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The Effects of DiCyanDiamide (DCD) Leaching and Runoff on Aquatic Environments

SLMACC project 30616

Final Project Report

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Prepared for MPI by

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Executive Summary

This research project investigated the potential non-targeted (unintended) effects of the nitrification inhibitor DiCyanDiamide (DCD) on farmland streams and wetlands. The project was a collaboration between scientists from the University of Otago (effects on streams) and NIWA (effects on wetlands).

Stage 1 of the stream research included surveys to identify current levels of DCD in New Zealand's farmland streams. Surveys were planned in different regions but only one of them, in Southland, could be carried out before DCD was withdrawn from the market in early 2013.

Stage 2 of the stream research aimed at experimentally testing the individual and combined effects of DCD and other important agricultural stressors (i.e. eutrophication, fine sediment inputs, and flow reduction) on stream ecosystems. We achieved this goal by running three strictly controlled, statistically powerful experiments with a high degree of realism in the *ExStream* System, an innovative field research facility developed at the University of Otago.

The wetlands part of the project consisted of a field experiment testing the effects of DCD application to a headwater seepage wetland on a North Island dairy farm.

In the Southland Survey, the water at none of the 43 relatively large stream/river sites (widths 3-50 m or more) contained detectable concentrations of DCD (the detection limit of the HPLC method used was 10 µg/L). However, because much higher DCD concentrations (1-5 mg/L) were found in small (width 0.2-0.5 m) dairy farming streams in Waikato, the non-detectable concentrations found at the Southland sites may be the result of downstream dilution and/or rapid degradation of DCD. Consequently, our subsequent experiments focused on DCD effects on small streams (simulated in the *ExStream* System) using realistic concentrations and also assessing indirect effects of DCD, for instance on the nutritional resources of aquatic organisms.

The combined findings of our three stream channel experiments indicate that DCD appears to be a relatively benign stressor (in terms of effect frequency and size of effect) when compared to the known agricultural stressors of deposited fine sediment addition, stream flow reduction and nutrient enrichment. Moreover, interactions of DCD with other stressors were uncommon and always weak, implying that DCD addition rarely made other stressor effects worse.

In Stream Channel Experiment 1, we examined the individual and combined effects of DCD and two key agricultural stressors (nutrient enrichment and fine sediment inputs) on stream invertebrate and algal communities. We manipulated six DCD concentrations (applied continuously) that spanned the entire range measured in New Zealand streams plus two higher levels, to simulate a "worst-case scenario", i.e. surface runoff during large floods affecting catchments where DCD had been applied shortly before. DCD had some significant ($P < 0.05$) single-stressor effects on stream communities; these were always negative but overall rare (invertebrates: 2 of 15 cases; algae: 2 of 24 cases) and very weak (mean effect size 0.05; range 0.0-1.0) compared to those of sediment (12/15 and 19/24; mean effect size 0.34) or nutrients (2/15 and 16/24; effect size 0.18; data analysis based on all 8 DCD levels). Some interactions of DCD with the other stressors occurred, but none of these were strongly synergistic.

In Stream Channel Experiment 2, we specifically tested the effects of DCD dynamics on aquatic communities and ecosystem functions (i.e. algal growth and leaf litter processing), mimicking dynamics measured in real streams where DCD concentrations peaked after rainfall events. Again we tested potential interactions of DCD with other agricultural stressors, in this case nutrient enrichment, fine sediment inputs and streamflow velocity reduction (due to water abstraction). DCD addition had weak positive or negative effects on one common algal taxon each and increased abundances of three common invertebrate taxa with low MCI sensitivity

scores, leading to a higher total invertebrate abundance. DCD also interacted weakly with nutrient enrichment to slightly aggravate the negative effect of enrichment on the abundance of EPT taxa (Ephemeroptera, Plecoptera and Trichoptera), which mostly represent pollution-sensitive invertebrates. DCD also weakly increased deciduous leaf litter processing rates. In this experiment, individual effects of DCD were in the same range as those of nutrient enrichment overall (in terms of pervasiveness and effect size), but rare (algae: 2 of 16 cases; invertebrates: 5/14) and weak (mean effect size 0.10) compared to the effects of fine sediment (11/16 and 12/14; mean effect size 0.36) and flow reduction (8/16 and 11/14; mean effect size 0.29).

In Stream Channel Experiment 3, we examined both individual and combined effects of DCD pulses along with effects of flow velocity reduction and nutrient enrichment with different N:P ratios. DCD addition had some significant (positive and negative) single-stressor effects on algae and invertebrates but, once again, these effects were rare (algae: 1 of 18 cases; invertebrates: 1/17) and weak (mean overall effect size 0.09). By contrast, nutrient enrichment and flow reduction had much more pervasive and stronger effects on algae (12 of 18 and 14 of 18 cases, respectively; effect sizes 0.37 and 0.26), whereas flow reduction was the key stressor for invertebrates (15/17; effect size 0.35). Survival rates of juvenile trout and changes in their condition from the start to the end of the experiment were not affected by DCD or nutrient enrichment, but responded negatively to flow reduction. There were also some 2-way or 3-way interactions of DCD effects with flow and nutrient effects, but all these were weak as well.

As a secondary aim of Stream Channel Experiments 2 and 3, we investigated the potential effects of DCD inputs on emissions of the greenhouse gas N_2O from streams. Our findings imply that DCD addition is unlikely to change N_2O emissions from small farmland streams. All observed N_2O concentrations were close to the atmospheric equilibrium, probably due to rapid water exchange in the channels (< 2 minutes), and the minor differences observed between DCD treatments may have been driven by diurnal changes in water temperature during sampling. Ammonium concentrations in the channels were also largely unaffected by DCD addition.

The wetlands part of the project focused on DCD effects on headwater wetland nitrogen dynamics (including nitrogen export downstream) and was conducted in 12 mesocosms installed in a seepage wetland (size ca. 100×20 m) at the head of a pastoral catchment (upstream catchment area about 5 hectares) near Hamilton. DCD concentrations in the mesocosms declined by about 50% within seven days, and DCD concentrations greater than 100-600 mg/m^3 caused a decrease in nitrate concentrations in the mesocosms. In contrast, ammonium concentrations did not seem to be affected by DCD. These results suggest that under stable weather conditions and in wetlands of this type with active growth of dense grasses, DCD is more likely to reduce than to increase export of inorganic nitrogen to streams, probably because any ammonium accumulating due to blockage of nitrification is rapidly taken up by grasses or other wetland plants. In addition, DCD probably reduces nitrous oxide emissions, though this effect may be small compared to other factors that create emission "hotspots."

Our report closes with a number of specific recommendations for future research needs on DCD effects on freshwater ecosystems in New Zealand. These recommendations include field-based research investigating DCD concentrations and potential effects on aquatic biota in real streams and wetlands draining farmland where DCD is used (to be performed if DCD comes back on the market at some point in the future), plus laboratory experiments aimed at elucidating the mechanisms behind DCD effects on stream invertebrates and algae.

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Background and Project Rationale

Prior to its withdrawal from the market in early 2013, application of the nitrification inhibitor dicyandiamide (DCD) to farmland had been increasing in New Zealand (Monaghan et al. 2008, Wilcock et al. 2008), as one of the strategies to conserve soil nitrogen (N) and enhance the efficiency of N supply to farm plants (MAF 2009). N can be lost from farmland soil in drainage as nitrate (NO_3^-) or as the greenhouse gas nitrous oxide (N_2O). To reduce these losses, DCD is applied to soils to slow conversion of N from the fairly immobile ammonium (NH_4^+) to the more mobile NO_3^- by inhibiting activity of NH_4^+ oxidising soil bacteria (Moir et al. 2007). DCD (which itself contains N; formula $C_2H_4N_4$) is readily water soluble (23 g/L at 13°C; Wilcock et al. 2008) and concentrations of 1-5 mg/L have been measured in two dairy farming streams in the North Island of New Zealand (R. Storey, NIWA, unpublished data); note that these concentrations are similar to NO_3^- levels in highly nutrient-enriched farmland streams (Matthaei et al. 2006, Wagenhoff et al. 2011, Wagenhoff et al. 2012).

The effects of DCD on the ecology of farmland streams in New Zealand were unknown when this research project started in August 2012. This was despite the fact that the most widely used DCD product in New Zealand has been classified as "harmful to aquatic life with long-lasting effects" in its safety data sheet (*Eco-N* safety data sheet 2010), and that another DCD product has received a similar classification in Germany (*Dicyandiamid T* safety datasheet 2008). In several European countries, DCD has been phased out and replaced by other nitrification inhibitor products, possibly as a result of this classification (Trenkel 2010, Ottow 2011). Note that, by contrast, an earlier OEDC report (OECD SIDS 2003) concluded, based on the findings of toxicological tests conducted by the Environmental Agency of Japan in 1998, that DCD had low toxicity for aquatic algae, invertebrates and fish. Given this conflicting information based on single-species tests in artificial laboratory settings and the complete lack of knowledge regarding potential off-farm effects of DCD on the ecology of real farmland streams and wetlands in New Zealand, there was a pressing need for research on this topic.

Based on this previous knowledge, DCD can be classified as a potential stressor for aquatic ecosystems. A stressor is a variable that, as a result of human activity, exceeds its range of normal variation and affects plant and/or animal communities (Townsend et al. 2008). While it is well known that intensive agriculture can impair stream health, the majority of research detecting such effects has focused on single stressors. In reality, however, most farmland streams are exposed to multiple stressors acting simultaneously, and the combined effects of these stressors are poorly understood. Therefore, if resource managers only consider effects of single stressors, their assessment of risk may be higher or lower than reality. The study of multiple stressors is a rapidly growing research focus worldwide, and the Stream Ecology Group at the University of Otago has conducted several pioneering studies in this area (e.g. Townsend et al. 2008, Matthaei et al. 2010, Wagenhoff et al. 2012, Piggott et al. 2012, 2014). Our research project builds on this expertise by combining the new potential stressor DCD with three other known agricultural stressors for stream ecosystems (elevated levels of dissolved nutrients, deposited fine sediment and stream flow reduction due to water abstraction).

Our project was a collaboration between University of Otago (DCD effects on streams/rivers) and NIWA scientists (DCD effects on wetlands). In Stage 1 of the stream research, we used a survey approach to identify current levels of DCD in New Zealand's farmland streams. Three stream surveys were planned but only the first, in Southland, could be carried out before DCD was withdrawn from the market. In Stage 2, we examined the individual and combined effects of DCD and other key agricultural stressors on stream ecosystems, by running three strictly controlled, statistically powerful yet highly realistic experiments in the *ExStream System*, an innovative field research facility developed at the University of Otago (Wagenhoff et al. 2012, 2013, Magbanua et al. 2013a, 2013b, Piggott et al. 2014). In the wetlands part of the project, we applied DCD to the catchment of a headwater seepage wetland on a North Island dairy farm.

Research Aim 1.1: Stream Surveys

Original research goals (*from Research Proposal*):

1. Determine if on-farm DCD use at industry-recommended levels results in elevated concentrations of dissolved DCD in streams adjacent to, or downstream of, agricultural fields or pastures treated with DCD.

2. Examine whether observed levels of DCD are significantly related to the distribution patterns of stream animals and plants.

Three stream surveys in different regions of New Zealand where DCD was regularly applied to dairy farm pastures (Southland, Canterbury and Waikato) were planned. However only the first of these, in Southland, could be carried out before DCD was withdrawn from the market in early 2013. Consequently, this research aim (and also aims 1.4 and 1.5, see below) had to be revised compared to the research contract signed before the DCD suspension.

Methods

- We surveyed 43 Southland streams (a subset of Environment Southland's annual stream health monitoring sites; stream orders mainly 4-6, stream widths ranging from 3 m to 50+ m) between mid-September and early October 2012, i.e. shortly after the second DCD application in late winter in Southland.

- These sites were selected because (i) they span wide gradients of catchment land-use intensity and known agricultural stressors such as fine sediment and nutrients, (ii) they are well-studied by our group (Wagenhoff et al. 2011, Liess et al. 2012, Blakemore 2012), and (iii) all are established sites with easy access that could be sampled within a period of just two weeks during which stable flow conditions prevailed at all sites.

- At the time of site selection (August 2012), we did not know yet in which of the site catchments DCD had been applied on farms. Therefore, we used a 'black box' approach by sampling across a broad gradient of farming intensity in the site catchments.

- In early 2013 a collaboration with Ravensdown (the sole supplier of DCD in Southland) was established, and we were able to determine which of our sites were close to dairy farms on which DCD had been applied in April/May and/or July/August 2012 (Fig. 1), the two application periods on Southland farms in the autumn and winter preceding our stream survey.

- We determined concentrations of DCD and nutrients (N and P; 4 replicate water samples per site), standing stocks of deposited fine sediment (Quorer method; Clapcott et al. 2011) and stream algal communities (quantitative samples on rocks) and invertebrate communities (semi-quantitative kick sampling). The latter two are standard methods used by New Zealand Regional Councils for sampling stream algae and invertebrates.

