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Analysis of potential impacts of DCD on ammonium concentrations in waterways

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Executive Summary

- A review of the DCD literature is covered with respect to studies examining the use, loss and degradation of DCD when applied to agricultural systems. It has been mooted that the loss of DCD to waterways could potentially cause a build-up of ammonia thereby potentially harming aquatic ecosystems.

- Two experiments were performed to examine the dynamics of ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) in water, while in the presence of DCD. A third experiment investigated if dicyandiamide (DCD) in water degraded when exposed to UV light.

- In experiment 1, stream water was incubated *in situ* in the LII River (mean 13.2ºC) in transparent columns for 150 hours. Treatments included four levels of DCD (0-7.1 μg mL^{-1}) with the upper concentrations intentionally exceeding any previously published DCD concentrations from drainage studies. Additions of a conservative tracer (bromide (Br^-)) and NH_4^+-N were also made. Concentrations of NH_4^+-N did not increase in the presence of DCD. In all treatments, both NH_4^+-N and NO_3^--N concentrations decreased at rates which exceeded that observed for the conservative inert tracer as indicated by NH_4^+-N/Br^- and NO_3^--N /Br^- ratio data. Concentrations of DCD did not change with time. Decreases in NH_4^+-N and NO_3-$^-$N with time are assumed to be the net result of nitrification and denitrification. Further ^{15}N labelling experiments are required to elucidate exact mechanisms.

- Experiment 2 was performed *in vitro* following DCD removal from the market place. In this experiment the incubations were kept aerobic and four DCD concentrations were used (0 to 0.2 μg mL^{-1}) along with added NH_4^+-N. Concentrations of NH_4^+-N did not increase in the presence of DCD. In all treatments, NH_4^+-N concentrations decreased and NO_3^--N concentrations increased. Again this is presumed to be the result of nitrification under the aerobic conditions.

- In experiment 3, deionised water or stream water had DCD added (1 μg mL^{-1}) *in vitro* and then these solutions were incubated in the dark or exposed to UV light. After 6 days no degradation of DCD was observed with DCD concentrations remaining constant. It was concluded that UV degradation of DCD was not significant in water bodies.

- It can be concluded from these preliminary studies that under a wide range of DCD concentrations that no increase in NH_4^+-N concentrations occurs.

Introduction

Dicyandiamide ($C_2H_4N_4$; DCD) is a white crystalline odourless powder in its pure state and is water-soluble (NCBI, 2009; NIST, 2011; UNEP, 2003). The DCD compound is used for various industrial applications such as metal extraction, electrical/electronic engineering, the refining and processing of metals, and pharmaceuticals. DCD is classified as a ”nontoxic substance" with an oral LD_{50} reported as being > 30,000 mg/kg body weight in female rats (Amberger, 1986; UNEP, 2003).

New Zealand farming has undergone intensification resulting in increased nitrogen (N) inputs as fertilizer-N and excreta-N and increased N losses via nitrate (NO_3^-) leaching and nitrous oxide (N_2O) emissions (Di et al., 2007; O'Callaghan et al., 2010). Nitrous oxide is a potent greenhouse gas and is responsible for approximately one-third of New Zealand’s total agricultural sector greenhouse gas emissions (Di et al., 2007). The leaching of NO_3^- increases the nutrient loading of aquatic systems and surface waterways contributing to eutrophication and indirect N_2O emissions.

As a nitrification inhibitor DCD is used as a mitigation tool to reduce N_2O emissions and NO_3^- leaching (Kelliher et al., 2008; Schwarzer and Haselwandter, 1996). The first stage of the nitrification process where ammonia (NH_3) is oxidised to nitrite (NO_2^-), is facilitated by the ammonia mono-oxygenase (AMO) enzyme of the autotrophic nitrifying bacteria. The DCD compound inhibits this enzyme. The action of DCD is bacteriostatic and not bactericidal meaning that it doesn’t kill the bacteria but inhibits the enzyme activity (Zacherl and Amberger, 1990). The inhibition of nitrification by DCD means less NO_2^- is produced and thus less NO_3^-, with the N remaining in the ammonium (NH_4^+) form longer. Ammonium is able to bind to negatively charged clay and organic matter surfaces due to its positive charge. In comparison NO_3^- has a negative charge meaning it is repelled from the binding sites within the soil causing it to readily leach through the soil profile. Therefore surplus N, as NO_3^-, is prone to leaching particularly over the autumn, winter and early spring periods when plant growth is low and rainfall is high. This is when the nitrification inhibitor, DCD, is most effectively used to prevent loss of N via N_2O emissions and NO_3^- leaching (Cameron et al., 2013; Singh et al., 2008; Smith and Schallenberg, 2013). The use of DCD as a nitrification inhibitor has also been shown to improve pasture productivity under certain conditions, possibly due to the longer residence time of N in the NH_4^+ form contributing to the additional pasture growth (Gillingham et al., 2012; Moir et al., 2007). The DCD compound is reported as one of the more environmentally benign nitrification inhibitors, having been shown to have no effect on the diversity of soil bacterial populations, earthworms or Collembola (O'Callaghan, 2010; Singh et al., 2008).

DCD is the most widely used nitrification inhibitor in New Zealand due to it being affordable, readily water-soluble and having minimal or no loss through volatilization (Zaman et al., 2009). There have been two formulations of DCD products marketed in New Zealand for agricultural use. The first, Eco-n (Ravensdown Fertiliser Co-operative) is a suspension preparation of DCD sprayed onto soils, while the second, DCn (Ballance Agri-Nutrients Ltd), was a granulated urea based product. The recommended application rate of DCD varies due to seasonal and regional variables but it is recommended that Eco-n is applied at 10 kg/ha as a fine particle suspension with two applications, one in autumn and one in late winter/early spring (Di and Cameron, 2005b). There has been an extensive amount of research done on the effects of DCD on nitrification in soil pastoral systems examining both N_2O emissions and NO_3^- leaching losses. Research into the degradation and movement of DCD in the environment once applied and its subsequent effects on the nitrification processes, especially in waterways, has received less attention.

In water DCD is abiotically stable, water-soluble and is not hydrolysed regardless of pH (UNEP, 2003). However, DCD is biodegradable and breaks down to form the relatively benign compounds of carbon dioxide, water and ammonia (Amberger, 1989; Schwarzer and Haselwandter, 1991). The degradation of DCD in soil was shown to be controlled by soil bacteria (Hallinger et al., 1990). This was also shown in a study by Schwarzer and Haselwandter (1991) where the degradation of DCD was enzyme catalysed, proving that it was not degraded from the interaction of metal oxides as previously suggested by Amberger (1986). Work by Rajbanshi et al. (1992) also showed that under sterile conditions at 30°C the applied DCD concentration remained constant over 36 days but when reinoculated with bacteria DCD degraded within 7 days.

Soil temperature is reported in the scientific literature as one of the main factors influencing the degradation of DCD. In the study by Amberger (1989) the decomposition of DCD was measured over 17 weeks at a range of temperatures. After 8 weeks the percentage of added DCD remaining in soil at a temperatures of 0, 4, 6 and 12°C was 80, 73, 68 and 40%, respectively, showing that the rate of DCD degradation increased with increasing temperature. This was also supported by the study performed by Bronson et al. (1989) which looked at the decomposition rate of DCD in two contrasting soils; a Decatur silt loam and a Norfolk (loamy sand). The results showed that at a soil temperature of 22°C the half-life of DCD in the Decatur soils was 7.4 while the half-life of DCD in the Norfolk soils was 14.7 days (with the 'half-life' of a substance being the time taken for the concentration of that substance to be reduced by half). At a soil temperature of 8°C the half-life of DCD in the Decatur soil was extended to 25.8 days while the half-life of DCD in the Norfolk soil was 52.2 days. This showed that DCD also degrades at different rates in different soil types. The study by Hauser and Haselwandter (1990) looked at the effect of temperature and aeration on the mineralization of DCD by the soil bacterium EK1. The study showed that DCD was degraded at a faster rate at temperatures of 25°C and 33°C than at 18°C. At a temperature of 10°C the DCD degradation rate was even slower. (Bronson et al., 1989) (Hauser and Haselwandter, 1990).

The laboratory incubation study by Guiraud and Marol (1992) looked at the influence of temperature on the action of DCD. Their results showed that at a temperature of 10°C only 10% of ^{15}N applied was nitrified over a 6 month period. However, when the temperature was increased to 20°C there was an increase in the decomposition of DCD. Guiraud and Marol (1992) concluded that the threshold temperature for rapid degradation of DCD was >15°C. The study by Rajbanshi et al. (1992) studied the decomposition kinetics of DCD at a soil water holding capacity of 80%, in pre-treated and non-pre-treated soils, using 3 different concentrations and at temperatures of 10, 20 and 30°C. The results clearly showed that an increase in soil temperature lead to an increase in the degradation rate of DCD and that the mineralization rates were independent of the initial concentrations of DCD applied and followed zero-order kinetics through metabolic degradation (Guiraud and Marol, 1992; Rajbanshi et al., 1992).

