5	569758.393	2.914	0.036
Error	4301681.513	22	195530.978		

Least Squares Means

Durbin-Watson D Statistic 1.888

First Order Autocorrelation -0.007

COL/

ROW TREATMENT

1	1
2	2
3	3
4	4
5	5
6	6

Using least squares means.

Post Hoc test of NITR_FLUX

Using model MSE of 195530.978 with 22 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	-102.738	0.000			
3	394.098	496.836	0.000		
4	880.403	983.141	486.305	0.000	
5	466.514	569.252	72.416	-413.889	0.000
6	427.780	530.518	33.682	-452.623	-38.734

6	
6	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.999	1.000			
3	0.767	0.561	1.000		
4	0.068	0.033	0.634	1.000	
5	0.565	0.355	1.000	0.729	1.000
6	0.650	0.430	1.000	0.652	1.000

6	
6	1.000

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

3 case(s) deleted due to missing data.

Dep Var: RATE_NITR N: 29 Multiple R: 0.532 Squared multiple R: 0.283

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONC	27567.303	5	5513.461	1.818	0.149
Error	69750.622	23	3032.636		

Least Squares Means

*** WARNING ***

Case 29 is an outlier (Studentized Residual = -2.825)

Durbin-Watson D Statistic 2.107

First Order Autocorrelation -0.184

COL/

ROW CONC

1 0
 2 0.5
 3 1
 4 1.5
 5 2
 6 2.5

Using least squares means.

Post Hoc test of RATE_NITR

Using model MSE of 3032.636 with 23 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	-35.025	0.000			
3	8.461	43.486	0.000		
4	-55.982	-20.958	-64.444	0.000	
5	-68.096	-33.071	-76.557	-12.114	0.000
6	-64.470	-29.445	-72.931	-8.487	3.626

6	
6	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.929	1.000			
3	1.000	0.843	1.000		
4	0.602	0.992	0.456	1.000	
5	0.396	0.944	0.276	0.999	1.000
6	0.455	0.965	0.325	1.000	1.000

6	
6	1.000

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

6 case(s) deleted due to missing data.

The following effects have lost degrees of freedom.

Effect	Initial df	Lost df	Final df
CONC	5	1	4

Dep Var: RATE_NITR N: 24 Multiple R: 0.433 Squared multiple R: 0.187

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CONC	15688.208	4	3922.052	1.096	0.387
Error	67988.373	19	3578.335		

Least Squares Means

Durbin-Watson D Statistic 2.073
 First Order Autocorrelation -0.170

COL/
 ROW CONC

1	0
2	0.5
3	1.5
4	2
5	2.5

Using least squares means.
 Post Hoc test of RATE_NITR
 Using model MSE of 3578.335 with 19 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	-35.025	0.000			
3	-55.982	-20.958	0.000		
4	-68.096	-33.071	-12.114	0.000	
5	-64.470	-29.445	-8.487	3.626	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.903	1.000			
3	0.587	0.984	1.000		
4	0.402	0.920	0.998	1.000	
5	0.455	0.946	0.999	1.000	1.000

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Nitrification_nitrification_all.syd, created Thu Mar 05, 2009 at 10:17:06, contains variables:

TREATMENT REPLICATE RATE_NITR CONC

WARNING

The file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Nitrification_nitrification_all.syd was read for processing, and its contents have been replaced by saving the processed data into it.

32 cases and 4 variables processed and saved.

	RATE_NITR
N of cases	29
Minimum	562.787
Maximum	826.751
Mean	713.237
Standard Dev	58.955

Kolmogorov-Smirnov one sample test using normal(713.24, 58.95) distribution

Variable N-of-Cases MaxDif Probability (2-tail)

RATE_NITR 29.000 0.083 0.988

3 case(s) deleted due to missing data.

Dep Var: RATE_NITR N: 29 Multiple R: 0.422 Squared multiple R: 0.178

Adjusted squared multiple R: 0.147 Standard error of estimate: 54.441

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	749.599	18.132	0.000	.	41.341	0.000
CONC	-28.501	11.798	-0.422	1.000	-2.416	0.023

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	17296.003	1	17296.003	5.836	0.023
Residual	80021.921	27	2963.775		

Durbin-Watson D Statistic 1.889
First Order Autocorrelation -0.049

Plot of residuals against predicted values

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Nitrification_nitrification_omit T3.syd, created Wed Mar 11, 2009 at 12:16:53, contains variables:

TREATMENT REPLICATE RATE_NITR CONC

WARNING

The file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Nitrification_nitrification_omit T3.syd was read for processing, and its contents have been replaced by saving the processed data into it.