- DCD concentrations in water samples were determined with the HPLC analysis method (see also Research Aim 1.2).

Results

- The water at none of the surveyed 43 stream or river sites contained detectable DCD concentrations (i.e. above the detection limit of the HPLC method of 10 μg/L).

- Because of the lack of detection of DCD in the stream water at all study sites, the second research objective (DCD effects on algae and invertebrates in the surveyed Southland streams/rivers) was abandoned.
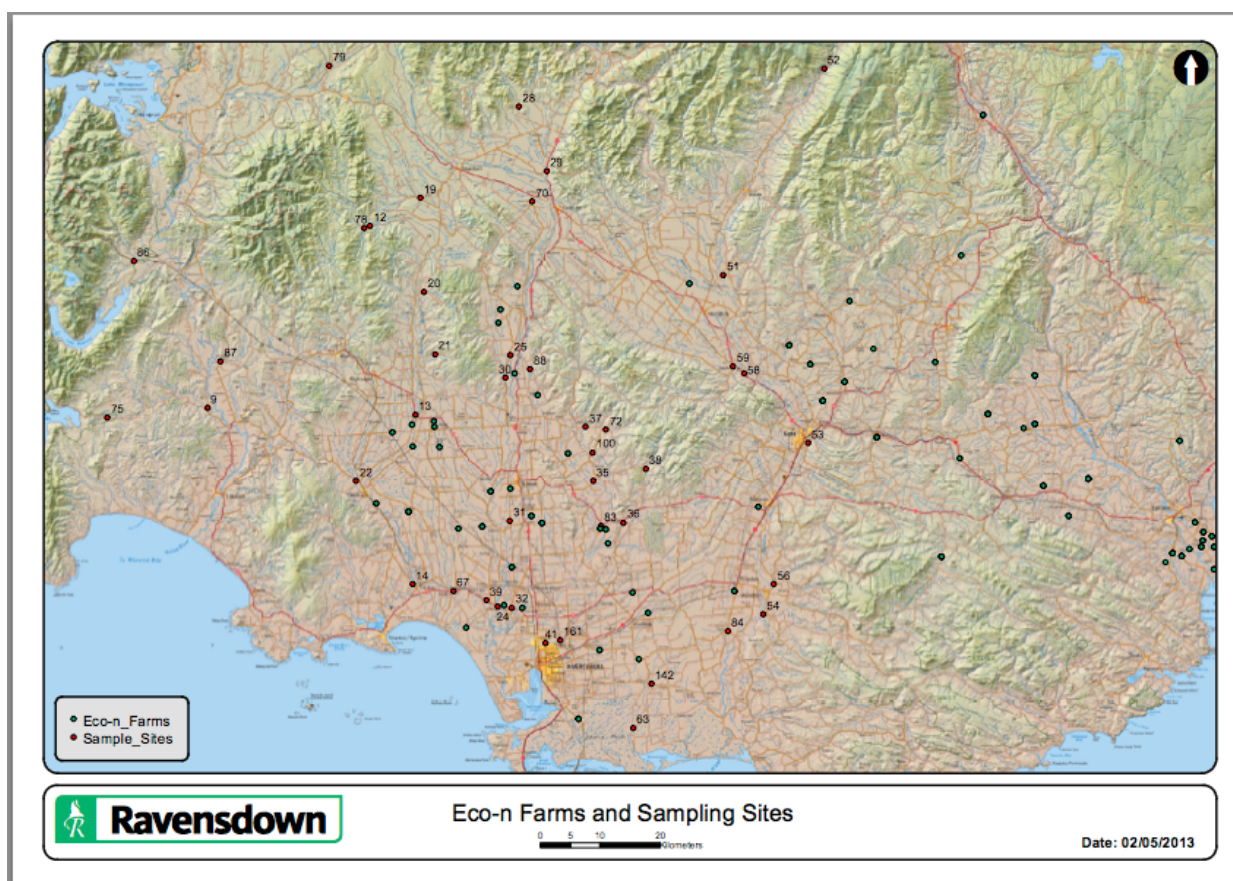
Figure 1. Southland Stream Survey – Locations of the 43 sampled stream/river sites (red dots, numbering according to Environment Southland's annual biomonitoring programme) and the dairy farms on which DCD (*Eco-N*) was applied by Ravensdown farm consultants (green dots) during the autumn/winter of 2012 that preceded sampling in September 2012. None of the sites contained DCD concentrations above the detection limit of the HPLC method of 10 μg/L.

Discussion

1) The toxicological laboratory tests summarized in the abovementioned OECD report (OECD SIDS 2003) found 'no observable adverse effects' of DCD on

    (i)   waterflea (*Daphnia magna*) reproduction rates at concentrations below 25 mg/L during a 21-day chronic toxicity test,

    (ii)   Japanese rice fish (*Oryzias latipes*) survival rates at concentrations below 100 mg/L during a 14-day chronic toxicity test, and

    (iii)   biomass of the green alga *Selenastrum capricornutum* at concentrations below 171 mg/L during a 72-hour acute toxicity test.

2) Given these eco-toxicological findings, the uniformly far lower concentrations (below 10 micrograms/L) observed in the surveyed Southland streams are expected to have negligible effects on the invertebrates, algae or fish in these streams. Therefore, we conclude that DCD pollution was unlikely to affect the stream communities at the surveyed sites in relatively large stream or river sections (with stream widths ranging from 3 m to more than 50 m).

3) These results contrast significantly with the much higher DCD concentrations (up to 5 mg/L) observed in two first-order streams (width typically 0.2 – 0.5 m) in the study of Richard Storey (NIWA, unpublished data) conducted on a Waikato dairy farm (Fig. 2).
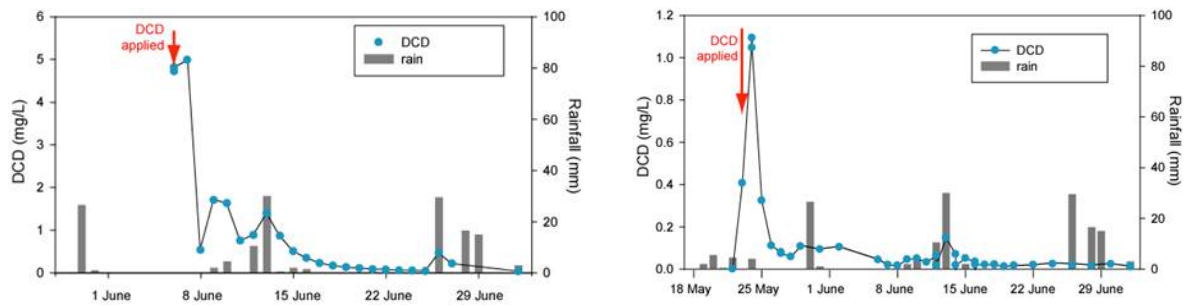
Figure 2. DCD concentrations (determined with HPLC) in two 1st order streams draining a dairy farm near Otorohanga, Waikato (R. Storey, NIWA, unpublished data). Note that DCD peaks were lower in the stream on the right (which was mostly spring-fed). In both streams DCD peaks lasted only for a few days shortly after DCD application to the catchment, followed by a rapid decline. However further, smaller DCD peaks occurred after rainfall events in both streams during the 4-6 weeks after DCD application.

4) Because DCD concentrations were so much higher in the two small dairy farming streams in Waikato, we suspect that the lack of detection of DCD at the fairly large stream/river sites in Southland was the result of downstream dilution and/or rapid degradation of DCD.

5) In spite of the absence of detectable DCD concentrations at the surveyed 43 Southland stream and river sites, the fact remains that DCD concentrations can be far higher in small farmland streams (1st and 2nd order streams; width typically 0.2 – 1.0 m). In terms of stream km, these small streams contribute at least 77% (or 326,793 km) of the total stream/river length in New Zealand (based on NIWA's River Environment Classification, REC; Snelder & Biggs 2002, Snelder et al. 2010). Note that this percentage is conservative because only 1st order streams above a certain catchment area (20 hectares) and with a length of at least 500 m are included in the REC. Recent work by NIWA (Storey & Wadhwa 2009) indicates that streams in the Auckland region may form in catchments as small as 1 hectare.

6) Moreover, because recolonisation in streams after natural or human-induced disturbances occurs primarily via drifting organisms from upstream (see reviews by Resh et al. 1988, Townsend 1989 and Mackay 1992), small headwater streams serve as the key source of recolonizing stream organisms (algae, bacteria, fungi, invertebrates and fish larvae) for downstream reaches after disturbance. This source of colonists is lost or impaired if small headwater streams are degraded. Consequently, the small headwater streams draining New Zealand farms are very important ecosystems, both in terms of their quantitative contribution to the total stream network and their quality as key habitats. Clearly they need to be investigated thoroughly when determining whether or not DCD is a potential new stressor for running water ecosystems in New Zealand.

7) Future research needs based on the key findings for the stream survey part of our project are identified on pages 28-29 of this report.

Research Aim 1.2: Stream Channel Experiment 1

Rationale

In New Zealand DCD is applied mainly on dairy farms. Streams draining dairy farms are already subject to several known stressors acting simultaneously, including increased concentrations of dissolved nutrients and increased quantities of deposited fine sediment (e.g. Matthaei et al. 2006, Townsend et al. 2008, Wagenhoff et al. 2011, 2012) and/or reduced stream flow due to water abstraction for farm irrigation (e.g. Matthaei et al. 2010, Lange et al. 2014). Consequently, our three stream channel experiments studied the off-farm effects of DCD on stream communities in a realistic multiple-stressors context simulating small streams draining New Zealand dairy farms with DCD application. In the context of this report, our research focused on the stream invertebrate and algal communities, because these groups of organisms are widely used in stream health biomonitoring around the world and also in New Zealand (Biggs & Kilroy 2000, Stark et al. 2001).

Against this background, our first stream channel experiment studied the effects of DCD on stream communities and its potential interactions with fine sediment and nutrient enrichment, two key agricultural stressors. Eight DCD treatment levels were selected. Six of these spanned the entire known range of DCD concentrations found in New Zealand streams based on the NIWA study in two North Island dairy farming streams (see Fig. 2). Further, as requested by MPI we added two even higher concentrations, to simulate „extreme" events such as uncontrolled surface runoff during large floods affecting farm catchments where DCD had been applied shortly before. In keeping with this "worst-case scenario" approach, DCD was added continuously during the entire manipulative period of the experiment (even though peak DCD concentrations in real streams are likely to last only for a few days – see Fig. 2).

Goal

- Examine the individual and combined effects of DCD and two key agricultural stressors (nutrients and fine sediment) on stream invertebrate and algal communities, using a study design that spans the entire known range of DCD concentrations in NZ streams (applied continuously) plus two even higher levels, to simulate a "worst-case scenario".

Methods

- All three stream channel experiments were conducted in the *ExStream System*, an innovative field research facility developed by C. Matthaei and J. Piggott at the University of Otago. The system comprises 128 circular stream channels and offers a rare combination of strict control of experimental variables, excellent statistical power and a high degree of realism, such as permitting natural immigration and emigration of stream organisms (invertebrates, algae and microbes) and achieving the same water temperature, light conditions and water chemistry as the adjoining river. This river, the Kauru River in North Otago, drains a low-intensity land use catchment (sheep/beef farming and native tussock grasslands) and contains a diverse aquatic fauna and flora. The channels are arranged in eight blocks of 16 units each, and each of these blocks is continuously supplied by stream water gravity-fed from one header tank via 16 individual supply pipes. The system has been used successfully in several major multiple-stressor experiments (e.g. Liess et al. 2009, Lange et al. 2011, Magbanua et al. 2013a, 2013b, Wagenhoff et al. 2012, 2013, Piggott et al. 2014).

- In Experiment 1, we applied the following stressor treatments (32 treatment combinations in total, with 4 replicates per treatment combination in 128 channels):
  - DCD concentrations: These comprised a log-linear gradient of 8 levels. These included the highest observed concentrations in NZ streams plus two treatments above these levels, with continuous, constant DCD addition simulating a worst-case scenario. Dissolved DCD concentrations were determined with HPLC analysis (calibration curve $R^2 = 0.997$). Concentrations achieved (in mg/L; means across four sampling dates during the manipulative period) were zero (control), 0.58, 1.27, 2.45, 4.94, 8.18, 18.13 and 30.68. All were in very good agreement with our targets. The final two levels represent "extreme" DCD concentrations.
  - Dissolved nutrients: 2 levels (enriched *vs* ambient, with enriched levels being similar to those in small dairy farming streams in Otago/Southland). Concentrations achieved (means across the same four sampling dates as above, in µg/L) were 2816 *vs* 40 for nitrate and 221 *vs* 5 for phosphate.
  - Deposited fine sediment: 2 levels (raised *vs* ambient, with raised levels being similar to those in small dairy farming streams in Otago/Southland). Sediment levels achieved (means across two sampling dates; Days 10 and 20) were 75 % fine sediment cover *vs* 0 % and 5 mm depth *vs* 0 mm.