The study by Corre and Zwart (1995) in the Netherlands found that the degradation of DCD, in topsoil (0-40 cm), was complete by spring following an autumn application. However, three months following the application DCD was still detectable at a depth of 90 cm. The decrease of DCD in the 0-40 cm layer was also shown to be slower during winter and faster in spring, showing a seasonal difference in the degradation. At a depth of 90-100 cm the degradation of DCD was shown to be very slow even in summer (Corre and Zwart, 1995). The study by Williamson et al. (1996) also showed that there was a clear relationship between the degradation of DCD and soil temperature following the application of dairy effluent. The DCD half-life ranged from >84 to 39 days at 6°C and 22°C, respectively (Williamson et al.,

1996). This relationship was also reflected in laboratory experiments performed by Puttanna et al. (1999) who showed that an increase in temperature from 10 to 30°C decreased the efficacy of DCD. The percentage of nitrification inhibition 120 days following application was 60% at 10°C, while it was 17% at 20°C. At a temperature of 30°C there was no nitrification inhibition 60 days following DCD application (Puttanna et al., 1999). In a study by Di and Cameron (2005a) DCD was applied at either, 7.5 or 15 kg/ha to a Lismore silt loam and incubated at a moisture content near field capacity under two temperatures 8 or 20°C. The results showed that at a soil temperature of 8°C the DCD concentrations at both application rates were relatively stable but that at the temperature of 20°C the DCD concentration in the soil decreased rapidly over (Fig. 1.4) (Di and Cameron, 2005a). The half-lives of DCD at 8°C were 111-116 days while at 20°C the half-lives were 18-25 days. The rate of DCD applied did not have a major effect on the half-lives calculated. The conclusion that was drawn from this study was that DCD would be most effective in New Zealand when average daily soil temperatures are <10°C i.e. late autumn-winter-early spring.

The average daily soil temperature in the study by Vallejo et al. (2005) varied between 17 and 28°C from June to September and between 5 and 16°C from October to January. They observed a short-term nitrification inhibitory effect of DCD during the first 20-30 days following application, which was attributed to the drainage conditions and the high temperatures following application. They concluded that the use of DCD was still beneficial in the days following the application of animal slurries (Vallejo et al., 2005). In the study by Singh et al. (2008) three different rates of DCD (0, 10 and 20 mg/kg) were added to three varying soil types that were incubated at 25°C for 58 days. The half-life of DCD ranged from 6-15 days, and was longer at the higher rate of DCD application (Singh et al., 2008). The results from a trial by Menneer et al. (2008b) in the Bay of Plenty indicated that autumn-applied DCD had a limited nitrification inhibition period, of 50 and 80 days (Menneer et al., 2008b). In comparison, winter-applied DCD showed greater effectiveness of inhibition, with NO_3^- concentrations in leachate 3 months after application still significantly less than in urine-treated plots without DCD applied (Menneer et al., 2008a). This suggested that a difference in soil temperature may have caused more rapid degradation of DCD in the autumn treatment implying that the persistence of DCD varies by region and the season (autumn to winter). A data synthesis by Kelliher et al. (2008) looked at the temperature dependence of DCD degradation in soils based on published data from controlled environment studies. They quantified the effect of soil temperature on the half-life of DCD and concluded that DCD should be applied when soil temperatures are low (at <10°C the half-life > 72 ± 14 days) to extend the persistence of DCD and in turn the effectiveness of DCD as a nitrification inhibitor. The study by Gillingham et al. (2012) was a 3-year research program, which began in autumn 2009 and took place around New Zealand in four different regions (Waikato, Manawatu, Canterbury and South Otago). DCD applied in autumn persisted in the soil for periods of 83-84 days in North Island trials, and from 40 -160 days in South Island trials. DCD applied in mid-winter generally showed a longer residency time than that applied in late autumn. There were a range of DCD residence times due to both temperature and rainfall varying between sites and between years. Winter temperatures in South Otago are below 5°C until early September and reach 12°C in early November while in the Waikato the winter temperatures are above 5°C and above 12°C from early November (Gillingham et al., 2012).

In the study by Kim et al. (2012) on a poorly drained New Zealand dairy-grazed pasture soil the half-life of DCD was longer when the soil temperature was lower. The half-life DCD showed a linear decrease with increased temperature over the observed range of average seasonal temperatures (10.7 to 16.5°C) (Kim et al., 2012). The study by Watkins et al. (2012) estimated a half-life of 5.5 days for the degradation of DCD in a volcanic ash soil at an average soil temperature of 16°C at a soil depth of 10 cm with a DCD application rate of 3.7

mg/g soil. The degradation of DCD fitted an exponential decay curve as it declined in concentration over the 0-15 days of sampling (Fig. 1.7). A cut plot experiment by O'Connor et al. (2012) in Ireland looked at the effect of DCD at 10 kg/ha on herbage production on two different soil types one a free-draining acid brown earth at Moorepark and one a fine loam soil with imperfect drainage at Johnstown Castle. They suggested that low rainfall could have resulted in DCD remaining on the soil surface exposed to high soil temperatures >14°C causing rapid degradation. As a possible solution to reduce the risk of DCD degradation they suggested the development of a technology that would allow the slow release of DCD into the soil over a period of time and this is currently under further investigation (O'Connor et al., 2012). Thus in summary it is clear from the studies outlined above that higher soil temperatures lead to faster degradation in DCD.

In comparison to the effect of soil temperature on the degradation of DCD the effect of soil moisture has not been as well reported in the literature. The study by Puttanna et al. (1999) showed that at a water holding capacity (WHC) of 40% DCD inhibited nitrification by 52% after 15 days, while at WHC of 60% and 80% DCD inhibited nitrification by 39% and 32%. This showed that DCD had a higher efficacy at lower soil moisture levels (Puttanna et al., 1999). However, in comparison a study by Kim et al. (2011) showed that the half-life of DCD varied with the seasonal variation in soil moisture over a 0-10 cm soil depth (7 days in March to 12 days in December). Their results showed that there was a strong correlation between soil moisture and half-life with a longer half-life in wetter conditions (Kim et al., 2011). This shows that the relationship of the environmental factors effecting DCD degradation are complex and not easily distinguished due to the large number of variables involved. Further studies to examine the effect of soil moisture are required.

Soil type, soil pH and soil organic matter content have been shown to affect the degradation of DCD in soil. The study by Rodgers et al. (1985) compared the mineralization of DCD in a near-neutral soil (pH 6.8) and 5 acidic soils (pH 4.0-4.3) and showed that the mineralization was significantly correlated with the soil pH. They found that a smaller proportion of the DCD (3.8-10.6%) was mineralized in the acidic soil compared to the neutral soil (41.6%) after 60 days (Rodgers et al., 1985). The results by Zhang et al. (2004) showed that a mollisol soil type with a comparatively high organic matter content and CEC sorbed more DCD than an alfisol with a lower organic matter content and CEC. This was supported by Singh et al. (2008) who observed a relatively low recovery (92%) of DCD due to the sorption of DCD caused by the higher organic matter concentration and CEC found in the allophanic Egmont soil. Singh et al. (2008) also observed that DCD degradation was faster in the brown loam allophanic soil and slowest in the silt loam non-allophanic soil. The differences in DCD degradation in the soils were attributed to the differences in the sorption of DCD and in the microbial activities of the soils (Zhang et al., 2004).

The study by Zhang et al. (2004) quantified the sorption-desorption behaviour of DCD in four different soil types and showed that DCD sorption takes place mainly on organic matter surfaces, as the DCD molecule contains 2 active functional groups (-NH_2 and -NH) and can bind to the carboxyl (COOH) functional groups of organic matter through hydrogen bonds. The sorption of DCD on peat humus was higher than that on the phaeozem and the burozem, with much lower sorption observed on soils with organic matter removed. This indicated that soil organic matter was the main site of DCD sorption. The DCD molecule is amphipathic, meaning it has both hydrophilic and hydrophobic functional groups. As the pH increased the sorption of DCD on the phaeozem and burozem soils decreased (from pH 2-5), while a further increase in pH caused a rise in DCD sorption. The study suggested that the hydrophobic domains of organic matter could play an important role in DCD sorption (Zhang et al., 2004).

There have been very few studies that have looked at the potential for the leaching of DCD and its movement to groundwater and the potential environmental impacts that DCD would have in waterways. Corre and Zwart (1995) detected the presence of DCD in leachates sampled at 90-100 cm deep 2-3 months following application. The amount of DCD leached was not large, with 7% leached in November and 2% in December following application**.** With DCD present in leachate at 1 m and the slow degradation of DCD in the deeper soil layers the study concluded that it was likely that DCD could be leached into ground waters. The high water solubility of DCD has been suggested to increase its potential for leaching which reduces its effectiveness as a nitrification inhibitor (Di and Cameron, 2002; Vogeler et al., 2007). The modeling study by Vogeler *et al.* (2007) showed that with an assumed half-life of 20 days only 80% of applied DCD should remain after an 8 day period and that DCD could be easily leached to depth in soil.

The study by Menneer et al. (2008a) suggested that the contribution of greater drainage after the autumn application could lead to separation of DCD from charged N compounds such as NH_4^+, as it is a non-charged and mobile compound and that this would result in its subsequent movement through the soil profile. The study by Menneer et al. (2008b) showed that the leaching of DCD was strongly influenced by macropore flow processes with a rapid emergence in leachate during the first 76 days. Of the DCD applied an average of 5.8 kg N/ha DCD was leached which represented approximately 58% of the applied DCD. The DCD concentrations in the leachate were greatest by day 52 at 3.2 mg L^{-1} (Menneer et al., 2008b)**.** The study by Monaghan et al. (2009) detected small amounts of DCD in drainage waters collected from DCD treated soil. They noted that elevated concentrations were observed in late autumn/early winter drainage and much lower concentrations were evident in the spring drainage**.** On an annual basis they calculated that the amounts of DCD lost in drainage were 2% of the DCD applied in 2004, 6% in 2005, 7% in 2006 and 16% in 2007**.** The results therefore showed that losses were largest when there was high rainfall (high drainage) shortly after application. They calculated that the cumulative losses of DCD in the drainage over the 4 years represented approximately 7% of applied DCD. The highest recorded concentration of DCD in drainage during the study was 3 mg L^{-1} in March 2007. This was suggested to have been possibly high enough to have an effect on the N processes within wetlands and streams (Monaghan et al., 2009).