30 cases and 4 variables processed and saved.

	RATE_NITR
N of cases	24
Minimum	562.787
Maximum	826.751
Mean	703.999
Standard Dev	60.317

Kolmogorov-Smirnov one sample test using normal(704.00, 60.32) distribution

Variable	N-of-Cases	MaxDif	Probability (2-tail)
RATE_NITR	24.000	0.123	0.817

6 case(s) deleted due to missing data.

Dep Var: RATE_NITR N: 24 Multiple R: 0.404 Squared multiple R: 0.163

Adjusted squared multiple R: 0.125 Standard error of estimate: 56.425

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	738.108	20.108	0.000	.	36.707	0.000
CONC	-25.582	12.362	-0.404	1.000	-2.069	0.050

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	13634.347	1	13634.347	4.282	0.050
Residual	70042.234	22	3183.738		

Durbin-Watson D Statistic 2.024
 First Order Autocorrelation -0.116

Plot of residuals against predicted values

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Ammonification_ammonification_all.syd, created Wed Mar 11, 2009 at 18:20:50, contains variables:

TREATMENT REPLICATE RATE_AMMONIF CIPRO_CONC

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

Dep Var: RATE_AMMONIF N: 30 Multiple R: 0.524 Squared multiple R: 0.275

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	1.059	5	0.212	1.821	0.147
Error	2.791	24	0.116		

Least Squares Means

*** WARNING ***

Case 15 is an outlier (Studentized Residual = 3.151)

Durbin-Watson D Statistic 2.578

First Order Autocorrelation -0.300

COL/

ROW CIPRO_CONC

1 0
 2 0.5
 3 1
 4 1.5
 5 2
 6 2.5

Using least squares means.

Post Hoc test of RATE_AMMONI

Using model MSE of 0.116 with 24 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	0.114	0.000			
3	0.342	0.227	0.000		
4	-0.241	-0.355	-0.582	0.000	
5	-0.116	-0.230	-0.458	0.125	0.000
6	-0.103	-0.217	-0.445	0.138	0.013

6	
6	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.994	1.000			
3	0.616	0.895	1.000		
4	0.870	0.578	0.112	1.000	
5	0.994	0.889	0.310	0.992	1.000
6	0.997	0.911	0.340	0.987	1.000

6	
6	1.000

File: C:\Documents and Settings\Monisa\My Documents\Systat files\Ammonification\1 way ANOVA_omit T3.syo
 File: C:\Documents and Settings\Monisa\My Documents\Systat files\Ammonification\1 way ANOVA_omit T3.syo
 File: C:\Documents and Settings\Monisa\My Documents\Systat files\Ammonification\1 way ANOVA_omit T3.syo

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Ammonification_ammonification_all.syd, created Wed Mar 11, 2009 at 18:20:50, contains variables:

TREATMENT REPLICATE RATE_AMMONIF CIPRO_CONC

30 cases and 4 variables processed and saved.

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Ammonification_ammonification_omit T3.syd, created Wed Mar 11, 2009 at 18:25:19, contains variables:

TREATMENT REPLICATE RATE_AMMONIF CIPRO_CONC

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

5 case(s) deleted due to missing data.

The following effects have lost degrees of freedom.

Effect	Initial df	Lost df	Final df
CIPRO_CONC	5	1	4

Dep Var: RATE_AMMONIF N: 25 Multiple R: 0.432 Squared multiple R: 0.187

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	0.356	4	0.089	1.146	0.364
Error	1.553	20	0.078		

Least Squares Means

Durbin-Watson D Statistic 2.234
 First Order Autocorrelation -0.138
 COL/

ROW CIPRO_CONC

1 0
 2 0.5
 3 1.5
 4 2
 5 2.5

Using least squares means.
 Post Hoc test of RATE_AMMONI
 Using model MSE of 0.078 with 20 df.