- The experiment was conducted in spring, after the winter DCD application period. A 23-day colonization period was followed by a 22-day manipulative period from late October to mid-December 2012. Dissolved DCD and nutrient concentrations plus surface cover and depth of deposited fine sediment were determined repeatedly during the manipulative period (see above for sampling dates). Invertebrate and algal communities were sampled once at the end of the manipulative period over two consecutive days (Day 21 and Day 22). On each of these dates, two randomly chosen replicate channels of the 32 treatment combinations were sampled (64 channels per day).

- Individual and combined stressor effects on invertebrate and algal communities were determined. The 15 studied invertebrate response variables (see Table S1 in the Appendix for a complete list) included 8 community-level metrics (e.g. total abundance, total taxon richness, invertebrate diversity and evenness, EPT richness = number of pollution-sensitive mayfly, stonefly and caddis fly taxa per channel, New Zealand Macroinvertebrate Community Index) and the abundances of the 7 most common individual invertebrate taxa (each contributing at least 0.5% to the total invertebrates counted and comprising 98.6% of all individuals counted when combined).

- Similarly, the 24 studied algal response variables (see Table S3 in the Appendix for a complete list) included 8 community-level metrics (e.g. algal biomass as chlorophyll a, algal taxon richness, algal diversity and evenness) and the abundances of the 16 most common individual algal taxa (each contributing at least 1.0% to the total algal cells counted and comprising 88.4% of all cells counted when combined).

- All these biological response variables were statistically analysed using a General Linear Model, with 'sediment' and 'nutrients' as the two categorical predictor variables, 'DCD concentration" (log-transformed) as a continuous linear predictor variable, plus all interactions among the predictor variables (see Piggott et al. 2014 for a very similar analysis). "Sampling day" (Day 21 versus Day 22, see above) was included as a block factor (without interaction terms with the predictor variables of primary interest). DCD, nutrient and sediment data were analysed with the repeated-measures equivalent (with 'sampling date' as within-subjects factor) of the same General Linear Model (focusing on the overall effects). Any existing block factor effects (there were few significant ones) represent merely background variation unrelated to our research objectives and are therefore not presented in this report.

Results

- Table 1 provides an overview of all significant findings for stream invertebrates, and Table 2 does the same for stream algae. The appendix of our report contains expanded versions of these tables (Tables S1-S4) with the statistical details of these results.

- The data were analysed in two ways: 1) The six lower DCD levels (based on the range of DCD concentrations observed in NZ streams) (96 channels), and 2) all eight DCD levels including the two highest ones simulating "extreme runoff" events (all 128 channels).

- DCD had few significant single-stressor or interactive effects on stream invertebrates (Table 1). DCD single-stressor effects were negative (see Fig. 3 for an example) but their effect size was small. Sediment was the most influential stressor (in terms of effect frequency, direction and size), and DCD the least influential one. Nutrient effect frequency and size were intermediate, with a positive effect direction. Including the two "extreme" DCD levels (which were well above the highest DCD concentrations observed in NZ farmland streams) did not change the key conclusions.

Table 1. Stream Channel Experiment 1 – Overview of significant effects on stream invertebrates (including effect frequencies, directions and sizes). Standardized effect sizes (partial eta-squared values; Garson 2012) range from 0.0 to 1.0 and can be categorized (after Nakagawa & Cuthill 2007) as follows: < 0.10 very weak, > 0.10 weak, > 0.30 medium, > 0.50 strong.

Dependent variables	DCD	Nutrients (N)	Sediment (S)	N × DCD	S × DCD	N × S	N × S × DCD
Invertebrates *(15 response variables)* **Six lower DCD levels**	2 = **13%** of all response variables	2 **13%**	12 **80%**	3 **20%**	0 **0%**	3 **19%**	0 **0%**
Direction	2-	2+	10-, 2+				
Effect size	0.10	0.21	0.36	0.07		0.07	
Invertebrates *(15 response variables)* **All 8 DCD levels**	2 **13%**	2 **13%**	12 **80%**	1 **7%**	0 **0%**	3 **19%**	0 **0%**
Direction	2-	1+, 1-	10-, 2+				
Effect size	0.04	0.15	0.37	0.03		0.06	

- Similarly, DCD had some significant effects on stream algae, but these were much less pervasive and smaller than those of the other stressors (Table 2). Single-stressor DCD effects were negative but their size was very small. The most influential stressors were fine sediment, nutrients and their interactions, whereas DCD was the least influential stressor. Including the two "extreme" DCD levels reduced the frequency of DCD main effects slightly while increasing the number of interactive effects by a similar percentage.

Table 2. Stream Channel Experiment 1 – Overview of significant effects on stream algae. See Table 1 for details.

Dependent variables	DCD	Nutrients (N)	Sediment (S)	N × DCD	S × DCD	N × S	N × S × DCD
Algae *(24 response variables)*	4	15	20	0	2	14	0
	16%	**63%**	**83%**	**0%**	**8%**	**58%**	**0%**
Six lower DCD levels							
Direction	4-	15+	19-, 1+				
Effect size	0.07	0.23	0.30		0.07	0.17	
Algae *(24 response variables)*	2	16	19	2	1	15	1
	8%	**75%**	**79%**	**8%**	**4%**	**63%**	**4%**
All 8 DCD levels							
Direction	2 -	15+, 1-	18-, 1+				
Effect size	0.05	0.20	0.30	0.04	0.04	0.15	0.04
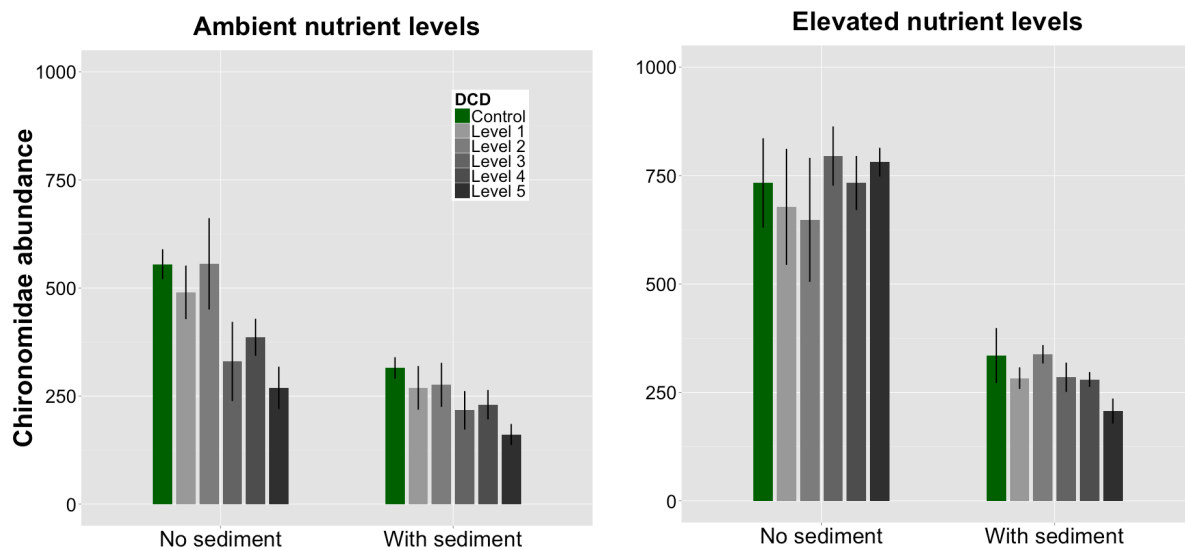
Figure 3. Stream Channel Experiment 1 - Effects of the six lower DCD concentrations, sediment and nutrients on the common invertebrate taxon Chironomidae (midges; 26% of the total invertebrate community in the stream channels). DCD (effect size 0.14) and sediment addition (effect size 0.58) both had negative effects, but the DCD effect was much smaller. Nutrient enrichment had a positive effect with an intermediate size (0.33). The effects of DCD and nutrients also interacted very weakly (effect size 0.08), with DCD effects being slightly more negative at ambient than at enriched nutrients. Adding the two "extreme" DCD levels did not change these results fundamentally.

Discussion

In the context of this report, which focuses on potential DCD effects on freshwater ecosystems, the combined findings of Experiment 1 for stream invertebrates and algae can be summarized and interpreted as follows:

1) DCD had some significant effects on stream communities in this "worst-case scenario" multiple-stressor experiment where DCD was added to stream channels continuously for 21 days at six concentrations spanning the entire range of known concentrations in New Zealand dairy farming streams, in combination with nutrient and fine sediment addition.

2) For both single-stressor effects and interactive effects of DCD, effect frequencies were rare compared to those of sediment or nutrients, and DCD effect sizes were always small or very small. By contrast, nutrients and especially sediment effects were much stronger.

3) The direction of single-stressor DCD effects was generally negative when determined as a linear relationship across the entire DCD gradient. No obvious non-linear patterns indicating potential subsidy effects of DCD at lower concentrations occurred.

4) Some complex interactions with other stressors occurred, but none of these were strongly synergistic; i.e. DCD did not make the effects of other stressors noticeably worse, or vice versa. By contrast, synergistic interactions between fine sediment and other stressors (nutrients, flow reduction, increased water temperature) were common in our previous related multiple-stressor research (e.g. Townsend et al. 2008, Matthaei et al. 2010).

5) Inclusion of two even higher DCD concentrations (18.1 and 30.7 mg/L; simulating "extreme" runoff events) did not change the overall impact of DCD, indicating that no obvious threshold of harm was crossed when including these two highest values.

6) Overall, DCD was a relatively benign stressor (compared to nutrient enrichment and especially to added deposited fine sediment) in this "worst-case scenario" experiment. It is worth mentioning in this context that the highest DCD concentration added (30.7 mg/L) was slightly above the threshold of 25 mg/L below which a toxicological laboratory experiment found 'no observable adverse effects' of DCD on waterflea (*Daphnia magna*) reproduction rates (OECD SIDS 2003) during a chronic toxicity test that spanned a similar length (21 days) as our 22-day manipulative period.

7) Several open research questions remain (e.g. effects of DCD pulses, responses of ecosystem functions and fish); these will be addressed in Stream Channel Experiments 2 and 3.

Research Aim 1.3: Stream Channel Experiment 2

Rationale

Our second stream channel experiment specifically aimed at testing the effects of DCD application mimicking concentration pulses observed in small agricultural streams (Fig. 2) instead of constant concentrations as in Experiment 1. In a fully crossed four-stressor experiment, we tested the effects of DCD in combination with three other agricultural stressors, nutrient enrichment, deposited fine sediment and flow reduction, on benthic stream communities (algae and invertebrates) and the processing of terrestrial leaf litter. The latter is a functional indicator that allows simulating the situation in small streams with intact riparian vegetation and is used increasingly around the world as a bioindicator complementing structural indices (Gessner & Chauvet 2002, Young et al. 2008), and also to detect effects of eutrophication (Woodward et al. 2012).

A secondary objective of this experiment (and also Stream Channel Experiment 3) was to determine if DCD affects the amounts of the greenhouse gas N_2O emitted from stream ecosystems, by inhibiting formation of N_2O to varying degrees depending on DCD concentrations, nitrate concentrations, and presence/absence of redox gradients and suboxic zones (in collaboration with B. Wilcock, NIWA). The findings of this objective, which were inconsistent and not very informative, are summarized in the description of Experiment 3.

Goals

- Test if realistic pulses of DCD application affect algal and invertebrate communities differently than constant concentrations.

- Test the effects of flow velocity reduction in combination with DCD, nutrient enrichment and fine sediment.

- Test if functional indicators provide useful findings and whether they complement structural indicators to detect DCD effects on aquatic ecosystems.

Methods

- The second experiment ran from 3rd April to 27th May 2013 using the experimental facility described in the previous section.

- We applied DCD in pulses (two different peak concentrations and frequencies, with a maximum of 4 and 2.68 mg/L, respectively; Fig. 4), and compared these treatments to constant application (1.63 mg/L) and to a control containing no DCD. All three DCD application treatments contained the same average concentration of DCD over the course of the experiment. We also manipulated flow velocity (0.12 *vs* 0.013 m/s), dissolved nutrient levels (enrichment to 2.8 mg/L nitrogen and 0.22 mg/L phosphorus *vs* ambient) and fine sediment levels (almost complete cover of the channel substratum *vs* control), resulting in 32 treatment combinations with 4 replicates each. Sediment and nutrient addition treatments were the same as in Experiment 1.

- The experiment consisted of a 4-week colonization period followed by a 4-week manipulative period during which we repeatedly monitored DCD and nutrient concentrations as well as the sediment and flow treatment levels.