In the study by Sprosen et al. (2009) 37% of applied DCD was measured in the top 45 cm of soil at day 48 of sampling with most of this DCD found below 15 cm**.** The results show that DCD was leached more readily than NH_4^+ down the profile (Sprosen et al., 2009). The study by O'Connor et al. (2012) suggested that there was a high risk of infiltration of DCD below the top 100 mm of the soil profile in free-draining soils or when high rainfall occurred. The study also reported that an increase in the level of rainfall affected DCD persistence in the top horizon of the soil profile and increased the amount of DCD leached. In the study by Kim et al. (2012) in a poorly drained soil a small amount of the applied DCD leached below 10 cm depth. However, this was not quantified in the results of the paper. The study by Smith et al. (2012) looked at the effectiveness of DCD in the mitigation of N leaching losses from a winter grazed forage crop on a free draining soil. They measured small amounts of DCD in leachate with annual losses in 2009 and 2010 representing 3-7% of the DCD applied**.** A large amount of DCD was measured in leachate collected during the winter/spring of 2011, representing 38% of the DCD applied. The reason given for the increase in DCD loss was linked to the higher than normal rainfall and drainage in the month following DCD application (Smith et al., 2012).

Shepherd et al. (2012) looked at the effect of soil type (clay, silt loam, or sandy loam) and precipitation on the movement of DCD in drainage water collected from lysimeters. DCD was

applied in May and July at a rate of 10 kg/ha and natural rainfall was supplemented with irrigation. The DCD leaching data indicated that the movement of DCD in the sandy loam and silt loam soils was the result of convective-dispersive flow. The results of the clay soil also showed that the main transport mechanism was convective-dispersive flow but there was some preferential flow of DCD from the soil surface to depth**.** The differences in the soil types caused differences in the drainage volume between the soils. The leaching losses ranged from 12 to 46% of applied DCD, with annual drainage in the range 422–1292 mm. DCD was detected in drainage up to 15 months after application. As an average of all soil types, DCD leaching was 4.6 kg/ha (1140 mm target precipitation) and 7.7 kg/ha (2280 mm target precipitation)**.** This represented 23% and 39% of the applied DCD lost via leaching over 12 months. Most of this was lost in the May–October drainage period when most of the drainage occurred.

The presence of DCD in leachate has raised concern around the transmission of DCD into waterways and wetlands. Although DCD in water is abiotically stable it should ultimately degrade in waterways. However, the duration and impact of the DCD degradation process in waterways is not understood.

Wilcock *et al.* (2008) reported that the N contained in DCD itself could be of environmental significance. Wilcock *et al.* (2008) mooted that DCD leached into waterways might block nitrification in waterways prior to DCD degradation. This would be of environmental significance as the inhibition of nitrification in a waterway could potentially cause an increase in ammonia (NH_3) concentrations.

Ammonia (NH_3) is toxic for aquatic organisms when concentrations reach species specific critical levels. For example Hickey and Vickers (1994) in New Zealand studied the toxic concentration of un-ionised ammonia for nine native invertebrate species (crustaceans, shrimp, caddis, mayfly, stonefly and snails) and found that harmful concentrations ranged from 0.18 to >0.8 g/m^3 (Hickey and Vickers, 1994). A study by Richardson (1991) found that NH_3 concentrations of 1.60 g/m^3 were harmful to native New Zealand fish. In salmonid containing waters the value given by the US Environmental Protection Agency (EPA) is 0.52 g/m^3 (Richardson, 1997; U.S.EPA, 1985). This means that if the presence of DCD in waterways causes a build-up of NH_3, there is the potential for harmful effects on the aquatic ecosystems.

There is a significant knowledge gap surrounding the effect(s) of DCD on nitrification in waterways. Therefore this study explores the effect of DCD on nitrification in lowland river water.

Experiment 1 –in situ effect of DCD

Experiment Site and set up

The experiment was carried out in the LII stream near English's Road, Lincoln, Canterbury, New Zealand (43°40'42.69"S, 172°28'35.88"E; Figure 1, 2a). The stream was fenced off from the surrounding farmland. The columns used in the experiment were transparent polycarbonate with PVC end caps. The total length of the columns was 108 cm and the diameter was 7.5 cm. The columns were set up in the stream on the 19th March 2013 and were pushed into the stream sediment (which exceeded 40 cm depth); with the water level 40 cm deep. This resulted in an approximate volume of 1767 mL in each column. The columns were placed approximately 10-

15 cm apart in a semi-circular pattern which facilitated sampling while standing in one position, since moving within the deep sediment was not possible. (Figure 2b). A metal rod was placed upstream from the columns to catch any debris floating on the river thus preventing any dislodgement of the columns.

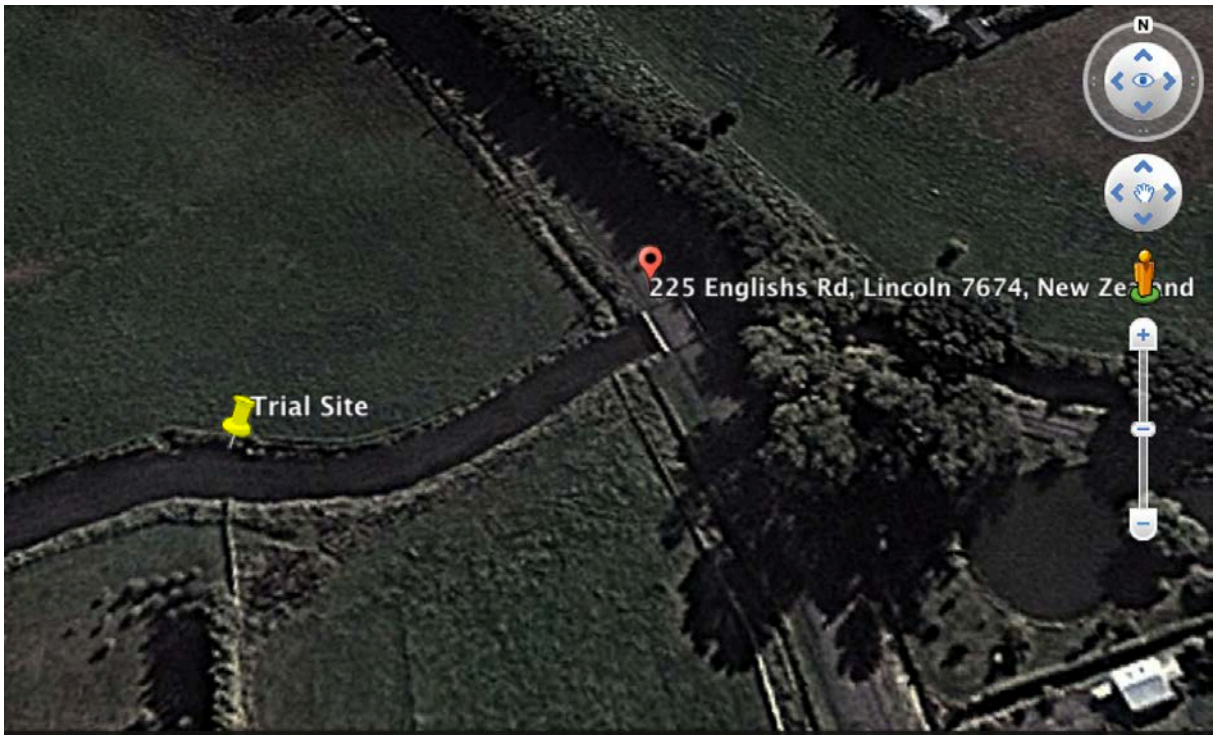


Figure 1. Map showing the field experimental site location in the Liffey Stream, English's Road, Lincoln, Canterbury, New Zealand (GoogleEarth, 2013).

Figure 2a. LII River trial site, facing south-west.

Figure 2b. Set up of the 12 columns in situ.

The initial DCD treatments examined were chosen on the basis that they should encompass the maximum range of DCD concentrations measured in drainage waters. Thus the four DCD treatments consisted of 0, 1.6, 3.5 and 7.1 μg DCD mL^{-1} at time zero which encompassed the 3 μg DCD mL^{-1} previously reported by Monaghan et al. 2009. These were established by adding 10 mL of DCD stock solutions. These treatments also had added to them Br^- as KBr, which served as a conservative tracer (i.e. it is unamended by biological activity), and ammonium sulphate ($(NH_4)_2SO_4$). The latter served to elevate the concentration of NH_4^+ to above detectable levels. Following addition of treatment solutions the water column was well circulated using a 60 mL syringe with tubing attached to pump water through the column water body.

Sampling

Water in the columns was sub-sampled at 0, 3, 6, 23, 26, 29, 47, 53, 71, 74, 141 and 146 hours. This was achieved using syringes with tubing attached to first gently circulate the water in the column and then to take the sub-sample. At the end of the study the transparent columns were removed from the stream bed with the sediment plug intact. To prevent release of DCD into the stream the water contained in the column was decanted off and stored, while the sediment was also bagged for further analysis.

Analyses

All samples were brought back from the field site and refrigerated at 4°C until analysis within 24 hours.

Sediment samples were analysed for pH. Air-dried sediment samples (10 g ± 0.05 were weighed into 70 mL vials and 25 mL of deionised water was added. Then samples were stirred and left to stabilise overnight. The pH meter was calibrated using pH 4 and pH 7 buffers and the samples were kept at the same room temperature as the pH meter at 20°C (Blakemore et al., 1987).