Matrix of pairwise mean differences:

File: C:\Documents and Settings\Monisa\My Documents\Systat files\Ammonification\1 way ANOVA_omit T3.syo

File: C:\Documents and Settings\Monisa\My Documents\Systat files\Ammonification\1 way ANOVA_omit T3.syo

File: C:\Documents and Settings\Monisa\My Documents\Systat files\Ammonification\1 way ANOVA_omit T3.syo

	1	2	3	4	5
1	0.000				
2	0.114	0.000			
3	-0.241	-0.355	0.000		
4	-0.116	-0.230	0.125	0.000	
5	-0.103	-0.217	0.138	0.013	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.965	1.000			
3	0.656	0.295	1.000		
4	0.963	0.690	0.952	1.000	
5	0.976	0.733	0.933	1.000	1.000

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Ammonification_ammonification_all.syd, created Wed Mar 11, 2009 at 18:20:50, contains variables:

TREATMENT REPLICATE RATE_AMMONIF CIPRO_CONC

	RATE_AMMONIF
N of cases	30
Minimum	0.336
Maximum	2.012
Mean	0.849
Standard Dev	0.364

Kolmogorov-Smirnov one sample test using normal(0.85, 0.36) distribution

Variable	N-of-Cases	MaxDif	Probability (2-tail)
RATE_AMMONIF	30.000	0.131	0.685

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)
 0, 0.5, 1, 1.5, 2, 2.5

Dep Var: RATE_AMMONIF N: 30 Multiple R: 0.524 Squared multiple R: 0.275

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	1.059	5	0.212	1.821	0.147
Error	2.791	24	0.116		

Least Squares Means

*** WARNING ***

Case 15 is an outlier (Studentized Residual = 3.151)

Durbin-Watson D Statistic 2.578

First Order Autocorrelation -0.300

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Ammonification_ammonification_all.syd, created Wed Mar 11, 2009 at 18:20:50, contains variables:

TREATMENT REPLICATE RATE_AMMONIF CIPRO_CONC

30 cases and 4 variables processed and saved.

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Ammonification_ammonification_omit T3.syd, created Wed Mar 11, 2009 at 18:25:19, contains variables:

TREATMENT REPLICATE RATE_AMMONIF CIPRO_CONC

RATE_AMMONIF	
N of cases	25
Minimum	0.336
Maximum	1.360
Mean	0.781
Standard Dev	0.282

Kolmogorov-Smirnov one sample test using normal(0.78, 0.28) distribution

Variable	N-of-Cases	MaxDif	Probability (2-tail)
RATE_AMMONIF	25.000	0.124	0.796

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)
 0, 0.5, 1, 1.5, 2, 2.5
 5 case(s) deleted due to missing data.

The following effects have lost degrees of freedom.

Effect	Initial df	Lost df	Final df
CIPRO_CONC	5	1	4

Dep Var: RATE_AMMONIF N: 25 Multiple R: 0.432 Squared multiple R: 0.187

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	0.356	4	0.089	1.146	0.364
Error	1.553	20	0.078		

Least Squares Means

Durbin-Watson D Statistic 2.234

First Order Autocorrelation -0.138

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files_denitrification_expt1.syd,
 created Wed Mar 11, 2009 at 10:41:45, contains variables:

SAMPLENO\$ TOTALDNR PCTCONTRDIFF EXPT RESP TREATMENT
 CIPRO_CONC

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

4 case(s) deleted due to missing data.

Dep Var: TOTALDNR N: 26 Multiple R: 0.905 Squared multiple R: 0.819

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	96400.994	5	19280.199	18.107	0.000
Error	21295.438	20	1064.772		

Least Squares Means

*** WARNING ***

Case 11 is an outlier (Studentized Residual = 3.607)

Durbin-Watson D Statistic 1.835

First Order Autocorrelation 0.036

COL/

ROW CIPRO_CONC

1	0
2	0.5
3	1
4	1.5
5	2
6	2.5

Using least squares means.

Post Hoc test of TOTALDNR

Using model MSE of 1064.772 with 20 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	4.861	0.000			
3	22.487	17.626	0.000		
4	-90.866	-95.727	-113.353	0.000	
5	42.348	37.487	19.861	133.214	0.000
6	-126.710	-131.570	-149.197	-35.843	-169.057

6	
6	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	1.000	1.000			
3	0.880	0.953	1.000		
4	0.006	0.003	0.001	1.000	
5	0.637	0.742	0.976	0.002	1.000
6	0.000	0.000	0.000	0.585	0.000

6	
6	1.000

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

4 case(s) deleted due to missing data.