- At the end of the experiment, we sampled the algal and invertebrate communities (16 and 13 response variables, respectively, including community metrics and abundances of the common taxa, each comprising at least 1% of the community). Algal biomass accrual and the mass loss of deciduous and evergreen leaf litter, i.e. the litter of exotic birch (*Betula pendula*) and native mahoe (*Melicytus ramiflorus*), were also sampled on the

same occasion. This final sampling was carried out over two consecutive days (with 2 replicates per treatment combination, or 64 channels, sampled per day).

- During the final sampling occasion, we also measured N₂O-concentrations in each stream channel, in collaboration with NIWA (described in Experiment 3).

- We statistically analysed the effects of the stressors and their interactions in an ANOVA with 'sediment', 'nutrients', 'flow' and 'DCD pulse' as categorical predictor variables. Since the final sampling occasion spanned two days, we included 'sampling day' as a blocking factor but refrain from reporting its effects here because they represent background variation in the data that is unrelated to our research objectives.
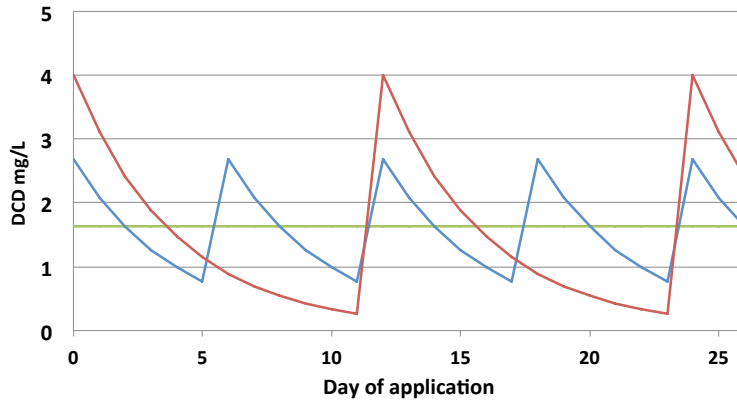
Figure 4: Stream Channel Experiment 2 - Concentrations of DCD used in the manipulative period. Note that the same DCD treatments were also applied in Experiment 3.

Results

Overall, the invertebrate community was dominated by *Potamopyrgus antipodarum* (35% of all individuals counted), oligochaetes (27%) and chironomids (10%), and EPT taxa (mayflies, stoneflies and caddis flies) contributed 9% when combined. DCD addition significantly affected 5 of the 14 invertebrate variables studied (6 community metrics and the abundances of the 8 most common taxa comprising at least 1% of the community each), the same effect frequency as for nutrient enrichment (Table 3). Compared to flow velocity reduction and fine sediment addition, however, DCD addition effects were less common and weaker based on a comparison of their average effect sizes. DCD application as such (the three addition treatments *vs* control) reduced invertebrate community evenness (range 0-100%) by 10%, mainly via increased abundances of *P. antipodarum* (+24%), oligochaetes (+41%; strongest increase at constant DCD addition) and chironomids (+29%; strongest increase at the low and frequent DCD pulses). The increases in these taxa also led to an increase of total invertebrate abundance (+21%; Fig. 5).

Elevated nutrients reduced the total abundance of EPT taxa (-15%) and *Deleatidium* spp. abundance in particular (-32%), whereas positive effects ensued on the abundances of copepods (+18%), chironomids (+18%) and total invertebrates (+9%). Flow velocity reduction decreased the abundance of EPT taxa (-10%), especially *Psilochorema* spp. (-27%), but also those of *P. antipodarum* (-46%) and chironomids (-33%). By contrast, it increased invertebrate taxon richness (+6%) and evenness (+17%), plus the abundances of *Deleatidium* (+28%), oligochaetes (+65%), and crustaceans. Sediment addition significantly reduced all community metrics tested (strongest effect on the abundance of EPT taxa: -55%) and the abundance of 5 of the 8 common taxa (reductions of 34-65%); however, it increased the abundance of *P. antipodarum* (+15%).

DCD effects interacted with the effects of nutrient enrichment in one case and with flow reduction in two cases, both with small effect sizes. High pulses of DCD weakly increased the negative effect of nutrients on EPT abundance. Further, DCD addition in general nullified the

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positive effect of flow velocity reduction on *Deleatidium* spp. and enhanced the negative effect of flow reduction on EPT abundance slightly.

Table 3. Stream Channel Experiment 2 - Overview of significant invertebrate responses (above) and algal responses (below), their direction and average effect size (ES) to the four manipulated stressors and their interactions. To improve clarity, only two-way interactions are shown because higher order interactions were rarely significant (4 cases in total) and had small effect sizes (ES ≤ 0.12). See Tables S5-S6 for detailed statistical results.

Treatments	DCD	Nutrients (N)	Flow (F)	Sediment (S)	DCD × N	DCD × F	N × F	DCD × S	N × S	F × S
Invertebrates (14 response variables)										
Significant responses	5	5	11	12	1	2	1	0	0	2
(% of all responses)	36%	36%	79%	86%	7%	14%	7%	0%	0%	14%
Direction	4+,1-	3+, 2-	7+,4-	1+, 11-						
Average ES	0.11	0.09	0.26	0.31	0.11	0.10	0.05			0.07
Algae (16 response variables)										
Significant responses	2	3	8	11	0	1	0	0	0	3
(% of all responses)	13%	19%	50%	69%	0%	6%	0%	0%	0%	19%
Direction	1+,1-	3+	1+,7-	11-						
Average ES	0.09	0.13	0.32	0.40		0.09	0.05			0.07

The benthic algal community was dominated by diatoms (93% of all algal cells counted), with smaller contributions by filamentous green algae (3.7%), blue-green algae (2.5%) and non-filamentous green algae (0.8%). Paralleling our findings for stream invertebrates (Table 3), the studied algal variables (3 community metrics and cell densities of the 13 most common taxa comprising at least 1% of the community each) responded to DCD addition in a similar magnitude and frequency as to nutrient addition (Table 3). Once again, algal variables were much more often (effect frequency) and much more strongly (effect size) affected by flow velocity reduction and fine sediment addition than by DCD addition.

DCD addition slightly reduced cell densities of the diatom species *Cymbella kappii* (in the constant and high pulse treatments by 4% each) but increased densities of *Fragillaria vaucheriae* (in the low pulse treatment by 6%). A positive trend of DCD addition on algal biomass (measured as chlorophyll a) was observed but this effect was not statistically significant ($P = 0.11$).

Nutrient enrichment increased overall algal biomass (+9%) mainly through an increase of *Melosira varians* cell densities (+17%). Flow reduction resulted in lower algal biomass (-18%) and cell densities of the most common taxa (between 5 and 24%) but it increased algal taxon richness (+9%). Similarly, fine sediment addition reduced algal biomass (-18%) via reducing the cell densities of most of the common algal taxa (by between 9 and 23%).

DCD interacted with the other stressors tested in just a single case. In this very weak interaction, DCD addition turned a small positive effect of flow reduction on algal taxon evenness into a small negative one.

The processing (mass loss due to decomposition) of birch leaf litter responded weakly positively to DCD addition ($P < 0.001$; effect size = 0.23; effect most noticeable for the high DCD pulses and constant addition; Fig. 5) and moderately negatively to flow reduction ($P < 0.001$; effect size = 0.34). Mahoe litter processing was not affected by DCD but increased very weakly with sediment addition ($P = 0.015$; effect size = 0.06). DCD did not interact with any of the other stressors in mediating processing rates of birch or mahoe litter.
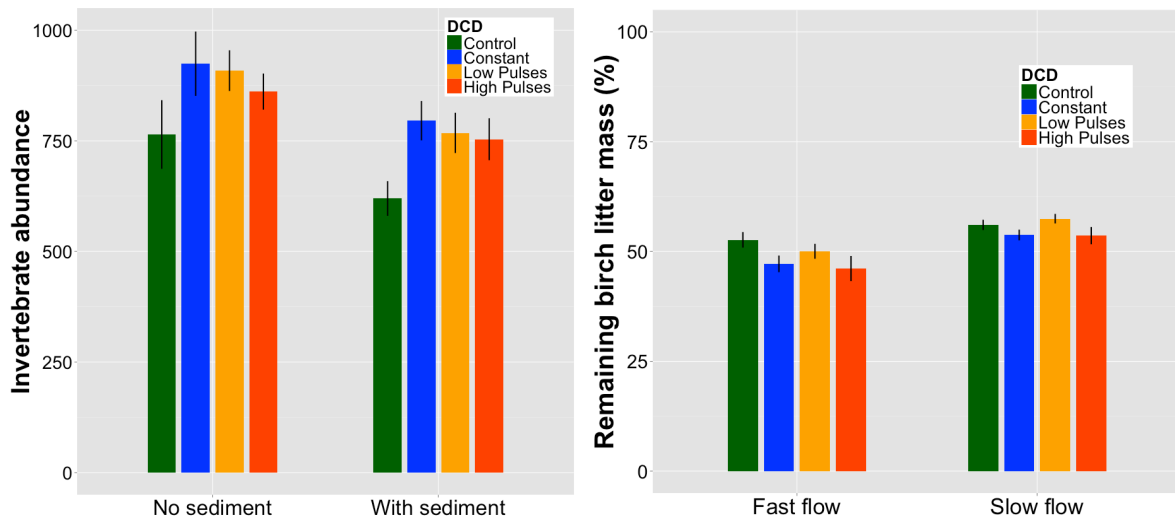
Figure 5. Stream Channel Experiment 2 - Effects of DCD and sediment additions on total invertebrate abundance (left panel) and of DCD addition and flow velocity reduction on birch litter mass remaining (right panel) at the end of the experiment. Mass loss due to decomposition (expressed as a percentage of initial birch leaf litter mass) is equivalent to 100% minus the litter mass percentage remaining at the end of the experiment. Shown are means ± 1 SE.

Discussion

Overall, the effects of DCD on the invertebrate and algal communities and on leaf litter processing during Experiment 2, which was conducted in late autumn, were similarly pervasive and of similar size as the effects of nutrient enrichment with relatively high concentrations of inorganic nitrogen and phosphorus. However, the effects of DCD were much smaller and less common than those of fine sediment addition and flow velocity reduction.

DCD changed invertebrate community composition and reduced community evenness via increased abundances of *P. antipodarum*, oligochaetes and chironomids. It is unlikely that these taxa profited directly from DCD addition, but they may have been able to more efficiently exploit the resources in the biofilm that increased in response to DCD additions, such as certain algal taxa, fungi (see next paragraph) and other microorganisms not assessed in our study. Although these invertebrate taxa have low MCI sensitivity scores (4, 1, and 2 out of 10, respectively), caution is warranted when drawing a parallel between these DCD effects and the presence of organic pollution implied by these low MCI scores (Stark et al. 2001). Reasons for caution include the absence of a direct response in abundance or taxon richness of the EPT taxa, which are particularly pollution-sensitive according to their MCI scores, to DCD addition.

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Instead, we observed only an indirect effect of DCD on EPT taxa, as DCD significantly increased the negative effect of elevated nutrients (but with a small effect size), possibly via changes in the ratio of nitrogen species. Moreover, the invertebrate taxa that benefitted from DCD addition in our experiment were mostly represented by smaller individuals compared to the EPT taxa. Therefore their importance (and also their increase with DCD addition) relative to the EPT taxa would be lower when expressed in terms of their contribution to total invertebrate biomass in the stream channels.

DCD addition increased processing of deciduous birch litter. Because the process of birch litter breakdown is qualitatively similar to that of other deciduous tree species such as willows (Haapala et al. 2001), which are more dominant in the riparian vegetation of New Zealand streams, our findings can be extrapolated to willow litter breakdown. In the absence of invertebrate shredders (which was the situation in our experiment), processing of leaf litter is governed by microbial fungi (Niyogi et al. 2003). Fungal activity depends on the supply of dissolved nutrients (Suberkropp et al. 2010) and on other chemicals and pollutants in the water. It is likely that these fungi were able to exploit nutrients (e.g. ammonium) that may have been augmented at the leaf surface as a consequence of DCD addition. The fact that we found minimal effects of DCD addition on the ratio of the nitrogen species (i.e. a relative increase in ammonium) in the water flowing through our channels may be due to the rapid water exchange in the channels (see discussion of greenhouse gas results below). Alternatively, this lack of effects on ammonium concentrations could be a consequence of our inability to take nutrient samples at the spatial scale most relevant for growth and activity of biofilm fungi (within the boundary layer of the leaf surface). Detecting such small-scale processes requires experiments specifically designed for this purpose. Our experiments focussed on a much larger scale, i.e. establishing realistic benthic stream ecosystems and testing processes at the community level (but see *Future Research Needs*).