Before analysis all water samples were filtered (0.45 µm Micro-Analytix Pty Ltd). Flow injection analysis was used to analyse for inorganic-N concentrations. The equipment used for FIA analysis was the ALPKEM FS3000, O.I. analytical, twin channel analyser with a standard curve range for ammonium-N and nitrate-N (ppm): 0.00, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and the standard curve range for nitrite-N (ppm): 0.0, 0.0, 0.1, 0.5, 1.0, 2.0. Ammonium-N was analysed using a gas diffusion membrane. The pH of the stream water samples was increased using 0.5M NaOH, where any ammonium ions present were converted to ammonia gas which was analysed colorimetrically at 590 nm. Nitrate-N was analysed by reduction of nitrate-N to nitrite-N using a cadmium reduction coil (OTCR-open tubular cadmium reactor). Nitrite-N reacts with sulphalnilamide/NED to form an azodye compound which was determined spectrophotometrically at 540nm.

To analyse anion concentrations (Br^- and SO_4^{2-}) a Dionex DX-2100 ion chromatograph (Thermo Scientific) and AS-AP Autosampler (Thermo Scientific) were used. The columns used for analysis were the IonPac AS9-SC analytical column 250 x 4 mm (Thermo Scientific) and IonPac AG9-SC guard column 50 x 4 mm (Thermo Scientific) with an anion self-regenerating suppressor (ASRS 300, 4 mm) (Thermo Scientific). The eluent comprised 200 mM Na_2CO_3/75 mM $NaHCO_3$ at a flow rate of 1.4 mL min^{-1}. The analysis temperature was 30°C and the inject volume was 25 µL. The standards were stock mixed standard (Cl, Br, NO_3-N, PO_4-P, and SO_4-S, Alltech Associates Inc), and NO_2^- stock standard (Merck).

To analyse for dicyandiamide an HPLC system was used which comprised of a Prominence Degasser (DGU-20A3); LC-20AB/Prominence Liquid Chromatograph (LC-20AB); Prominence Auto Sampler (SIL-20A HT); Prominence UV/Vis Detector (SPD-20A); Prominence Column Oven (CTO-20A). The column was a Rezex RHM-Monosaccharid (50 x 7.80 mm, Phenomenex) and the eluent was 0.0025M H_2SO_4. The flow rate was 1 mL min^{-1} and the analysis temperature was 45°C. The detector wavelength was 210 nm and the inject volume was 50 µL. The standards were made from high purity dicyandiamide (99%, Sigma-Aldrich) with deionised water to establish a standard curve range comprising: 0, 0.005, 0.008, 0.012, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 5.0, and 10 µg ml^{-1}.

Statistical analyses were performed using Minitab®16. Analysis of variance was used to determine if treatments differed and if differences occurred a Tukey's test was used to determine treatment effects. Effects of time were tested for using a repeated measures test using the General Linear Model in Minitab®16.

Results

River water temperatures averaged 13.2°C during the course of the study.

Concentrations of DCD varied with treatment ($p<0.01$) averaging 0, 1.6, 3.5 and 7.1 µg mL^{-1} at time zero (Figure 3a). These DCD concentrations remained significantly different ($p<0.01$) as they declined with time ($p<0.01$) to be 0, 1.3, 2.6, and 5.5 µg mL^{-1}, respectively after 146 hours (Figure 3a).

Neither Br^-, NO_3^--N or NH_4^+-N concentrations differed due to DCD treatment at time zero averaging 3.6 ± 0.2 (stdev)) 3.4 ± 0.1 and 4.00 ± 0.3 μg mL^{-1}, respectively. Bromide concentrations decreased with time ($p<0.01$) and after 146 hours averaged 2.6 ± 0.2 μg mL^{-1} with no DCD treatment effect (Figure 3b). Concentrations of NO_3^--N also declined with time ($p<0.01$) to average 0.6 ± 0.2 μg mL^{-1} after 146 hours with no DCD treatment effect (figure 3c). The NH_4^+-N concentrations also declined ($p<0.01$) to equal 2.4, 2.8, 2.3, 2.5 μg mL^{-1} after 146 hours with no significant DCD effect on concentration (Figure 4).

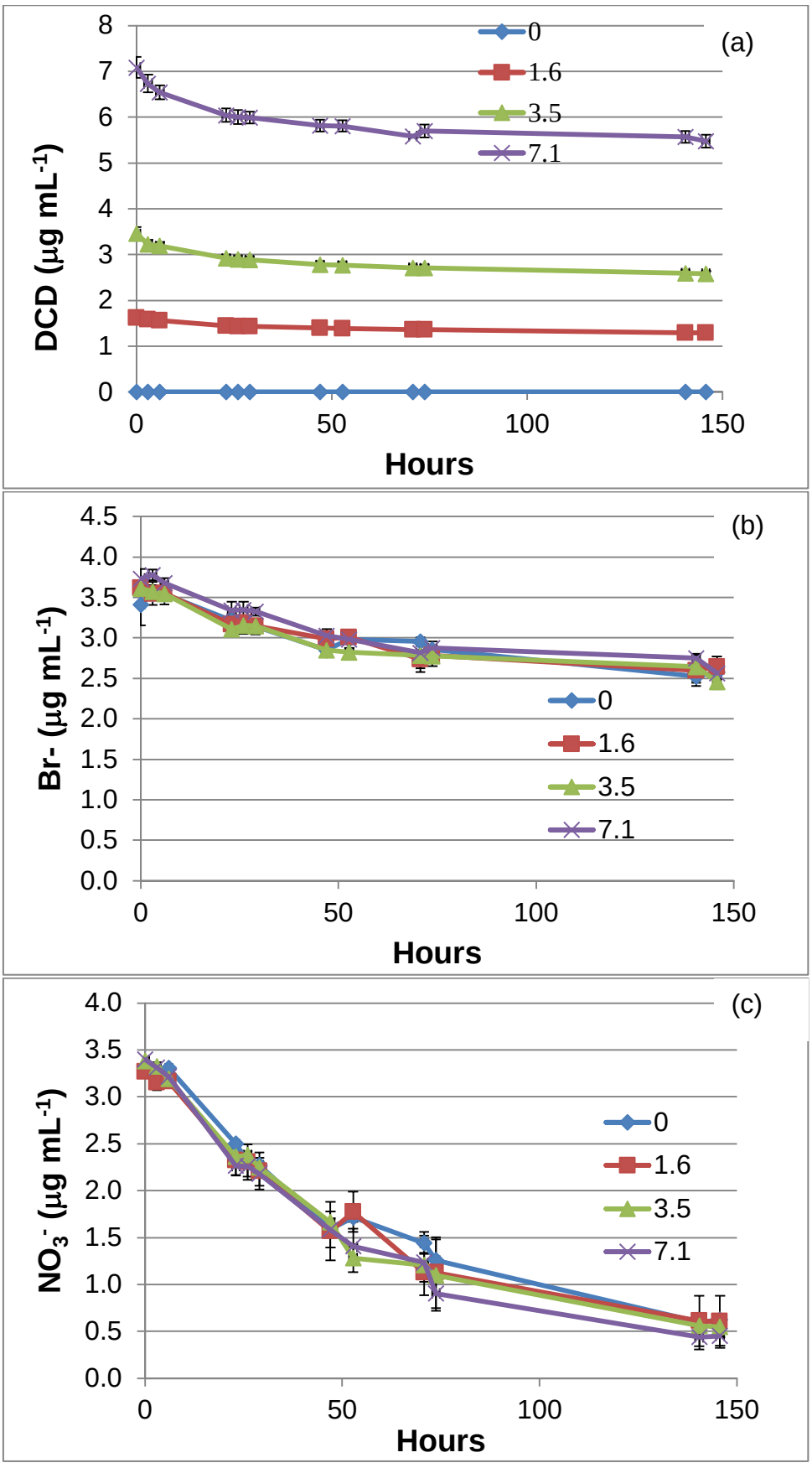


Figure 3. Mean DCD, Br^- and NO_3^- concentrations versus time (error bars are s.e.m, n=3)

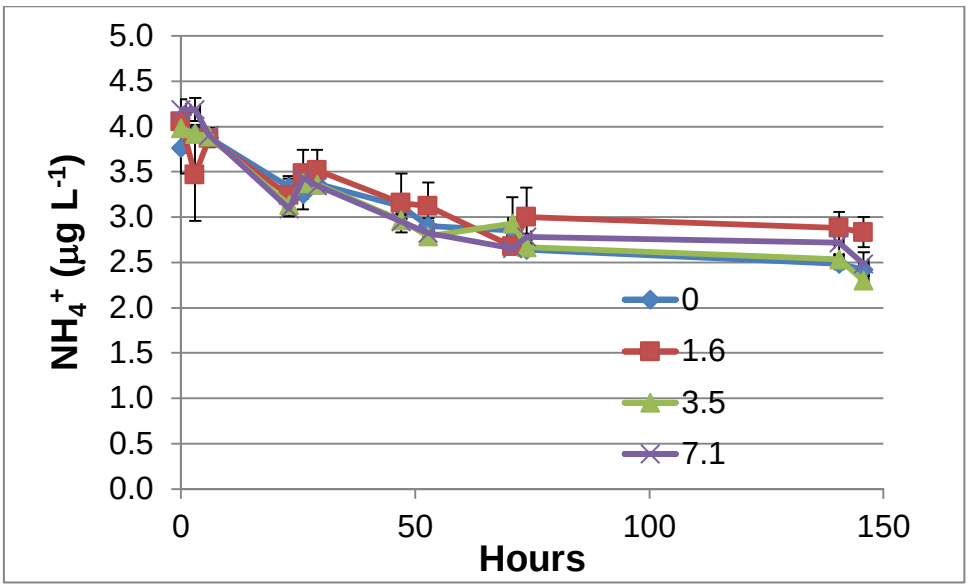


Figure 4. Mean NH_4^+ concentrations versus time for the four DCD concentrations shown in the legend with units of µg mL^{-1} (error bars are s.e.m, n=3)

A comparison of water chemistry, over time, based simply on concentration does not allow for diffusion of elements and compounds from the columns into the sediment. Bromide is considered a biologically inert tracer and any loss of Br^- can therefore be considered to be due to physical processes. Thus by comparing the relative ratios of the biologically influenced nutrient concerned to Br^-, over time, an assessment can be made as to whether biological effects are promoting loss of non-bromide nutrients.