Dep Var: PCTCONTRDIFF N: 26 Multiple R: 0.671 Squared multiple R: 0.450

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	135.221	5	27.044	3.279	0.025
Error	164.949	20	8.247		

Least Squares Means

*** WARNING ***
 Case 26 is an outlier (Studentized Residual = -3.174)
 Case 30 is an outlier (Studentized Residual = 5.577)

Durbin-Watson D Statistic 1.825
 First Order Autocorrelation -0.165
 COL/
 ROW CIPRO_CONC
 1 0
 2 0.5
 3 1

4 1.5
 5 2
 6 2.5

Using least squares means.

Post Hoc test of PCTCONTRDIF

Using model MSE of 8.247 with 20 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	-2.310	0.000			
3	-4.134	-1.824	0.000		
4	-1.709	0.601	2.425	0.000	
5	-4.930	-2.620	-0.796	-3.221	0.000
6	2.030	4.340	6.164	3.739	6.960

6	
6	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.796	1.000			
3	0.249	0.911	1.000		
4	0.945	1.000	0.803	1.000	
5	0.350	0.879	0.999	0.784	1.000
6	0.868	0.207	0.030	0.408	0.082

6	
6	1.000

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files_denitrification_expt1.syd,
 created Wed Mar 11, 2009 at 10:41:45, contains variables:

SAMPLENO\$	TOTALDNR	PCTCONTRDIFF	EXPT	RESP	TREATMENT
CIPRO_CONC					

30 cases and 7 variables processed and saved.

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files_denitrification_expt1_omit T5.syd,
 created Wed Mar 11, 2009 at 11:09:36, contains variables:

SAMPLENO\$	TOTALDNR	PCTCONTRDIFF	EXPT	RESP	TREATMENT
CIPRO_CONC					

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

6 case(s) deleted due to missing data.

The following effects have lost degrees of freedom.

Effect	Initial df	Lost df	Final df
CIPRO_CONC	5	1	4

Dep Var: TOTALDNR N: 24 Multiple R: 0.897 Squared multiple R: 0.805

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	85113.486	4	21278.371	19.584	0.000
Error	20643.909	19	1086.522		

1 0
 2 0.5
 3 1
 4 1.5
 5 2
 6 2.5

Using least squares means.

Post Hoc test of RESP

Using model MSE of 6880.724 with 22 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	85.774	0.000			
3	7.011	-78.763	0.000		
4	14.055	-71.719	7.044	0.000	
5	85.583	-0.191	78.572	71.528	0.000
6	90.794	5.020	83.783	76.739	5.211

6	
6	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.586	1.000			
3	1.000	0.667	1.000		
4	1.000	0.840	1.000	1.000	
5	0.588	1.000	0.669	0.841	1.000
6	0.527	1.000	0.609	0.799	1.000

6	
6	1.000

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files_denitrification_expt1.syd,
 created Wed Mar 11, 2009 at 10:41:45, contains variables:

SAMPLENO\$	TOTALDNR	PCTCONTRDIFF	EXPT	RESP	TREATMENT
CIPRO_CONC					

30 cases and 7 variables processed and saved.

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files_denitrification_expt1_omit T5.syd,
 created Wed Mar 11, 2009 at 11:09:36, contains variables:

SAMPLENO\$	TOTALDNR	PCTCONTRDIFF	EXPT	RESP	TREATMENT
CIPRO_CONC					

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

6 case(s) deleted due to missing data.

The following effects have lost degrees of freedom.

Effect	Initial df	Lost df	Final df
CIPRO_CONC		5	1
			4

Dep Var: TOTALDNR N: 24 Multiple R: 0.897 Squared multiple R: 0.805

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	85113.486	4	21278.371	19.584	0.000
Error	20643.909	19	1086.522		

Least Squares Means

*** WARNING ***

Case 11 is an outlier (Studentized Residual = 3.605)

Durbin-Watson D Statistic 1.688

First Order Autocorrelation 0.108

COL/

ROW CIPRO_CONC

1 0
 2 0.5
 3 1
 4 1.5
 5 2.5

Using least squares means.