The functional indicator used in this experiment, leaf litter processing, responded to DCD addition in a magnitude similar to that of structural indicators such as invertebrate and algal community metrics. The functional indicator allowed us to examine stressor effects on processes not covered by structural indicators but obviously linked to them because they govern the quantity and type of the available nutritional resources. Since assessing these functional responses takes a fraction of the time (approximately 20%) required to identify invertebrate and algal communities, we recommend assessing them in combination with structural indictors to allow a more complete understanding of stressor effects on stream ecosystems.

Research Aim 1.4: Stream Channel Experiment 3

Rationale

Our final stream channel experiment examined the effects of DCD pulses in combination with flow reduction and nutrient enrichment spanning a range of nitrogen to phosphorus (N:P) ratios on benthic algal and invertebrate communities as well as fish. In this experiment, fish (juvenile brown trout) were included as an additional bioindicator because elevated DCD levels could have indirect adverse effects on fish, for instance due to high ammonia levels toxic to salmonids (Richardson 1997), or via effects on their nutritional resources. The effect of enrichment with different N:P ratios was investigated because these ratios have been shown to be an important driver of algal community composition (Stevenson et al. 1996). Thus, algal taxonomic groups often respond differently to a range of N:P-ratios because of having different limitation levels to these two key nutrients (e.g. Fairchild et al. 1985). In this context, we wanted to determine whether nutrient enrichment with certain N:P ratios favoured toxin-producing blue-green algae (as observed in a recent survey of 58 New Zealand streams and rivers by A. Wagenhoff, Cawthron Institute) and, if so, whether this effect was modified by DCD addition.

Goal

- Examine the effects of nutrient enrichment with different N:P ratios in combination with DCD pulses and flow velocity reduction on stream algae, invertebrates and fish.

Methods

- Experiment 3 was conducted in spring/early summer 2013 (5 November - 17 December) using the same stream channel setup as the first two experiments.

- The experiment consisted of an initial period of 15 days with the different nutrient and flow treatments in place, to establish distinct algal communities via natural colonisation. This was followed by a period of 27 days with DCD application in addition to the nutrient and flow treatments.

- DCD treatments were the same as in Experiment 2, with two pulsed treatments (high and low) compared to constant DCD addition and a control where no DCD was added (Fig. 4). As in Experiment 2, DCD concentrations were monitored frequently during the manipulative period.

- Flow treatments were also the same as in Experiment 2, consisting of 2 flow velocity levels, fast (0.065 m/s) *vs* slow (0.013 m/s).

- Nutrient treatments (measured on three sampling dates) consisted of ambient values (90 µg/L nitrogen [NO_3^- and NH_4^+ combined as dissolved inorganic nitrogen] and 15 µg/L phosphorus [as dissolved reactive phosphorus, PO_4^{3-}]; molar ratio of N:P = 13.3) *vs* three enriched nutrient treatments: a low molar N:P ratio (600 µg/L N, 120 µg/L P; N:P = 11.1), a medium ratio (600 µg/L N, 40 µg/L P; N:P = 33.2) and a high ratio (600 µg/L N, 15 µg/L P; N:P = 89.3).

- All biological samples were collected on a single day at the end of the experiment, after 27 days of DCD addition. Invertebrate (17 response variables) and algal communities (18 response variables) were investigated, focusing on community metrics and abundances of the most common taxa (each contributing >1% of the community), as in Experiments 1 and 2. One juvenile trout was added per channel and fish survival and change in condition (compared to initial condition) were determined at the end of the experiment. Trout condition was measured using Fulton's condition factor, an index of the weight of the fish in relation to its length (Fulton 1902).

- During each of the three final days of the DCD addition period, we also measured N_2O concentrations in all stream channels with constant DCD addition and in all control channels, in collaboration with NIWA.

- Individual and combined stressor effects on these response variables were statistically analysed in an ANOVA with 'DCD', 'nutrients' and 'flow' as categorical predictor variables. A block factor was included in the model to account for any potential variation between the two spatial blocks present in the experiment. Statistical results for this block factor are not presented here because they merely represent background variation in the data that is unrelated to our research objectives.

Results

In this experiment, the invertebrate community was dominated in abundance by chironomid midges (*Tanitarsus* spp., 36%, and Orthocladiinae, 33%). EPT taxa made up 15% of the community, with the mayfly *Deleatidium* spp. (8.5%) being the most abundant of these pollution-sensitive taxa. DCD addition had a significant single-stressor effect on only one invertebrate response variable (Table 4), with a very weak negative effect on EPT abundance (Table S7, Fig. 6). Nutrient enrichment had weak positive effects on total invertebrate abundance and five of the common taxa. Flow velocity reduction was the most influential of the three manipulated stressors, with medium to strong negative effects on almost all invertebrate response variables (Table 4).

DCD also interacted weakly with the effects of flow reduction on total invertebrate taxon richness (no figure) and EPT abundance (Fig. 6). DCD had a minor negative effect on total taxon richness at slow flow velocity but not at fast flow velocity. Under fast flow conditions, the abundance of EPT taxa was reduced by constant DCD addition but not by the pulsed DCD treatments. By contrast, when flow was reduced all three DCD addition treatments negatively affected EPT taxa abundance. There were also some weak interactions among all three stressors (for total invertebrate abundance, community evenness and two of the common taxa).
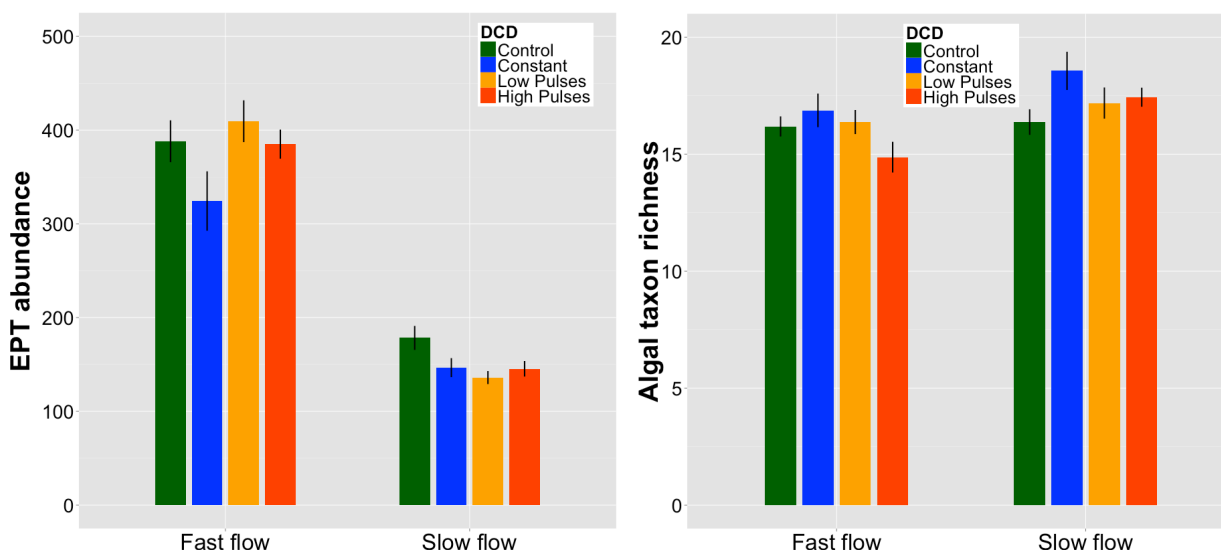
Figure 6. Stream Channel Experiment 3 - Effects of DCD addition and flow velocity reduction on the abundance of EPT taxa (mayflies, stoneflies and caddis flies; left panel) and algal taxon richness (right panel). Shown are means ± 1 SE.

The algal community was dominated by diatoms (86%), with non-filamentous green algae, filamentous greens and blue-green algae contributing 11.1%, 1.8% and 1.5% of all algal cells, respectively. Overall nutrient enrichment was the most influential stressor for algae (in terms of effect frequency, direction and size), followed by flow reduction, with both stressors having

frequent, medium to strong effects (Table 4). DCD was the least influential stressor with just a few, weak effects. DCD addition had a weak positive effect on algal taxon richness, which was higher in the constant DCD addition treatment compared to the control (Table S8, Fig. 6).

Nutrient enrichment had positive effects on algal biomass (with the highest biomass at lower N:P ratios), reflecting increases in diatoms (highest at high N:P) and non-filamentous green algae (highest at low N:P). By contrast, nutrient enrichment had negative effects on species richness, community diversity and evenness, and individual common taxa showed mixed responses. Flow reduction had mainly positive effects on algal metrics in this experiment.

DCD interacted weakly with the effects of nutrients on filamentous green algae (constant DCD addition increased cell density in ambient but not in enriched nutrient treatments) and with the effects of flow reduction on the common taxon *Scenedesmus* spp. (10.1% of all algal cells counted; Table S8). However, the most common interactive effect on the algal community was between nutrient enrichment and flow reduction, with weak to medium-sized effects on algal taxon richness, community diversity and evenness, as well as on several of the common taxa.

There were no significant effects of DCD or nutrient addition on fish condition or survival (Table 4). However, flow reduction had negative effects on both. Thus, fish survival averaged 22.1 days (of 28) at fast flow and 18.6 days at slow flow.

Table 4. Experiment 3 - Overview of all significant invertebrate, algal and fish responses to the three manipulated stressors and their interactions, showing the number of responses, their direction and average effect size (ES). An expanded version of this table detailing the statistical results can be found in the Appendix (Tables S7-8).

Treatments	DCD	Nutrients (N)	Flow (F)	DCD × N	DCD × F	N × F	DCD × N × F
Invertebrates *(17 response variables)*							
Significant responses	1	6	15	0	2	2	5
(% of all responses)	6%	35%	88%	0%	12%	12%	29%
Direction	1-	6+	13-,2+				
Average ES	0.08	0.12	0.35		0.09	0.10	0.19
Algae *(18 response variables)*							
Significant responses	1	12	14	1	1	11	0
(% of all responses)	6%	67%	78%	6%	6%	61%	0%
Direction	1+	6-,6+	11+,3-				
Average ES	0.10	0.37	0.26	0.21	0.10	0.14	
Fish *(2 response variables)*							
Significant responses	0	0	2	0	0	0	0
(% of all responses)	0%	0%	100%	0%	0%	0%	0%
Direction			2-				
Average ES			0.05				

Discussion

In this third stream channel experiment, the second conducted in spring (the same season as Experiment 1), effects of DCD addition on invertebrates and algae were weaker and far less common than the effects of flow reduction and nutrient enrichment, with only one invertebrate and one algal variable responding significantly. DCD addition slightly increased algal taxon richness, whereas it caused a very minor reduction in the abundance of the pollution-sensitive EPT invertebrate taxa. Flow reduction was the most influential stressor for invertebrates, while nutrient enrichment was the most influential stressor for algae, with changes in the ratio of nitrogen to phosphorus (N:P) influencing algal community composition.

None of the different N:P ratios in our three enrichment treatments (N:P 11, 33 and 89) caused an increase in the abundance of toxin-producing blue-green algae, in contrast with the findings of a recent survey of 58 New Zealand streams and rivers (A. Wagenhoff, Cawthron Institute, unpublished data) where cyanobacteria were most prevalent at N:P ratios between 15 and 40. Adding DCD as well as nutrients did not change this non-significant result.

Juvenile trout survival and condition were also unaffected by DCD addition and showed significant but very weak (in terms of effect size, see Table 4) negative responses to flow reduction. A possible reason for this general paucity of stressor effects on fish is that they may be too far from the actual cause of the stressor effects. We would expect the strongest responses in the algal community, which indirectly affect the invertebrates and subsequently the fish. Consequently, indirect effects of these stressors on fish via effects on their food resources, i.e. invertebrates, could be quite subtle and/or require a longer time frame to become apparent than a 6-8 week experiment. Moreover, in the present experiment fish were also impacted by methodological constraints such as high water temperatures (during a 1-week period of unseasonably hot weather), restricted movement in the stream channels (5-cm-long fish in 25-cm diameter circular channels), and possibly insufficient food supply (drifting and benthic invertebrates) because fish densities per unit area in our channels where very high compared to real streams. The effects of the three manipulated stressors (DCD, nutrients and flow) were probably quite benign compared to these constraints, leading to largely undetectable changes. Therefore, in these types of experiments in relatively small stream channels, fish such as juvenile trout may be less useful as bioindicators compared to the much smaller invertebrates and algae.

Greenhouse gas emissions during Experiments 2 and 3: Results and Discussion

As a secondary aim of these two stream channel experiments, we investigated the potential effects of DCD inputs on emissions of the greenhouse gas N_2O from simulated small farmland streams.

In Experiment 2 (all 128 channels sampled over two days at the end of experiment,), DCD addition had a significant ($P < 0.001$) but small effect (effect size 0.23) on N_2O emissions. Constant DCD addition reduced N_2O emissions compared to control channels, whereas pulsed DCD addition did not. None of the other stressors (sediment, nutrients, flow reduction) affected N_2O emissions.