The DCD/Br^- ratios at time zero were 0.45, 0.96, and 1.90 for the 1.6, 3.5 and 7.1 µg mL^{-1} DCD treatments, respectively, and varied with DCD treatment ($p<0.01$). After 146 hours the DCD/ Br^- ratios for the 1.6, 3.5 and 7.1 µg mL^{-1} treatments had increased ($p<0.01$) to be 0.49, 1.05, and 2.13, respectively (Figure 5a). At time zero the NO_3^--N/Br^- ratio averaged 0.9 ± 0.1 with no DCD treatment effect and after 146 hours this had decreased ($p<0.01$) to average 0.21± 0.08 with no DCD treatment effect occurring (Figure 5b). The NH_4^+-N/Br^- treatment averaged 1.1± 0.02 at time zero, again with no DCD treatment, but after 146 hours this ratio had decreased ($p<0.01$) to be 0.98± 0.09) with no difference due to DCD treatment (Figure 5c).

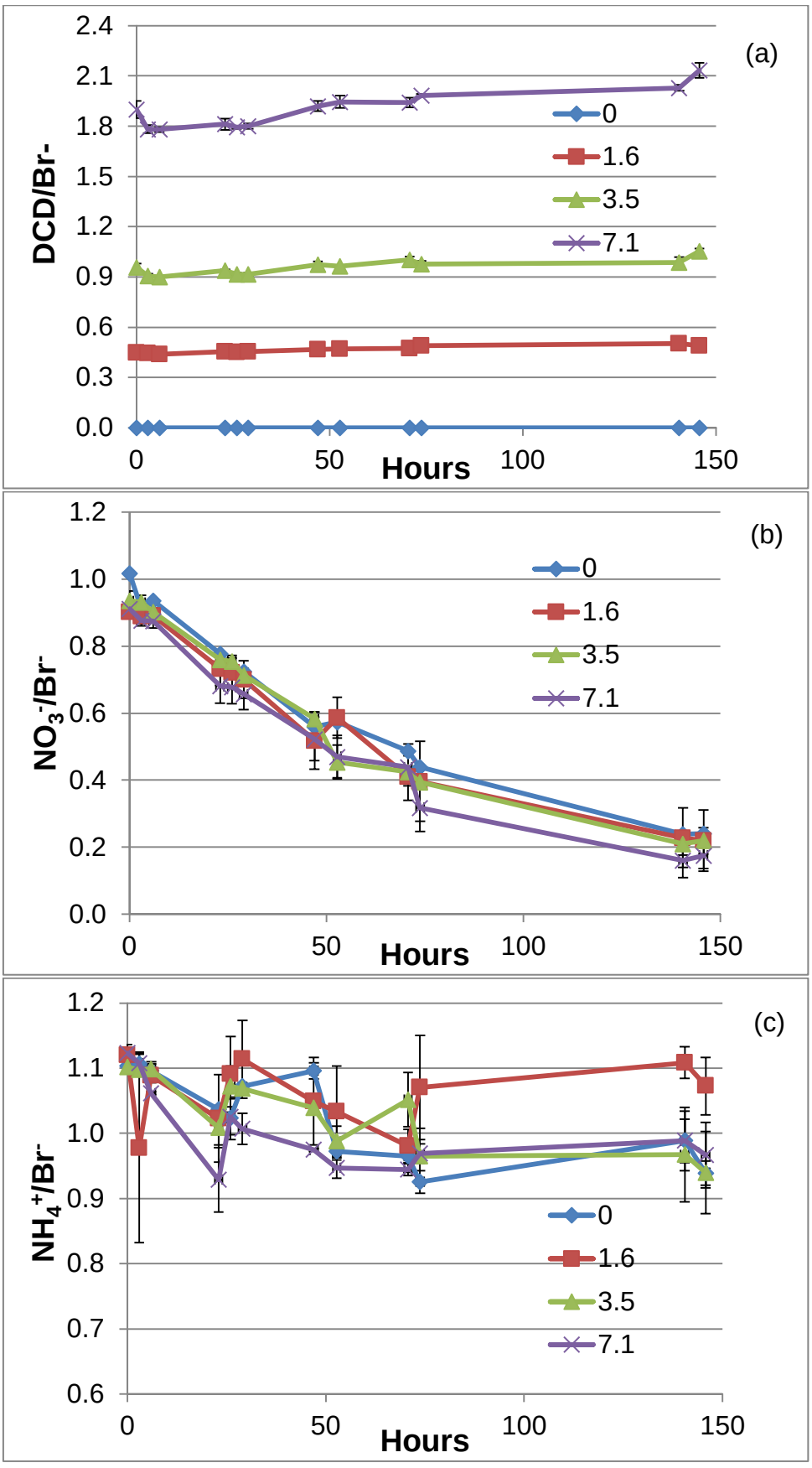


Figure 5 Mean DCD/Br^-, NO_3^--N /Br and NH_4^+-N/Br^- ratios versus time for the four DCD concentrations shown in the legend with units of μg mL^{-1} (n=3, error bars = s.e.m).

A further method to examine relative temporal changes in nutrient concentrations is to look at relative ratios of concentrations i.e. the ratio of the concentration at a given time (C) relative to the concentration at time zero (Co). This ratio can provide a rate of decrease in a given concentration or when plotted against the same ratio for another nutrient can show if the rate of decrease is equal or dissimilar. Figure 6 shows the C/Co ratios for Br^- and NH_4^+ for each of the DCD treatments. After 146 hours the C/Co ratio of the NH_4^+ concentration is lower, relative to time zero, when compared to the Br^- concentration, for all treatments except the 1.6 µg mL^{-1} treatment, where there is more noise in the data.

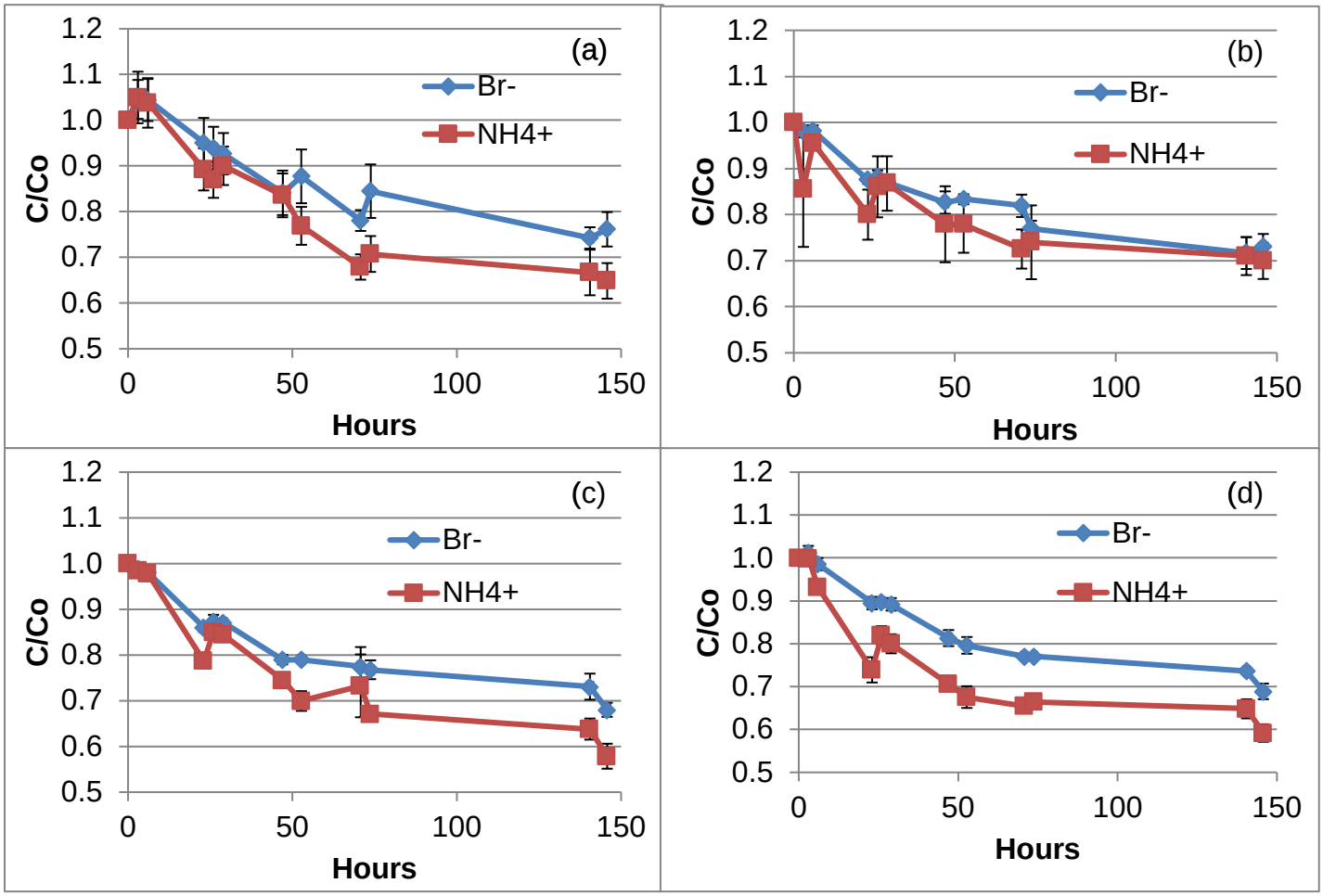


Figure 6 Mean values for C/Co for Br^- and NH_4^+ for the four DCD concentrations at time zero of (a) 0 µg mL^{-1}, (b) 1.6 µg mL^{-1}, (c) 3.5 µg mL^{-1} and (d) 7.1 µg mL^{-1} versus time (n=3, error bars = s.e.m).