Post Hoc test of TOTALDNR

Using model MSE of 1086.522 with 19 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	4.861	0.000			
3	22.487	17.626	0.000		
4	-90.866	-95.727	-113.353	0.000	
5	-126.710	-131.570	-149.197	-35.843	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.999	1.000			
3	0.815	0.913	1.000		
4	0.005	0.003	0.001	1.000	
5	0.000	0.000	0.000	0.503	1.000

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Denitrification_denitrification_E4_July08.syd, created Wed Mar 11, 2009 at 10:41:45, contains variables:

SAMPLENO\$ TOTALDNR PCTCONTRDIFF EXPT RESP TREATMENT
 CIPRO_CONC

	TOTALDNR
N of cases	26
Minimum	45.987
Maximum	315.891
Mean	180.357
Standard Dev	68.614

Kolmogorov-Smirnov one sample test using normal(180.36, 68.61) distribution

Variable	N-of-Cases	MaxDif	Probability (2-tail)
TOTALDNR	26.000	0.164	0.485

4 case(s) deleted due to missing data.

Dep Var: TOTALDNR N: 26 Multiple R: 0.615 Squared multiple R: 0.378

Adjusted squared multiple R: 0.352 Standard error of estimate: 55.243

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	234.914	17.936	0.000	.	13.097	0.000
CIPRO_CONC	-47.282	12.388	-0.615	1.000	-3.817	0.001

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	44454.505	1	44454.505	14.567	0.001
Residual	73241.927	24	3051.747		

Durbin-Watson D Statistic 1.095
First Order Autocorrelation 0.418

Plot of residuals against predicted values

PCTCONTRDIFF	
N of cases	26
Minimum	4.532
Maximum	22.242
Mean	9.670
Standard Dev	3.465

Kolmogorov-Smirnov one sample test using normal(9.67, 3.46) distribution

Variable N-of-Cases MaxDif Probability (2-tail)

PCTCONTRDIFF 26.000 0.160 0.519

4 case(s) deleted due to missing data.

Dep Var: PCTCONTRDIFF N: 26 Multiple R: 0.177 Squared multiple R: 0.031

Adjusted squared multiple R: 0.000 Standard error of estimate: 3.481

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	8.876	1.130	0.000	.	7.854	0.000
CIPRO_CONC	0.688	0.781	0.177	1.000	0.882	0.387

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	9.419	1	9.419	0.777	0.387
Residual	290.751	24	12.115		

*** WARNING ***

Case 30 is an outlier (Studentized Residual = 5.153)

Durbin-Watson D Statistic 0.912
 First Order Autocorrelation 0.309

Plot of residuals against predicted values

	RESP
N of cases	28
Minimum	-360.998
Maximum	-54.175
Mean	-238.862
Standard Dev	85.643

Kolmogorov-Smirnov one sample test using normal(-238.86, 85.64) distribution

Variable	N-of-Cases	MaxDif	Probability (2-tail)
----------	------------	--------	----------------------

RESP	28.000	0.128	0.752
------	--------	-------	-------

2 case(s) deleted due to missing data.

Dep Var: RESP N: 28 Multiple R: 0.286 Squared multiple R: 0.082

Adjusted squared multiple R: 0.046 Standard error of estimate: 83.631

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	-272.487	27.170	0.000	.	-10.029	0.000
CIPRO_CONC	27.290	17.936	0.286	1.000	1.522	0.140

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	16191.313	1	16191.313	2.315	0.140
Residual	181846.228	26	6994.086		

Durbin-Watson D Statistic 2.176

First Order Autocorrelation -0.109

Plot of residuals against predicted values

File: C:\Documents and Settings\Monisa\My Documents\Systat files\Denitrification\Linear regression_E4_omit T5 for DNR.syo
 File: C:\Documents and Settings\Monisa\My Documents\Systat files\Denitrification\Linear regression_E4_omit T5 for DNR.syo
 File: C:\Documents and Settings\Monisa\My Documents\Systat files\Denitrification\Linear regression_E4_omit T5 for DNR.syo

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Denitrification_denitrification_E4_omit T5 for DNR.syd, created Wed Mar 11, 2009 at 11:09:36, contains variables:

SAMPLENO\$ CIPRO_CONC	TOTALDNR	PCTCONTRDIFF	EXPT	RESP	TREATMENT
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	TOTALDNR
N of cases	24
Minimum	45.987
Maximum	315.891
Mean	174.342
Standard Dev	67.810

Kolmogorov-Smirnov one sample test using normal(174.34, 67.81) distribution

Variable	N-of-Cases	MaxDif	Probability (2-tail)
TOTALDNR	24.000	0.164	0.487

6 case(s) deleted due to missing data.