In Experiment 3, we determined N_2O emissions three times on consecutive days (Days 25-27) near the end of the DCD addition period. This time we collected water samples only from constant DCD addition and control channels because these two treatments had shown the strongest difference in Experiment 2. Data were analysed with a repeated-measures ANOVA to account for the fact that they were temporally non-independent. On the first sampling day, constant DCD addition reduced N_2O emissions compared to control channels, as in Experiment 2. However, this effect was reversed for the two consecutive sampling days, resulting in an overall weakly positive effect (effect size 0.18; $P = 0.002$) of DCD addition on N_2O emissions

compared to the control. In this experiment, flow reduction also had a weak effect (size 0.23; *P* < 0.001), with N_2O emissions from slow-velocity channels being slightly higher than those from fast-velocity channels. This effect remained similar across sampling dates.

When combined, these results imply that DCD addition to small farmland streams is unlikely to change N_2O emissions from these running-water ecosystems, due to at least two reasons. First, the results from our two experiments were inconsistent and showed no clear overall pattern. Second, all observed N_2O concentrations were close to the atmospheric equilibrium. For an atmospheric concentration of 0.33 ppm, the water concentrations in equilibrium at 10, 15 and 20 °C are 0.363, 0.306 and 0.261 μg/L, respectively. Our N_2O concentrations were all within this range and water temperatures changed by several degrees Celsius during the course of each sampling day (from morning to late afternoon), reflecting the natural diurnal water temperature dynamics in the river feeding the channel setup. Therefore, any observed differences between our experimental DCD treatments could have been driven at least partly by these changes in water temperature. Because of the very short water retention period in our streams channels (less than 2 minutes), which simulates the situation in small streams realistically, it seems more likely that DCD can affect N_2O emissions from standing water bodies such as wetlands (see next research aim).

Paralleling the results for N_2O concentrations, ammonium concentrations in the stream channels were also affected very little by DCD addition, again presumably due to the short water retention period. Thus, in Experiment 2 ammonium concentrations in the two pulsed DCD treatments (29.0 μg/L and 28.5 μg/L) were only slightly higher than in control channels (24.0 μg/L) when averaged across all sampling dates (*P* = 0.005, effect size 0.12), with no significant differences between constant DCD addition (27.0 μg/L) and the remaining three treatments (repeated-measures ANOVA and Tukey *post hoc* tests). In Experiment 3, time-averaged ammonium concentrations were virtually identical across the four DCD treatments (range 29.7 to 31.3 μg/L; P = 0.87, effect size = 0.007).

Research Aim 1.5: DCD effects on wetlands (NIWA)

Rationale

Many small streams in pasture catchments have "wetlands" at their source or adjacent to the main channel along their length. Typically these wetlands are saturated areas of low-lying ground overgrown with thick grasses, where water seeps through the soil and across its surface rather than flowing in a confined channel. The low oxygen and high organic matter content of such "seepage wetlands" makes them ideal environments for denitrification. Studies have confirmed that in many cases they do indeed have high denitrification rates and are able to reduce the dissolved nitrogen concentration of pasture runoff before it enters streams (Rutherford & Nguyen 2004). Such wetlands can also be significant sources of nitrous oxide (Wilcock et al. 2008).

The effects of DCD on nitrogen processes have been studied at the plot scale (metres) in unsaturated pasture soils (e.g. Di & Cameron 2002; Monaghan et al. 2009). These studies have found reduced leaching of nitrate, improved uptake of nitrogen by grasses and reductions in nitrous oxide emissions. However, because oxygen concentrations and the carbon to nitrogen ratio have a strong influence on the various nitrogen transformation processes occurring in soils, parallel studies are required in wetlands to determine whether DCD has a similar effect on nitrogen species (nitrate, ammonium and total nitrogen) in wetlands as it does in pastures. In other words, even if DCD reduces total export of dissolved nitrogen and nitrous oxide from pasture soils, it may not have the same effect in wetlands. One previous study (Smith & Schallenberg 2013) has examined wetland sediments, but study this was confined to a laboratory and used sieved sediments with all plant material removed. Consequently, it is important to determine whether results from this rather artificial environment are similar to what occurs under field conditions in pastoral headwater wetlands.

If DCD disrupts the closely linked processes of nitrification and denitrification that remove nitrogen from runoff passing through wetlands, then potentially the use of DCD in pasture catchments could increase nitrogen export to streams. In addition, DCD itself has a high nitrogen content and may contribute to total nitrogen export if it passes through a wetland (Wilcock et al. 2008). The effects on nitrous oxide emissions in this environment are unknown.

Therefore the aims of this research were to answer the following questions:

1. Could DCD in wetlands increase dissolved nitrogen export to streams?

    a. determine whether DCD affects nitrate, ammonium and total dissolved nitrogen in wetlands (saturated, anaerobic soils) in the same way as in pasture (unsaturated, aerobic soils).

    b. determine whether DCD effects on nitrogen species in anaerobic wetland soils are the same in the field as in laboratory experiments

2. Does DCD decrease nitrous oxide emissions from wetland soils as it does from pasture soils?

3. At what concentrations of DCD do these effects occur?

4. How long does DCD take to decompose in Waikato pastoral headwater wetlands?

Methods

- We set up 12 mesocosms in a seepage wetland at the head of a pastoral catchment near Hamilton (Fig. 7). The wetland was about 20 m wide by 100 m long, with an upstream catchment area of about 5 hectares. The mescosms, spaced about 2 m apart, were areas of the wetland where the overlying water was contained by a 0.6 m-diameter cylinder

pressed into the soil. Within each mesocosm, a submersible pump kept the water moving, mimicking water movement in the wetland and maintaining dissolved oxygen levels.

Figure 7. A. Array of 12 mesocosms in the headwater wetland. B. Inside view of mesocosm showing pump to circulate water, piezometer to sample subsurface water and chamber to collect emitted nitrous oxide.

- DCD was added initially to the mesocosms in four different concentrations: 0, 100, 600 and 2000 mg/m^3, with three replicate mesocosms for each concentration.

- Concentrations of DCD, ammonium, nitrate, total dissolved nitrogen and relevant physico-chemical variables (oxygen, temperature, pH and conductivity) were measured in surface and subsurface water (10 cm below ground level) inside the mesocosms. Additional samples and measurements were taken outside the mesocosms at the four corners of the mesocosm array to check whether the conditions and processes inside the mesocosms were similar to those outside. Measurements were taken once every two to four days for 12 days before, and 15 days after, DCD application.

- After 15 days, DCD was applied a second time, this time together with 1000 mg/m^3 ammonium, and samples were taken for a further 10 days.

- Nitrous oxide emissions from the mesocosms were measured in 10 cm-diameter closed-end tubes that were pressed into the soil. Gas samples from the headspace were taken with a syringe up to four times over a 96-hour period. Nitrous oxide concentrations in the samples were measured on a gas chromatograph.

Results
- In surface water, temperatures remained between 10 and 16 °C and dissolved oxygen concentrations were mostly about 80% saturation. Under these conditions, DCD concentrations declined over 15 days to about 350 mg/m^3 in the 2000 mg/m^3 treatment, to about 100 mg/m^3 in the 600 mg/m^3 treatment and to trace levels in the 100 mg/m^3 treatment.

- Nitrate concentrations in the mesocosms were initially moderately high (due to concentrations in the water added with the DCD), but declined in all mesocosms over 15 days (Fig. 8). The rate of decline (and therefore the concentration of nitrate in the various mesocosms) was lower at higher concentrations of DCD, with a clear difference between the 600 mg/m^3 and the 2000 mg/m^3 treatment (Fig. 9).
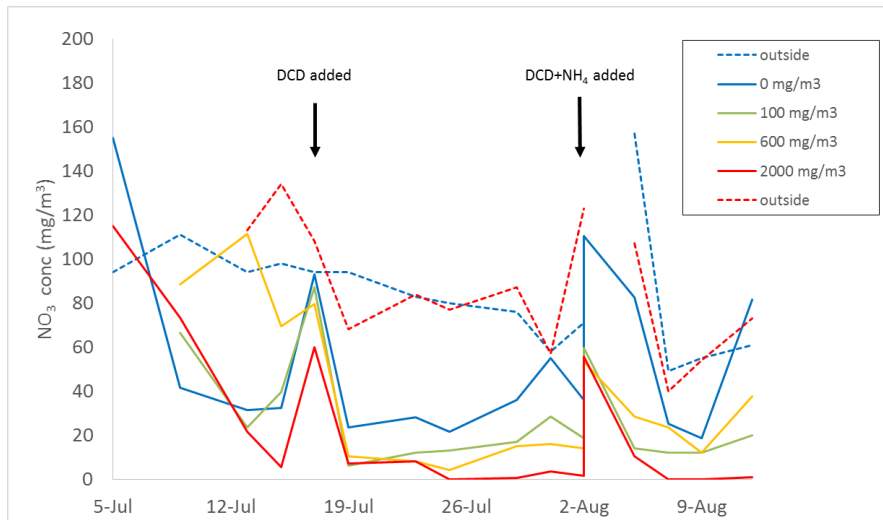
Figure 8. Nitrate concentrations over time inside the mesocosms with four different concentrations of DCD, and outside the mesocosms.
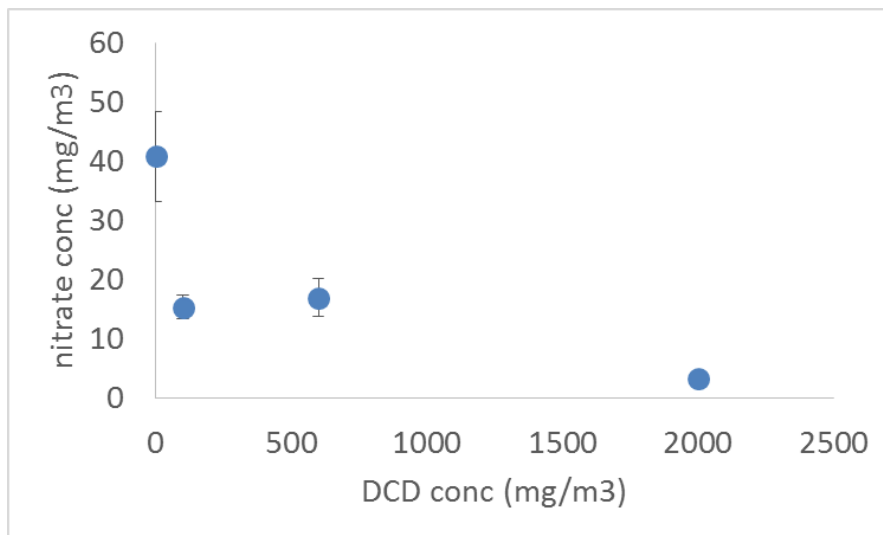
Figure 9. Nitrate concentrations in the four treatments (corresponding to four concentrations of DCD), averaged over the period following DCD application. Error bars represent standard errors.

- Ammonium concentrations rose slightly in most mesocosms after the first addition (DCD only), probably due to ammonium in the water added with the DCD, then declined to previous levels of less than 50 mg/m^3 within four days (Fig. 10). After the second addition (DCD+NH$_4^+$), ammonium concentrations declined from very high initial concentrations (1000-1300 mg/m^3) to previous concentrations of less than 50 mg/m^3 within three days (within five days in treatment 4). No correlation was seen between DCD concentration and the rate of decline in ammonium, nor between DCD concentration and the final concentration of ammonium.

Figure 10. Ammonium concentrations over time inside the mesocosms with four different concentrations of DCD, and outside the mesocosms.

- Total dissolved nitrogen (TDN) is composed of dissolved inorganic nitrogen (DIN; mainly ammonium and nitrate) and dissolved organic nitrogen (DON). In our study, TDN was largely composed of DCD (which is a form of DON), therefore patterns over time and among treatments (Fig. 11) largely reflected those of DCD. When DIN and DCD were subtracted from TDN, the remaining non-DCD DON showed no obvious pattern over time, but concentrations did appear to be a little lower in the high DCD treatments than in the low DCD treatments (no figure).