If both nutrients have identical decreases in concentration over time relative C/Co concentrations plot along a 1:1 line. Figure 6 presents the ratio of NH_4^+-N C/Co versus the ratio of Br^- C/Co where it can be seen that NH_4^+-N C/Co plots below the 1:1 line, and the rate of decrease in NH_4^+-N C/Co is greater ($p<0.01$), from 23 hours to the end of the experiment, than the rate of decrease in Br^-, when averaged across all DCD treatments.

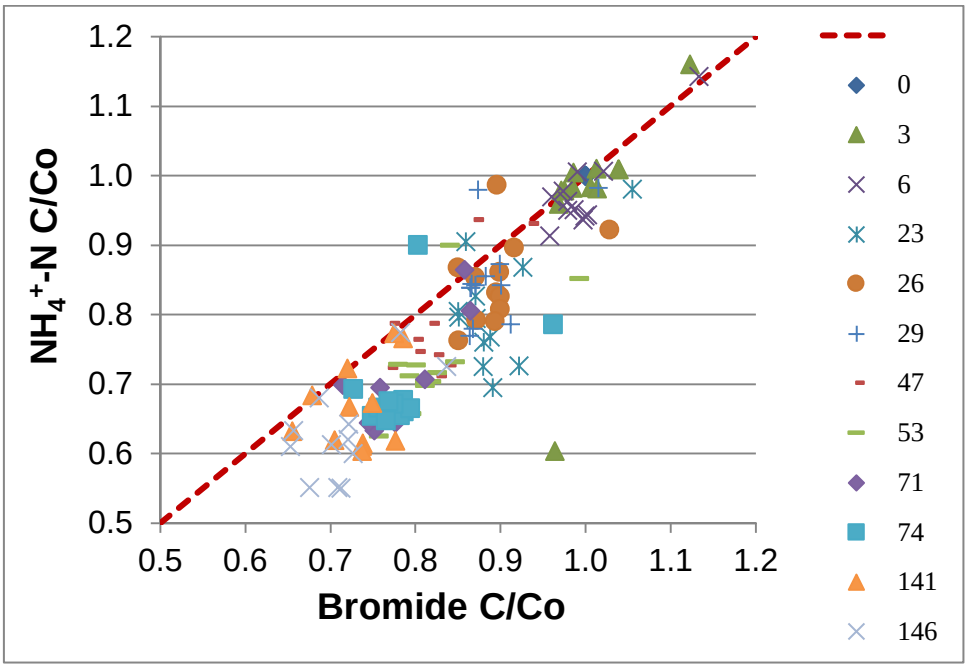


Figure 7 Individual data points for NH_4^+-N C/Co versus the ratio of Br^- C/Co at different sampling times (see legend (hours)), dashed line represents the 1:1 line.

Discussion - experiment 1

The decline in DCD concentrations with time suggests that DCD diffused from the system or was degraded. When comparing the DCD concentrations with Br^- concentrations using the DCD/Br^- ratios the ratio actually increased indicating that the Br-, a conservative tracer, was diffusing from the system faster than the DCD. Since DCD is not created in-situ its loss via diffusion must have been slower than the Br^- anion.

Nitrate declined steadily over time, at a faster rate than the Br^- anion, indicating biological transformation of the NO_3^- anion occurred. This decline was independent of DCD concentration. In this study plants were absent and plant uptake of NO_3^--N can be ruled out, and it is unlikely immobilisation accounted for the entire decrease in NO_3^- concentrations. The most likely explanation for the decrease in NO_3^--N concentrations is denitrification. Dissolved oxygen levels were unavailable for this experiment but it is likely that as time progressed the water in the columns would have become more anaerobic thus favouring denitrification.

Ammonium-N concentrations did not increase due to DCD addition, and in fact declined over time, with this rate of decline significantly greater than the decline in the conservative tracer (Br^-) as indicated by the NH_4^+/Br^- ratio and changes in C/Co over time. This indicates biological processing of the ammonium occurred. This may have been due to microbial uptake or nitrification. Nitrification may have been hampered by a lack of aeration in this study since no plants were present to provide more oxygen and circulation in the water columns was limited to mixing during sampling. Given that NO_3^--N concentrations were also declining, possibly as a result of anaerobic processes, it is important to remember that the measured NH_4^+-N concentrations were a net concentration and thus could have resulted from loss processes such as nitrification and inputs from processes such as dissimilatory reduction of NO_3^- to NH_4^+. Because NH_4^+ is a cation its potential rate of loss from the water column via diffusion through the sediment (pH 5.3) is lower, all things being equal, and so the decline in NH_4^+ concentrations is, in this case, more significant.

In summary, these high DCD concentrations did not lead to an increase in NH_4^+-N concentrations under the conditions of this experiment.

Further studies are required to pursue further interpretation of the results established here. Following this experiment DCD had been withdrawn from the market and so the next experiment was conducted in vitro. To examine DCD effects on NH_4^+ under lower DCD concentrations and more aerobic conditions.

Experiment 2 In-vitro nitrification and DCD effects

Rationale

The concentrations of DCD used in the *in situ* experiment encompassed maximum concentrations reported in drainage waters and are unlikely to persist due to further dilution by the time drainage reaches a stream. Thus a second experiment was performed using lower DCD concentrations. Given DCD was being removed from the market it was prudent to conduct further investigation in the laboratory. Therefore an *in vitro* experiment was set up to explore the effect (if any) of lower concentrations of DCD on nitrification dynamics.

Experimental design

A factorial experiment was set up in *vitro* to determine if ammonium (NH_4^+) was transformed in stream water samples at lower concentrations of DCD and under aerobic conditions. Erlenmeyer flasks were filled with 150 mL of stream water freshly collected from the LII River and four DCD treatments were set up with DCD concentrations of 0, 0.04, 0.1, and 0.2 μg mL^{-1}. Stream water was not filtered and contained 5 grams (wet weight) of sediment in 2.5 L of water. Sediment was included so that a microbial consortium, representative of what would be present *in situ*, was present. Stream water had a total organic carbon concentration of 2.25 ±0.27 μg mL^{-1} (±stdev, n=4). Ammonium was added to all treatments aiming for a concentration of 2 μg mL^{-1}, well in excess of the detection limit on the FIA (0.1 μg mL^{-1}). Once set up all treatments were immediately analysed by taking 30 mL sub-samples from the flasks (time zero (t_0). The flasks were sub-sampled again after 24, 48, 72, and 96 hours. The *in vitro* experiment was conducted at 20ºC and flasks were kept aerobic keeping them unsealed and agitated by placing them on an orbital shaker (Scientific Engineering Ltd).

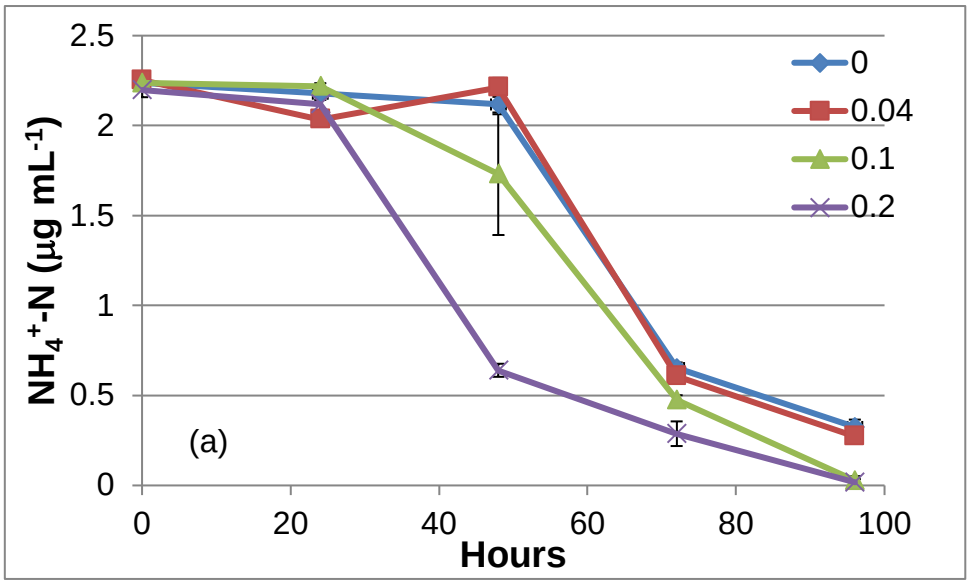
Analyses

Samples were analysed for DCD concentrations using a Rezex RHM-Monosaccharid column (50 x 7.8 mm, Phenomenex) and a 0.0025M H_2SO_4 eluent in conjunction with a Prominence HPLC system (DGU-20A3 Degasser; LC-20AB Liquid chromatograph; SIL-20A HT Autosampler; SPD-20A UV/Vis Detector; CTO-20A Column Oven). The eluent flow rate was 1 mL min^{-1} at a temperature of 45ºC. The UV detection wavelength was 210 nm and the inject volume was 50 μL. Dicyandianide standards were made in a matrix of deionised water and were 0, 0.005, 0.008, 0.012, 0.050, 0.10, 0.20, 0.50, 1.00, 2.00, 3.00, 5.00 and 10.00 μg mL^{-1}.