Dep Var: TOTALDNR N: 24 Multiple R: 0.770 Squared multiple R: 0.593

Adjusted squared multiple R: 0.575 Standard error of estimate: 44.217

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	237.718	14.375	0.000	.	16.537	0.000
CIPRO_CONC	-58.501	10.327	-0.770	1.000	-5.665	0.000

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	62743.312	1	62743.312	32.091	0.000
Residual	43014.083	22	1955.186		

*** WARNING ***

Case 11 is an outlier (Studentized Residual = 4.173)

Durbin-Watson D Statistic 1.156

First Order Autocorrelation 0.398

Plot of residuals against predicted values

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files_denitrification_expt2.syd,
 created Thu Feb 12, 2009 at 12:52:08, contains variables:

SAMPLENO\$ TOTALDNR PCTCONTRDIFF EXPT RESP TREATMENT

30 cases and 7 variables processed and saved.

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

3 case(s) deleted due to missing data.

Dep Var: TOTALDNR N: 27 Multiple R: 0.964 Squared multiple R: 0.930

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	252782.133	5	50556.427	55.419	0.000
Error	19157.404	21	912.257		

Least Squares Means

*** WARNING ***

Case 28 is an outlier (Studentized Residual = 6.147)

Durbin-Watson D Statistic 1.998

First Order Autocorrelation -0.038

COL/

ROW CIPRO_CONC

1	0
2	0.5
3	1
4	1.5
5	2
6	2.5

Using least squares means.

Post Hoc test of TOTALDNR

Using model MSE of 912.257 with 21 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	-22.198	0.000			
3	-44.711	-22.514	0.000		
4	63.707	85.905	108.418	0.000	
5	3.391	25.589	48.103	-60.316	0.000
6	238.769	260.966	283.480	175.062	235.377

6	0.000
---	-------

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.878	1.000			
3	0.408	0.906	1.000		
4	0.048	0.002	0.001	1.000	
5	1.000	0.760	0.287	0.047	1.000
6	0.000	0.000	0.000	0.000	0.000

6	1.000
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Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

3 case(s) deleted due to missing data.

Dep Var: PCTCONTRDIFF N: 27 Multiple R: 0.925 Squared multiple R: 0.856

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	23610.759	5	4722.152	24.976	0.000
Error	3970.455	21	189.069		

Least Squares Means

*** WARNING ***
 Case 5 is an outlier (Studentized Residual = 4.962)

Durbin-Watson D Statistic 2.062
 First Order Autocorrelation -0.125
 COL/

ROW CIPRO_CONC
 1 0
 2 0.5
 3 1
 4 1.5

5 2
 6 2.5

Using least squares means.

Post Hoc test of PCTCONTRDIF

Using model MSE of 189.069 with 21 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	25.999	0.000			
3	40.537	14.538	0.000		
4	-33.765	-59.764	-74.302	0.000	
5	-8.734	-34.733	-49.271	25.031	0.000
6	-45.972	-71.971	-86.509	-12.207	-37.238

6	6
0.000	

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.093	1.000			
3	0.010	0.699	1.000		
4	0.016	0.000	0.000	1.000	
5	0.929	0.008	0.001	0.083	1.000
6	0.001	0.000	0.000	0.725	0.004

6	6
1.000	

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

Dep Var: RESP N: 30 Multiple R: 0.469 Squared multiple R: 0.220

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	31028.511	5	6205.702	1.353	0.277
Error	110090.785	24	4587.116		

Of 6 cases, 2 were excluded by making graph range less than data range.

Least Squares Means

*** WARNING ***

Case 3 is an outlier (Studentized Residual = -4.461)

Durbin-Watson D Statistic 1.931

First Order Autocorrelation 0.016

COL/

ROW CIPRO_CONC

1 0
 2 0.5
 3 1
 4 1.5
 5 2
 6 2.5

Using least squares means.

Post Hoc test of RESP

Using model MSE of 4587.116 with 24 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	25.268	0.000			
3	-59.300	-84.568	0.000		
4	-35.651	-60.918	23.649	0.000	
5	-42.753	-68.021	16.547	-7.102	0.000
6	19.660	-5.608	78.960	55.311	62.413

6	
6	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.991	1.000			
3	0.736	0.385	1.000		
4	0.958	0.714	0.993	1.000	
5	0.914	0.614	0.999	1.000	1.000
6	0.997	1.000	0.459	0.787	0.693

6	
6	1.000

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Denitrification_denitrification_E5_Aug08.syd,
 created Wed Mar 11, 2009 at 10:49:49, contains variables:

SAMPLENO\$ TOTALDNR PCTCONTRDIFF EXPT RESP TREATMENT
 CIPRO_CONC

	TOTALDNR
N of cases	27
Minimum	5.553
Maximum	393.111
Mean	101.801
Standard Dev	102.270

Kolmogorov-Smirnov one sample test using normal(101.80, 102.27) distribution

Variable N-of-Cases MaxDif Probability (2-tail)

TOTALDNR 27.000 0.201 0.224

3 case(s) deleted due to missing data.