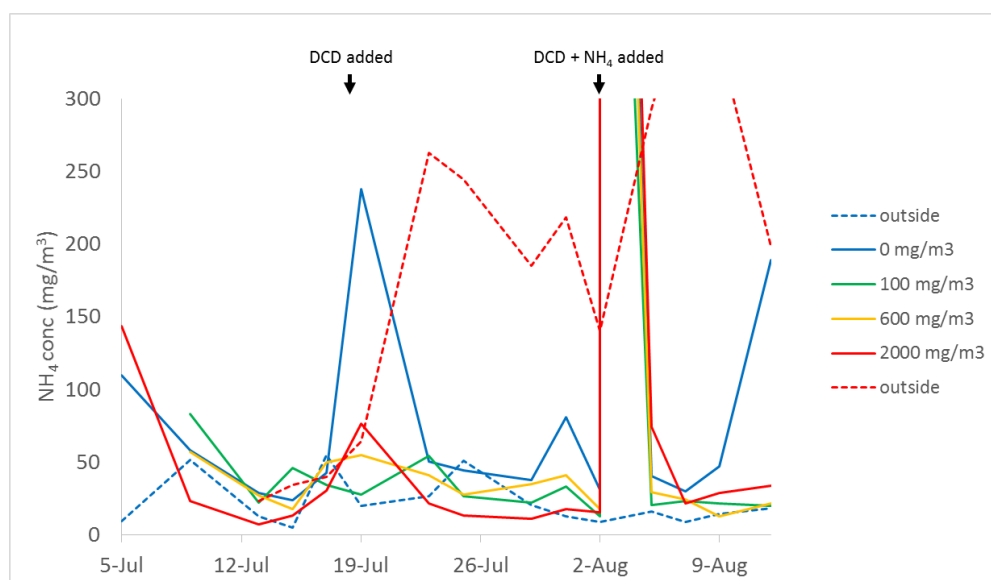**Figure 11.** Total dissolved nitrogen (TDN) concentrations over time inside the mesocosms with four different concentrations of DCD, and outside the mesocosms.

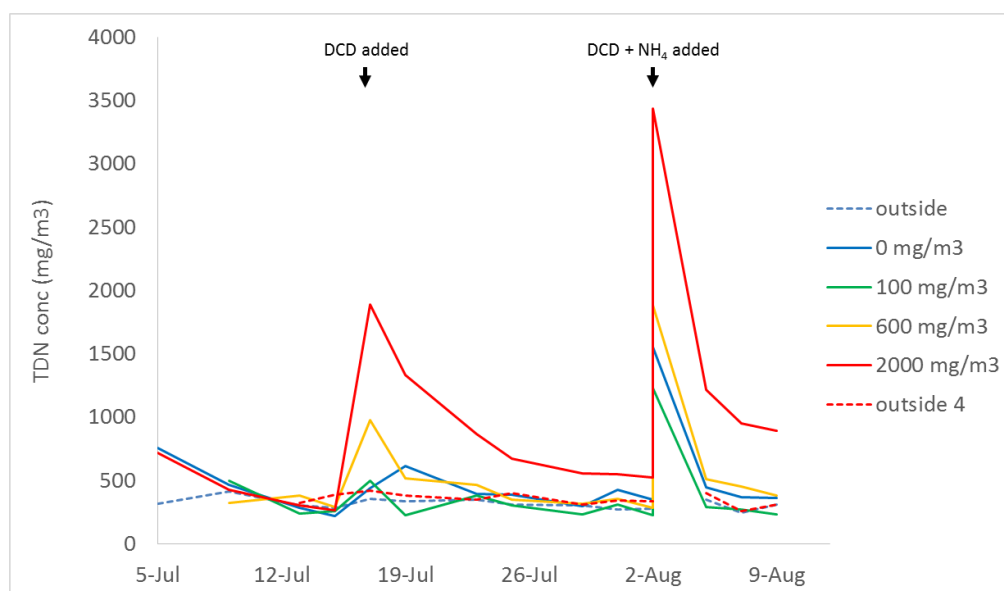- Nitrous oxide emissions appeared to be slightly reduced in mesocosms with greater concentrations of DCD (see Fig. S1 in the Appendix). However, emissions were patchy across the wetland, with "hotspots" of high emissions seemingly unrelated to DCD treatments.

Discussion

This experiment was conducted during a rather mild winter, when surface water temperatures remained between 10 and 16 °C. At these temperatures, and with dissolved oxygen concentrations mostly about 80% saturation, DCD concentrations in the mesocosms declined by about 50% within seven days. Previous studies have estimated the half-life of DCD as 111-116 days at 8 °C and 18-25 days at 20 °C (Di & Cameron 2004). It is not clear why the rate of decline in our study was much more rapid than in previous studies but the difference may be due to uptake by plants, which were not present in the study by Di & Cameron (2004).

DCD concentrations greater than 100-600 mg/m^3 appeared to cause a decrease in nitrate concentrations within the mesocosms. This is a similar result to those found in field studies of pasture soils and a laboratory study of wetland sediments (Smith & Schallenberg 2013), and it implies that nitrification was the main process producing nitrate in all these studies.

In contrast, ammonium concentrations did not seem to be affected by DCD addition. This result differs from that in the laboratory study of Smith & Schallenberg (2013), who found an accumulation of ammonium in the presence of DCD. Our result implies that in this wetland, the main process consuming ammonium was not nitrification. Instead, the most likely "sink" of ammonium in our study was uptake by grasses (see above). The wetland sediments in Smith & Schallenberg's (2013) study had no vegetation, and therefore no equivalent sink for ammonium.

Management implications

Our results suggest that, under stable weather conditions and in wetlands of this type with active growth of dense grasses, DCD is more likely to reduce than to increase export of inorganic nitrogen to streams. The most likely reason for this is that any ammonium accumulating due to blockage of nitrification is rapidly taken up by grasses or other wetland plants. In addition, DCD probably reduces nitrous oxide emissions, though this effect may be small compared to other factors that create emission "hotspots."

However, it should be noted that our experiment was conducted in a "closed" system. In an open system, with a through-flow of water, our results imply reduction in N export only if the water remains within the wetland for at least 2-4 days, long enough for plants to assimilate the ammonium. During storms, water residence time may be much shorter, and significant nitrogen export may occur.

Water residence time in a wetland may also determine whether DCD itself contributes significant nitrogen to downstream export. Although DCD appears to break down rapidly during warm conditions (and the ammonium that probably results from this breakdown would be taken up rapidly by wetland plants), even a 50% reduction in DCD concentration requires the water to remain within the wetland for 7 days. This may occur during stable weather, but is unlikely during rainy weather.

Future research needs based on these findings for a grassy seepage headwater wetland in a dairy farm are identified in the next section of this report.

Synthesis, Management Implications and Future Research

Please note: This section provides the basis for the user-friendly guide outlining the key risks associated with DCD use near waterways and their implications (see next research aim).

Key findings and management implications

- The combined findings of our three stream channel experiments indicate that DCD appears to be a relatively benign stressor (in terms of effect frequency and effect size) compared to the known agricultural stressors deposited fine sediment addition, stream flow reduction and nutrient enrichment. Moreover, interactions of DCD with other stressors were uncommon and always weak, implying that DCD addition rarely made other stressor effects worse. This finding contrasts with several strongly synergistic interactions between sediment and nutrients or sediment and flow reduction in previous multiple-stressor experiments in streams (e.g. Townsend et al. 2008, Matthaei et al. 2010, Wagenhoff et al. 2012, Piggott et al. 2012, 2014) and in the current experiments.

- Reasons for the observed differences in magnitude and pervasiveness of effects among stressors may arise from their different categories and likely pathways of effect. While DCD and nutrient additions affect the chemical composition of the water, with unlikely direct effects on the organisms at the concentrations tested, sediment addition and flow velocity reduction affect stream habitat conditions and may also have direct physical effects on algae, invertebrates and fish besides their effects on resource availability and quality. Managers have to be aware of the different stressor categories as well as the magnitude and pathways of their effects.

- Riparian buffer strips with intact riparian vegetation are likely to be a cost-efficient restoration measure for agricultural streams affected by three of the four stressors examined in this project. Establishing such buffer strips would reduce the inputs of DCD, nutrients and fine sediment from farmland, stabilize river and stream banks, and prevent access of livestock to the waterways.

- In grassy headwater wetlands on farms, DCD is more likely to reduce than to increase export of inorganic nitrogen to streams, probably because any ammonium accumulating due to blockage of nitrification is rapidly taken up by grasses or other wetland plants.

Future research needs

Based on the findings and experience gained during this project, we recommend the following future research aimed at addressing remaining knowledge gaps. These knowledge gaps concern processes and effects occurring at spatial scales that were beyond the scope of our experiments, i.e. at the reach scale (reach-scale surveys and experiments) or at the microhabitat scale (laboratory experiments).

I. Surveys in streams and wetlands on farms with DCD application

In collaboration with Ravensdown and Ballance Agri-Nutrients, scientists should sample small streams on farms with known DCD application at the catchment scale, in regions of New Zealand where DCD works well on farms (e.g. Southland and Waikato), to determine how high DCD peaks can get in these small streams draining dairy farms.

Note: These surveys require DCD application to the catchments of a large number of streams and wetlands with different background conditions and ideally a simultaneous gradient of other agricultural stressors to allow examining stressor interactions. As a consequence, they can only be conducted after DCD is back on the market in NZ and applied to farmland.

1) Sample several sites along each stream (e.g. 1st, 2nd, 3rd and 4th order) to determine how fast DCD gets diluted downstream.

2) Sample each stream several times, starting shortly after DCD application in each site catchment (e.g. 1, 5, 10, 20 days after application), to determine how quickly DCD peaks decrease after application.

3) Aim to include sampling after heavy/prolonged rain (e.g. 100 mm or more), to determine how high DCD peaks can get during such "extreme" rainfall events.

4) Our second original research objective in Research Aim 1.1 (*Examine whether observed levels of DCD are significantly related to the distribution patterns of stream animals and plants*), which had to be abandoned in the current project because we found no detectable concentrations of DCD in the surveyed 4th-6th order streams in Southland, should be investigated in small streams draining dairy farms. This should be done by sampling stream invertebrate and algal communities at each study site selected for the Future Research Aims 1-3 above using a similar spatial and temporal sampling schedule.

5) To determine whether DCD affects the aquatic communities in wetlands downstream of small streams draining dairy farms with DCD applications, such wetlands should be included in the study design (in addition to the stream sites) as far as logistically feasible. The aquatic invertebrate and algal communities in these receiving wetlands should also be investigated using a similar spatial and temporal sampling schedule as for the Future Research Aims 1-4.

6) To determine whether DCD increases or decreases nitrogen export from farmed headwater wetlands to streams and affects the algal and invertebrate communities as well as functional indicators in these ecosystems, surveys of such headwater wetlands should cover multiple sampling points downstream, before and after DCD is applied to the study catchments, and in comparison to catchments where DCD has not been applied.

II. Laboratory experiments

To complement our findings regarding DCD effects at the community level gained from outdoor stream channel experiments, laboratory studies are required to elucidate the mechanisms of observed DCD effects on algae, fungi and invertebrates at the population or individual level and to address effects of DCD on the micro-scale, i.e. directly on sediment surfaces or within the periphyton matrix. To this end, our research group will collaborate with Dr David Buchwalter, North Carolina State University. Dr Buchwalter is an ecotoxicologist with extensive experience in studying multiple-stressor effects on invertebrate physiology and thus provides the relevant expertise to elucidate the mechanisms behind DCD effects. He will work on this topic while visiting our research group at Otago for 3 months in early 2015 on a Fulbright Scholarship.

III. Reach scale experiments

As in our previous multiple-stressors research on sediment and nutrient effects, reach-scale experiments would complement our DCD experiments in small circular stream channels conducted to date. These experiments could have similar study designs to Matthaei et al. (2006; fine sediment addition to 50-m reaches in 12 small farmland streams) or Townsend et al. (2008; sediment and nutrient addition to 50-m reaches in 9 small farmland streams).

Note: Such reach-scale experiments require adding large quantities of DCD added to farmland streams, which will then be diluted downstream. Because DCD would enter the environment and thus possibly the food chain, such experiments can only be conducted after DCD is back on the market in NZ.

Research Aim 1.6: Extension of Results to Key Stakeholders

Goal

- Distil and communicate the key results and insights from the project to key stakeholders - including Central Government Ministries, Regional Councils, Resource Managers, and Industry - in a user-friendly fashion.

Methods

- We organised two workshops (presentations followed by ample time for discussion) with the key stakeholders listed above. These workshops were held at MPI in Wellington in August 2013 and June 2014.

- As part of Project Milestone 7, we have produced the draft version of a user-friendly guide (1-2 pages) outlining the key risks associated with DCD use near waterways and their implications. This draft version will be sent to all participants of the second workshop for their feedback.

Outcomes

- The first DCD Enduser Workshop was attended by 15-20 MPI staff members, and the second by a wide range of interest groups (see list of workshop participants below).

- No specific feedback regarding the contents of the Final Project Report was received from the participants of Workshop 2; however, there was an animated and prolonged discussion of the results presented. Moreover, all participants will be invited to provide their feedback on the user-friendly guide resulting from our project (see above).

List of attendees at the second DCD Enduser Workshop (25 June 2014)

Dr Philip Mladenov, Chief Executive, Fertiliser Association of New Zealand
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Dr Antony Roberts, Chief Scientific Officer, Ravensdown Ltd
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Aaron Stafford, Science Manager, Ballance Agri-Nutrients <Aaron.Stafford@ballance.co.nz>

Dr Stewart Ledgard, Principal Scientist, AgResearch <stewart.ledgard@agresearch.co.nz>

Prof Keith Cameron, Lincoln University <Keith.Cameron@lincoln.ac.nz>

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APPENDIX

Table S1-S4: Stream Channel Experiment 1 - Detailed results of statistical analyses of invertebrate and algal data.