Statistical analyses were performed using Minitab®16. Analysis of variance was used to determine if treatments differed and if differences occurred a Tukey's test was used to determine treatment effects. Effects of time were tested for using a repeated measures test using the General Linear Model in Minitab®16.

Results

Figure 8 shows the change in both NH_4^+-N and NO_3^--N concentrations after 96 hours of aerobic conditions. At time zero NH_4^+-N concentrations averaged 2.23 ± 0.06 μg mL^{-1} (± stdev) while NO_3^--N concentrations were 3.91 ± 0.02 μg mL^{-1}, with neither affected by DCD treatment. Concentrations of NH_4^+-N then proceeded to decrease over time with consistently lower concentrations of NH_4^+-N ($p<0.01$) when DCD concentrations were ≥ 0.1 μg mL^{-1} (Figure 8a). Final NH_4^+-N concentrations were 0.33, 0.27, 0.028, and 0.018 for the 0, 0.04, 0.10 and 0.20 μg mL^{-1} DCD treatments, respectively (Figure 8). As NH_4^+-N concentrations decreased NO_3^--N concentrations increased to a maximum of 4.70 μg mL^{-1}, with consistently higher concentrations of NO_3^--N ($p<0.01$) when DCD concentrations were ≥ 0.1 μg mL^{-1} (Figure 8a).



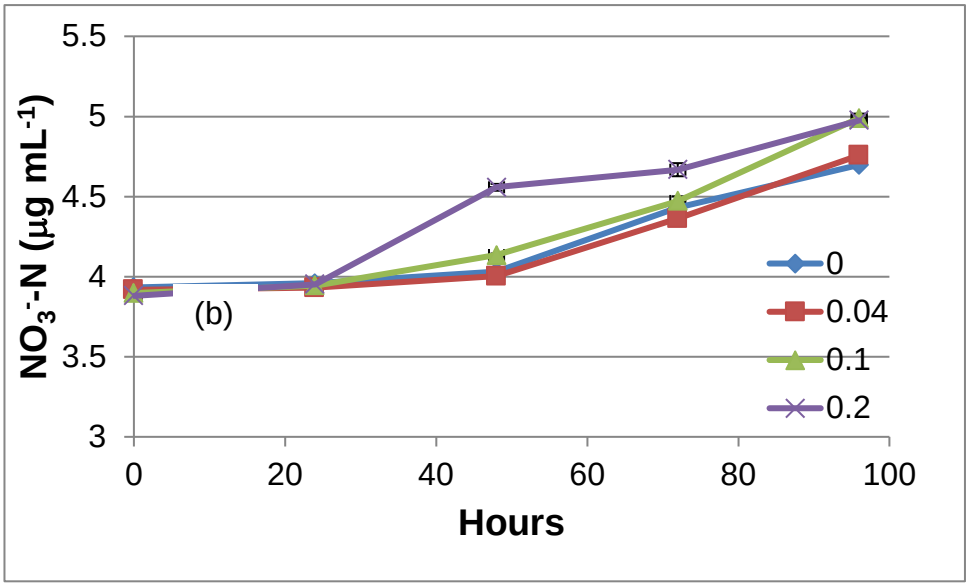


Figure 8: Mean inorganic-N concentrations in stream water treated with DCD at either 0, 0.04, 0.1 or 0.2 µg mL^{-1} DCD versus time (n=4; error bar = one s.e.m).

Over the study the concentration of DCD remained constant ($P > 0.91$) regardless of initial DCD concentration (Figure 9).

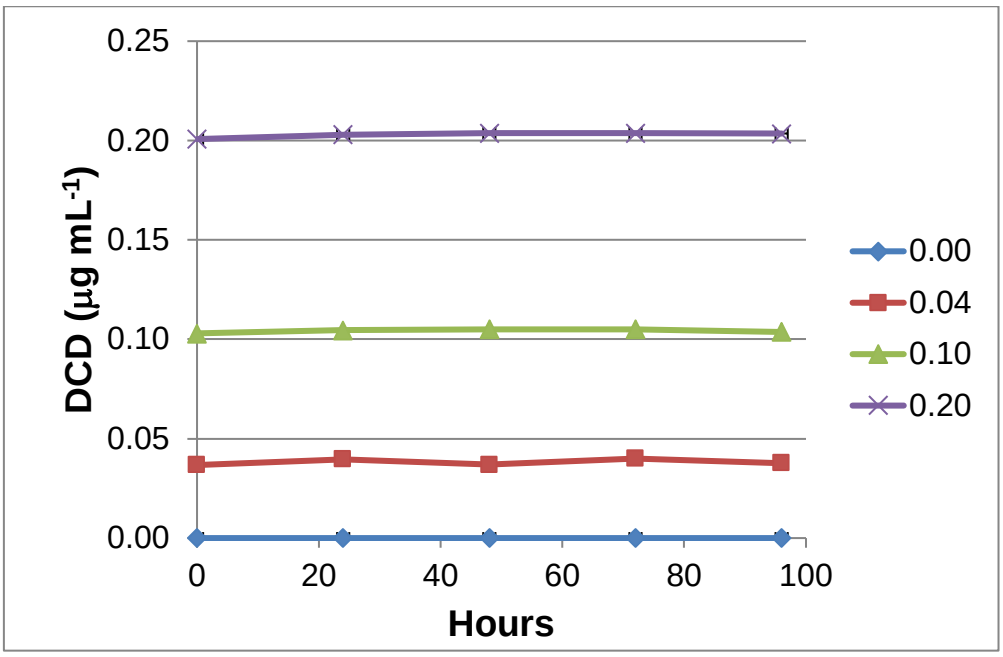


Figure 9: Mean DCD concentrations of in vitro stream water incubated over time when treated with DCD at either 0, 0.04, 0.1, or 0.2 µg DCD mL^{-1} versus time (n=4; error bar = one s.e.m).

Discussion - experiment 2

Under the conditions of this *in vitro* experiment NH_4^+-N concentrations were observed to decrease. The mechanism responsible for this dynamic in NH_4^+-N is presumed to be the result of nitrification. Supporting this assumption are the corresponding increases in the NO_3^--N concentrations. This increase in NO_3^--N concentration occurred clearly in experiment 2, as opposed to experiment 1, presumably as a result of the more aerobic conditions that reduced or prevented denitrification of NO_3^--N. However, NH_4^+-N concentrations decreased by almost 2 µg mL^{-1} while NO_3^--N concentrations increased by < 1 µg mL^{-1} indicating that other processes such as immobilisation, or other loss processes such as N_2O degasing may have contributed to the discrepancy between the decline in NH_4^+-N and the increasing NO_3^--N.

While it initially appears that DCD treatment had a statistically significant effect on the rate of decrease in the NH_4^+-N concentration, with more DCD enhancing NH_4^+-N disappearance, the reason for this is not obvious since DCD concentrations did not change. If DCD concentrations had also declined then heterotrophic nitrification may have been suspected to be occurring. Other uncontrolled variables in this experiment include microbial population numbers. It may be that in the higher DCD concentration treatment there were a greater number of nitrifying organisms promoting the more rapid decline in NH_4^+-N.

Experiment 3 Effect of UV light on DCD degradation in water

Rationale

Approximately 10% of the energy emitted from the sun is emitted as ultraviolet (UV) light (400-100 nm). This may be further classified as UVA (400 – 315 nm nm), UVB (315 – 280 nm), and UVC (280-100 nm). Ozone in the stratosphere prevents most of the UV reaching Earth's surface and the UV light reaching Earth's surface is predominantly UVA.

Photodegradation has been shown to lead to the loss of soil organic matter (Rutledge et al., 2010) and the degradation of dissolved organic carbon (DOC) in waterways (Moody et al., 2013). It is not known if photodegradation of DCD occurs in waterways. Thus, a factorial experiment was set up to determine if UV light caused the degradation of DCD in water over time.

Experimental design

Treatments consisted of two levels of DCD (1 or 0 μg mL^{-1}), two water treatments (deionised water (DI) or stream water taken from the LII River near Lincoln), and two light treatments (samples kept in the dark or exposed to UV light). The UV light was provided using a Blak-Ray® ultra violet lamp (100 Watt, Ultra Violet Products Inc. San Gabriel, Ca. USA) which provided long wave UV (365 nm) in the UVA range. This was placed 60 cm above the flasks containing the treated waters. The flux was measured using a MACAM UVA-sensor made by Macam Photometrics Ltd, Livingston, Scotland. All treatments were replicated four times.

Erlenmeyer flasks were filled with 150 mL of the treatment water and 'plus DCD' treatments were set up with a DCD concentration of 1 μg mL^{-1}. Stream water had a total organic carbon concentration of 2.25 ±0.27 μg mL^{-1} (± stdev, n=4). Once set up all treatments were immediately analysed by taking 30 mL sub-samples from the flasks (time zero (t_0). The flasks were sub-sampled again after three days (t_3) and six (t_6) days. It was considered that 6 days was more than adequate to cover the range of water residence times that might be expected to occur in New Zealand streams and rivers.