Dep Var: TOTALDNR N: 27 Multiple R: 0.693 Squared multiple R: 0.480

Adjusted squared multiple R: 0.459 Standard error of estimate: 75.232

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	-4.188	26.401	0.000	.	-0.159	0.875
CIPRO_CONC	80.611	16.791	0.693	1.000	4.801	0.000

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	130444.610	1	130444.610	23.048	0.000
Residual	141494.928	25	5659.797		

*** WARNING ***

Case 28 is an outlier (Studentized Residual = 3.233)

Durbin-Watson D Statistic 0.558

First Order Autocorrelation 0.686

Plot of residuals against predicted values

PCTCONTRDIFF	
N of cases	27
Minimum	9.749
Maximum	100.000
Mean	52.398
Standard Dev	32.570

Kolmogorov-Smirnov one sample test using normal(52.40, 32.57) distribution

Variable N-of-Cases MaxDif Probability (2-tail)

PCTCONTRDIFF 27.000 0.154 0.543

3 case(s) deleted due to missing data.

Dep Var: PCTCONTRDIFF N: 27 Multiple R: 0.642 Squared multiple R: 0.412

Adjusted squared multiple R: 0.389 Standard error of estimate: 25.462

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	83.694	8.935	0.000	.	9.367	0.000
CIPRO_CONC	-23.803	5.683	-0.642	1.000	-4.188	0.000

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	11373.462	1	11373.462	17.543	0.000
Residual	16207.752	25	648.310		

Durbin-Watson D Statistic 0.803

First Order Autocorrelation 0.514

Plot of residuals against predicted values

	RESP
N of cases	30
Minimum	-509.567
Maximum	-191.596
Mean	-322.914
Standard Dev	69.758

Kolmogorov-Smirnov one sample test using normal(-322.91, 69.76) distribution

Variable	N-of-Cases	MaxDif	Probability (2-tail)
RESP	30.000	0.099	0.928

Dep Var: RESP N: 30 Multiple R: 0.058 Squared multiple R: 0.003

Adjusted squared multiple R: 0.000 Standard error of estimate: 70.872

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	-317.049	22.939	0.000	.	-13.821	0.000
CIPRO_CONC	-4.692	15.153	-0.058	1.000	-0.310	0.759

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	481.603	1	481.603	0.096	0.759
Residual	140637.693	28	5022.775		

*** WARNING ***

Case 3 is an outlier (Studentized Residual = -3.356)

Durbin-Watson D Statistic	1.605
First Order Autocorrelation	0.188

Plot of residuals against predicted values

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

7 case(s) deleted due to missing data.

Dep Var: TOTALDNR N: 53 Multiple R: 0.486 Squared multiple R: 0.236

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	29374.126	5	5874.825	0.750	0.590
EXPT	75359.982	1	75359.982	9.622	0.003
Error	360261.843	46	7831.779		

Least Squares Means

*** WARNING ***

Case 58 is an outlier (Studentized Residual = 3.214)

Durbin-Watson D Statistic 0.521

First Order Autocorrelation 0.718

COL/

ROW CIPRO_CONC

1 0
2 0.5
3 1
4 1.5
5 2
6 2.5

Using least squares means.

Post Hoc test of TOTALDNR

Using model MSE of 7831.779 with 46 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	-13.061	0.000			
3	2.778	15.839	0.000		
4	-13.777	-0.716	-16.555	0.000	
5	-6.813	6.248	-9.591	6.964	0.000
6	51.637	64.698	48.859	65.414	58.450

6	
6	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	1.000	1.000			
3	1.000	0.999	1.000		
4	0.999	1.000	0.999	1.000	
5	1.000	1.000	1.000	1.000	1.000
6	0.800	0.581	0.853	0.598	0.767

6	
6	1.000

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

7 case(s) deleted due to missing data.