Analyses

Samples were analysed for DCD concentration using a Rezex RHM-Monosaccharid column (50 x 7.8 mm, Phenomenex) and a 0.0025M H_2SO_4 eluent in conjunction with a Prominence HPLC system (DGU-20A3 Degasser; LC-20AB Liquid chromatograph; SIL-20A HT Autosampler; SPD-20A UV/Vis Detector; CTO-20A Column Oven). The eluent flow rate was 1 mL min^{-1} at a temperature of 45ºC. The UV detection wavelength was 210 nm and the inject volume was 50 μL. Dicyandianide standards were made in a matrix of deionised water and were 0, 0.005, 0.008, 0.012, 0.050, 0.10, 0.20, 0.50, 1.00, 2.00, 3.00, 5.00 and 10.00 μg mL^{-1}.

Statistical analyses were performed using Minitab®16. Analysis of variance was used to determine if treatments differed and if differences occurred a Tukey's test was used to determine treatment effects. Effects of time were tested for using a repeated measures test using the General Linear Model in Minitab®16.

Results

As expected no DCD was found in the 0 μg mL^{-1} DCD treatment and this treatment is not discussed further. The mean DCD concentration in the plus DCD treatments, when averaged over 'light' and 'dark' treatments did not change ($P < 0.473$) over time or with water type ranging from 0.958 to 0.968 μg mL^{-1} DCD (Figure 10).

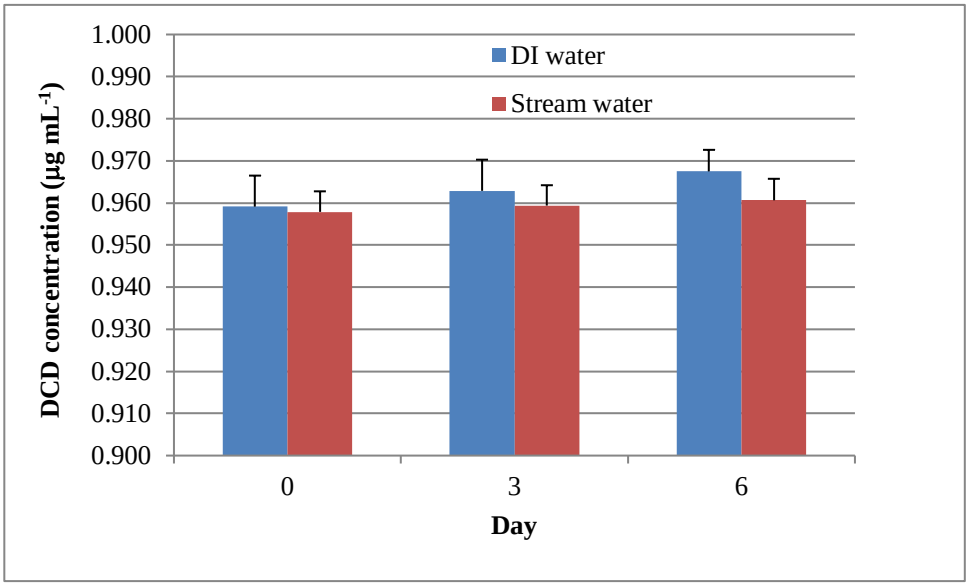


Figure 10: Mean DCD concentrations in either deionised water (DI) or stream water versus time (n=4; error bar = one s.e.m)

There was no effect of either keeping water samples in the dark or under UV light, when comparing the DCD concentrations over time, in either the DI or stream waters. At t_o, t_3 and t_6 the respective P values with respect to the light treatment were 0.396, 0.410, and 0.472 (Figure 11). In the DI water DCD concentrations ranged from 0.958 to 0.968 µg mL^{-1} DCD while in the stream water the range was 0.951 to 0.967 µg mL^{-1} DCD (Figure 11).

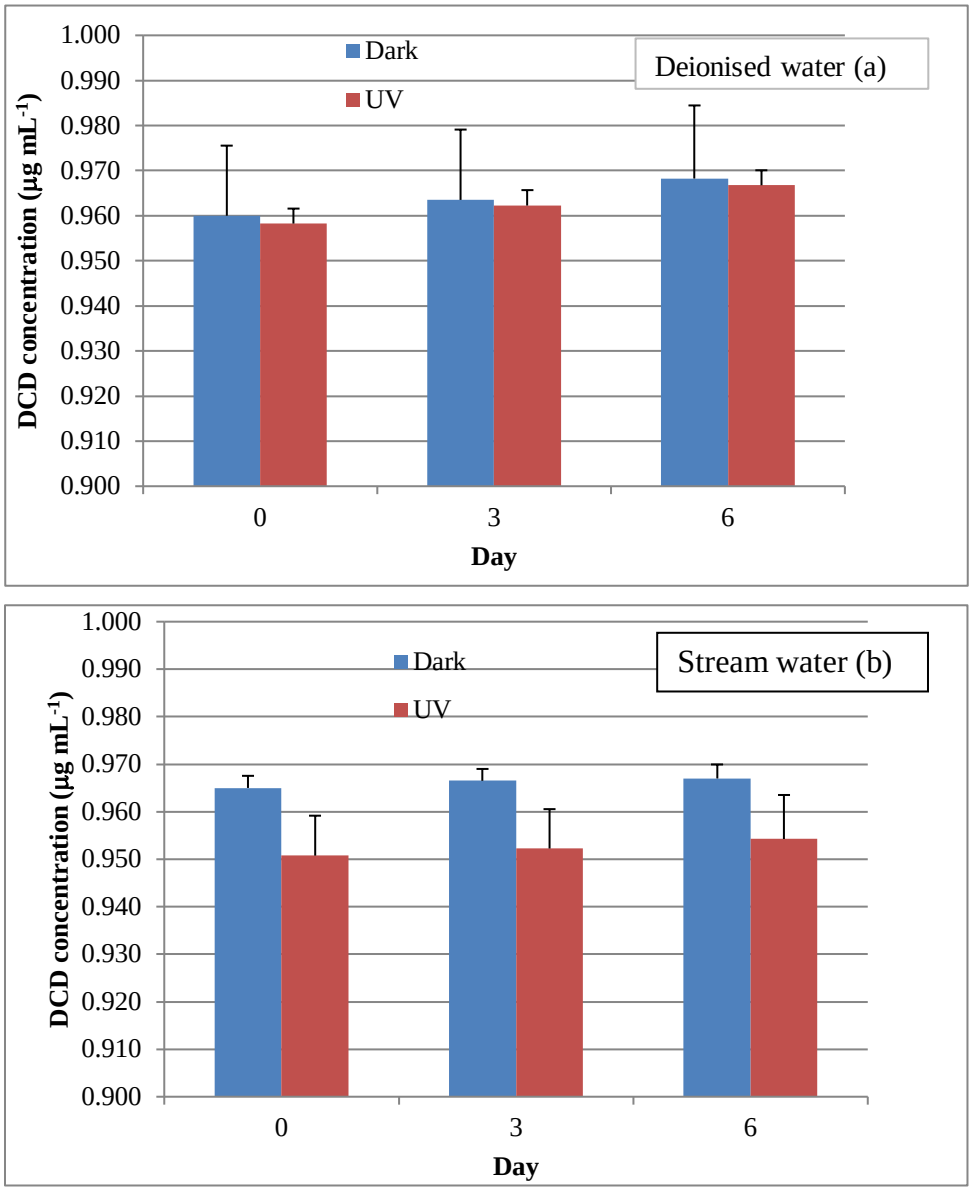


Figure 11: Mean DCD concentrations in deionised water (a) and stream water (b) versus time as influenced by either UV light or storage in the dark (n=4; error bar = one s.e.m)

Discussion – experiment 3

Clearly, the UV light treatment had no effect on the break-down of DCD in water. A Screening Information Dataset (SIDS) prepared under the auspices of the Organisation for Economic Co-operation and Development (OECD) states dicyandiamide may undergo indirect photo-oxidation by hydroxy radicals in the atmosphere, with it predicted to occur with a half-life of 3.1 hours (OECD SIDS 2003). This same report states that "In water DCD is abiotically stable, water-soluble and is not hydrolysed regardless of pH (UNEP, 2003)." This concurs with what was observed in this experiment, with the DCD being stable.

Conclusion

In conclusion the two incubation studies performed with additions of NH_4^+-N and DCD have shown that NH_4^+-N continues to decline in the presence of DCD at the concentrations used here. The concentrations chosen here were arbitrary and based on a reported drainage study. Further studies of DCD concentrations in drainage water are required to assist in future river study work. Scenarios can of course be considered. If we take, for example, the loss of DCD recorded by Sprosen et al. (2009) who found 37% of DCD applied remained in the top 45 cm of soil 48 days after application and if it is further assumed that the remaining 63% has leached, and that 10 kg ha^{-1} was initially applied. Then 6.3 kg is lost in drainage. Making further assumptions, that the soil bulk density is 1 g cm^{-3} and that the ground water is at 2 m depth below the soil surface, and no degradation of DCD occurs below 45 cm then 1240 mm of rainfall would be required to cause one pore volume of drainage to occur. Assuming 6.3 kg of DCD was equally distributed in 1240 mm of drainage over 1 ha (1.24×10^{10} cm^3) this equates to a DCD concentration of 0.5 μg mL^{-1}. Such a calculation is extremely coarse but it serves to show the likely magnitude of any DCD concentration in soil drainage prior to further dilution from groundwater or river waters and shows the factors influencing such a calculation: soil type, rainfall (total, amount relative to timing of DCD application), depth to water table, and soil porosity. At this concentration, based on the studies performed here, DCD would not prevent nitrification and it would not lead to the build-up of ammonium-N and ammonia toxicity.

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