Dep Var: PCTCONTRDIFF N: 53 Multiple R: 0.811 Squared multiple R: 0.658

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	10075.662	5	2015.132	5.206	0.001
EXPT	25810.246	1	25810.246	66.679	0.000
Error	17805.721	46	387.081		

Least Squares Means

Durbin-Watson D Statistic 0.866

First Order Autocorrelation 0.555

COL/

ROW CIPRO_CONC

1 0

2 0.5

3 1
 4 1.5
 5 2
 6 2.5

Using least squares means.
 Post Hoc test of PCTCONTRDIF

Using model MSE of 387.081 with 46 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	12.028	0.000			
3	12.388	0.360	0.000		
4	-19.151	-31.179	-31.539	0.000	
5	-6.755	-18.783	-19.143	12.396	0.000
6	-21.787	-33.815	-34.175	-2.637	-15.032

6	0.000
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Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.767	1.000			
3	0.786	1.000	1.000		
4	0.326	0.015	0.023	1.000	
5	0.984	0.402	0.446	0.812	1.000
6	0.175	0.005	0.008	1.000	0.641

6	1.000
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Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

2 case(s) deleted due to missing data.

Dep Var: RESP N: 58 Multiple R: 0.600 Squared multiple R: 0.360

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	56556.521	5	11311.304	2.041	0.088
EXPT	97241.991	1	97241.991	17.549	0.000
Error	282600.316	51	5541.183		

Of 6 cases, 3 were excluded by making graph range less than data range.

Least Squares Means

Durbin-Watson D Statistic 2.228
 First Order Autocorrelation -0.119

COL/
 ROW CIPRO_CONC

1 0
 2 0.5
 3 1
 4 1.5
 5 2
 6 2.5

Using least squares means.
 Post Hoc test of RESP

Using model MSE of 5541.183 with 51 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1		0.000			
2	55.521		0.000		
3	-26.144	-81.665		0.000	
4	-9.107	-64.628	17.037		0.000
5	21.415	-34.106	47.559	30.522	
6	55.227	-0.294	81.371	64.334	33.812

6	
6	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.559	1.000			
3	0.969	0.158	1.000		
4	1.000	0.459	0.997	1.000	
5	0.987	0.908	0.710	0.954	1.000
6	0.564	1.000	0.161	0.464	0.911

6	
6	1.000

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Denitrification_denitrification_all.syd,
 created Wed Mar 11, 2009 at 10:53:54, contains variables:

SAMPLENO\$	TOTALDNR	PCTCONTRDIFF	EXPT	RESP	TREATMENT
CIPRO_CONC					

60 cases and 7 variables processed and saved.

SYSTAT Rectangular file C:\Documents and Settings\mnandi\My Documents\School\Systat files\Denitrification_denitrification_all_omit T5 E4 for dnr.syd,
 created Wed Mar 11, 2009 at 11:12:07, contains variables:

SAMPLENO\$	TOTALDNR	PCTCONTRDIFF	EXPT	RESP	TREATMENT
CIPRO_CONC					

Effects coding used for categorical variables in model.

Categorical values encountered during processing are:

CIPRO_CONC (6 levels)

0, 0.5, 1, 1.5, 2, 2.5

9 case(s) deleted due to missing data.

Dep Var: TOTALDNR N: 51 Multiple R: 0.491 Squared multiple R: 0.241

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
CIPRO_CONC	40495.743	5	8099.149	1.057	0.397
EXPT	43500.293	1	43500.293	5.676	0.022
Error	337201.190	44	7663.663		

Least Squares Means

*** WARNING ***

Case 58 is an outlier (Studentized Residual = 3.154)

Durbin-Watson D Statistic 0.381

First Order Autocorrelation 0.787

COL/

ROW CIPRO_CONC

1 0
 2 0.5
 3 1
 4 1.5
 5 2
 6 2.5

Using least squares means.

Post Hoc test of TOTALDNR

Using model MSE of 7663.663 with 44 df.

Matrix of pairwise mean differences:

	1	2	3	4	5
1	0.000				
2	-13.888	0.000			
3	3.812	17.701	0.000		
4	-15.432	-1.544	-19.244	0.000	
5	-48.807	-34.919	-52.620	-33.375	0.000
6	50.810	64.698	46.997	66.242	99.617

6	
6	0.000

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	1	2	3	4	5
1	1.000				
2	0.999	1.000			
3	1.000	0.998	1.000		
4	0.999	1.000	0.998	1.000	
5	0.928	0.981	0.915	0.985	1.000
6	0.803	0.569	0.867	0.574	0.356

6	
6	1.000