Table S5-S6: Stream Channel Experiment 2 – Detailed results of statistical analyses of invertebrate and algal data.

Table S7-S8: Stream Channel Experiment 3 – Detailed results of statistical analyses of invertebrate and algal data.

Figure S1. Nitrous oxide concentrations in the DCD addition experiment to a North Island wetland (Research Aim 1.5).

Table S1. Experiment 1 – Invertebrate results for the lower six DCD levels (n = 96 channels). *P*-values for single-stressor effects (stressor main effects) plus 2-way or 3-way interactions among stressors are given for all response variables (8 community-level metrics and the 7 most common taxa). Effect sizes (partial eta squared values, range 0.0 – 1.0) are given in brackets for all significant (*P* < 0.05) results. Response variables were log(x)- or log(x+1) transformed where necessary (as indicated after each variable name) to improve normality and homoscedasticity. For each common taxon, "%" indicates its contribution to the total number of individuals counted in all 128 channels.

Dependent variable	%	Nutrients	Sediment	DCD	Nutrients × DCD	Sediment × DCD	Nutrients × Sediment	Nutrients × Sediment × DCD
Invertebrate abundance		0.43	0.11	0.55	0.76	0.73	0.75	0.24
Invertebrate taxon richness		0.95	0.61	0.28	0.41	0.60	0.86	0.36
EPT taxon richness		0.79	**0.003** (0.10) -	0.09	0.22	0.14	**0.02** (0.06)	0.69
EPT abundance *(log)*		0.35	**<0.001** (0.29) -	0.11	0.53	0.21	0.83	0.77
Invertebrate diversity (Simpson)		0.74	**<0.001** (0.57) -	0.10	0.12	0.08	0.87	0.18
Invertebrate evenness (Simpson)		0.54	**<0.001** (0.32) -	0.21	0.09	0.40	0.32	0.62
Macroinvertebrate Community Index (MCI)		0.24	**0.03** (0.05) -	0.17	0.06	0.39	0.50	0.16
Invertebrate body size		**0.005** (0.09) +	**<0.001** (0.45) +	**0.02** (0.06) -	**0.04** (0.05)	0.60	0.55	0.83
Oligochaeta	43.6	0.82	**<0.001** (0.22) +	0.99	**0.005** (0.09)	0.18	**0.04** (0.05)	0.51
Chironomidae *(log)*	25.5	**<0.001** (0.33) +	**<0.001** (0.58) -	**<0.001** (0.14) -	**0.008** (0.08)	0.34	**0.002** (0.11)	0.17
Copepoda *(log)*	21.8	0.26	**<0.001** (0.87) -	0.85	0.74	0.90	0.88	0.76
Potam. antipodarum (log)	3.5	0.73	0.50	0.79	0.73	0.81	0.20	0.19
Ostracoda	2.4	0.77	**<0.001** (0.17) -	0.95	0.83	0.73	0.70	0.57
Tanypodinae *(log)*	1.1	0.75	**<0.001** (0.45) -	0.34	0.60	0.34	0.43	0.88
Deleatidium spp.	0.7	0.995	**<0.001** (0.19) -	0.14	0.09	0.88	0.45	0.24

Table S2. Experiment 1 – Invertebrate results for all eight DCD levels (n = 128 channels). For more details see Table S1.

Dependent variable	%	Nutrients		Sediment		DCD		Nutrients × DCD	Sediment × DCD	Nutrients × Sediment	Nutrients × Sediment × DCD
Invertebrate abundance		0.53		0.81		0.81		0.91	0.09	0.88	0.24
Invertebrate taxon richness		0.79		0.79		0.17		0.16	0.77	0.70	0.26
EPT taxon richness		0.21		**0.001** (0.10)	-	0.19		0.58	0.10	**0.03** (0.04)	0.71
EPT abundance *(log)*		0.25		**<0.001** (0.29)	-	0.05	-	0.31	0.36	0.76	0.24
Invertebrate diversity (Simpson)		0.96		**<0.001** (0.61)	-	0.08	-	0.09	0.17	0.70	0.21
Invertebrate evenness (Simpson)		0.73		**<0.001** (0.37)	-	0.24		0.06	0.53	0.53	0.11
Macroinvertebrate Community Index (MCI)		0.71		**0.007** (0.06)	-	0.33		0.73	0.24	0.55	0.11
Invertebrate body size		0.056 (0.03)	+	**<0.001** (0.48)	+	0.45		0.38	0.63	0.15	0.20
Oligochaeta	43.6	0.04 (0.04)	-	**<0.001** (0.25)	+	0.86		**0.045** (0.03)	0.15	**0.002** (0.08)	0.22
Chironomidae *(log)*	25.5	**<0.001** (0.26)	+	**<0.001** (0.56)	-	**0.02** (0.04)	-	0.097 (0.02)	0.83	**0.003** (0.07)	0.42
Copepoda *(log)*	21.8	0.13		**<0.001** (0.87)	-	0.98		0.75	0.56	0.63	0.56
Potam. antipodarum (log)	3.5	0.68		0.31		0.70		0.59	0.55	0.57	0.51
Ostracoda	2.4	0.76		**<0.001** (0.22)	-	0.80		0.78	0.52	0.25	0.99
Tanypodinae *(log)*	1.1	0.47		**<0.001** (0.47)	-	0.46		0.74	0.33	0.32	0.96
Deleatidium spp.	0.7	0.38		**<0.001** (0.18)	-	**0.03** (0.04)	-	0.17	0.34	0.73	0.97

Table S3. Experiment 1 – Algal results for the lower six DCD levels (n = 96 channels). For more details see Table S1.

Dependent variable	%	Nutrients		Sediment		DCD		Nutrients × DCD	Sediment × DCD	Nutrients × Sediment	Nutrients × Sediment × DCD
Algal biomass		**<0.001** (0.49)	+	**<0.001** (0.35)	-	**0.02** (0.06)	-	0.59	**0.007** (0.08)	**<0.001** (0.33)	0.16
Algal cell density		**<0.001** (0.34)	+	**<0.001** (0.47)	-	0.12		0.27	0.54	**<0.001** (0.34)	0.22
Algal taxon richness		**0.001** (0.13)	+	0.76		0.75		0.91	0.97	0.73	0.56
Algal diversity (Simpson)		**0.045** (0.05)	+	**<0.001** (0.20)	-	0.53		0.20	0.66	0.27	0.49
Algal evenness (Simpson)		0.76		**<0.001** (0.27)	-	0.28		0.15	0.62	0.22	0.17
High growth form		**<0.001** (0.26)	+	**<0.001** (0.47)	-	0.19		0.38	0.39	**<0.001** (0.35)	0.41
Low growth form *(log)*		**<0.001** (0.39)	+	**<0.001** (0.75)	-	**0.003** (0.10)	-	0.96	0.38	**<0.001** (0.27)	0.18
Motile growth form *(log)*		**0.001** (0.12)	+	**0.03** (0.05)	+	0.94		0.19	0.47	0.55	0.14
Cymbella kappii (log)	14.3	0.21		**<0.001** (0.41)	-	0.06		0.85	0.12	**0.04** (0.05)	0.26
Fragilaria vaucheriae (log)	13.6	**<0.001** (0.31)	+	**<0.001** (0.14)	-	0.90		0.91	0.99	**0.04** (0.05)	0.53
Gomphon. minutum (log)	11.9	**<0.001** (0.21)	+	**<0.001** (0.74)	-	0.051		0.74	0.37	**<0.001** (0.22)	0.43
Nitzschia palea (log)	11.8	**0.02** (0.06)	+	0.43		0.74		0.30	0.91	0.07	0.22
Encyonema minuta (log)	6.2	**<0.001** (0.13)	+	**<0.001** (0.17)	-	**0.02** (0.06)	-	0.53	0.67	**<0.001** (0.18)	0.25
Synedra arcus/ulna (log)	5.9	0.09		**<0.001** (0.33)	-	0.35		0.44	0.69	**0.001** (0.11)	0.20
Melosira varians (log)	3.7	**<0.001** (0.35)	+	**0.006** (0.09)	-	0.057		0.07	0.60	**0.04** (0.05)	0.36
Achnanthidium minutissimum (log)	3.6	0.26		**<0.001** (0.35)	-	**0.01** (0.07)	-	0.49	0.70	**0.01** (0.07)	0.64
Fragilaria cap. / Synedra rump. (log)	3.1	0.87		0.69		0.59		0.99	0.67	**0.02** (0.06)	0.26
Tabellaria flocculosa (log)	2.9	0.34		**<0.001** (0.19)	-	0.30		0.08	0.39	0.48	0.76
Phormidium spp. *(log)*	2.8	0.55		0.25		0.06		0.93	0.74	**<0.001** (0.16)	0.67
Filamentous green spp. *(log)*	2.6	0.58		**<0.001** (0.33)	-	0.95		0.82	0.42	0.57	0.61
Rossithidium spp. *(log)*	2.1	**0.001** (0.12)	+	**<0.001** (0.32)	-	0.37		0.07	0.87	0.12	0.19
Gomphon. clavatum (log)	1.4	0.80		**0.01** (0.07)	-	0.39		0.52	0.28	0.20	0.27
Scenedesmus spp. *(log)*	1.3	**<0.001** (0.40)	+	**0.001** (0.13)	-	0.09		0.72	0.26	**<0.001** (0.14)	0.81
Gomphoneis minuta var. *cass. (log)*	1.2	**0.02** (0.06)	+	**0.005** (0.09)	-	0.85		0.99	**0.03** (0.05)	0.60	0.62

Table S4. Experiment 1 – Algal results for all eight DCD levels (n = 128 channels). For more details see Table S1.

Dependent variable	%	Nutrients		Sediment		DCD		Nutrients × DCD	Sediment × DCD	Nutrients × Sediment	Nutrients × Sediment × DCD
Algal biomass *(log)*		**<0.001** (0.40)	+	**<0.001** (0.18)	-	0.53		0.43	0.48	**0.002** (0.08)	0.83
Algal cell density		**<0.001** (0.34)	+	**<0.001** (0.48)	-	0.48		**0.04** (0.03)	0.08	**<0.001** (0.26)	0.66
Algal taxon richness		**0.003** (0.07)	+	0.83		0.57		0.11	0.50	0.46	**0.03** (0.04)
Algal diversity (Simpson)		0.25		**<0.001** (0.29)	-	0.54	-	0.98	0.68	**0.002** (0.08)	0.29
Algal evenness (Simpson)		0.28		**<0.001** (0.34)	-	0.68	-	0.30	0.98	**0.006** (0.06)	0.67
High growth form		**<0.001** (0.23)	+	**<0.001** (0.46)	-	0.65		0.06 (0.03)	**0.03** (0.04)	**<0.001** (0.26)	0.28
Low growth form *(log)*		**<0.001** (0.37)	+	**<0.001** (0.74)	-	**0.02** (0.05)	-	0.66	0.82	**<0.001** (0.24)	0.32
Motile growth form *(log)*		**<0.001** (0.12)	+	**0.04** (0.04)	+	0.24		**0.02** (0.05)	0.82	0.79	0.33
Cymbella kappii (log)	14.3	0.39		**<0.001** (0.30)	-	0.45		0.36	0.53	0.28	0.71
Fragilaria vaucheriae (log)	13.6	**<0.001** (0.31)	+	**<0.001** (0.13)	-	0.22		0.48	0.33	**0.001** (0.08)	0.69
Gomphon. minutum (log)	11.9	**<0.001** (0.20)	+	**<0.001** (0.71)	-	0.097		0.88	0.64	**<0.001** (0.15)	0.95
Nitzschia palea (log)	11.8	**0.002** (0.08)	+	0.32		0.45		0.09	0.88	0.15	0.54
Encyonema minuta (log)	6.2	**<0.001** (0.10)	+	**<0.001** (0.17)	-	0.08		0.39	0.87	**<0.001** (0.14)	0.35
Synedra arcus/ulna (log)	5.9	**0.03** (0.04)	+	**<0.001** (0.42)	-	0.93		0.21	0.76	**0.001** (0.08)	0.65
Melosira varians (log)	3.7	**<0.001** (0.23)	+	**<0.001** (0.10)	-	0.07		0.54	0.61	**0.001** (0.09)	0.92
Achnanthidium minutissimum (log)	3.6	0.67		**<0.001** (0.33)	-	**0.02** (0.05)	-	0.77	0.75	**0.001** (0.09)	0.23
Fragilaria cap. / Synedra rump. (log)	3.1	0.97		0.06		0.40		0.96	0.16	**0.03** (0.04)	0.48
Tabellaria flocculosa (log)	2.9	**0.01** (0.05)	-	**<0.001** (0.21)	-	0.50		0.45	0.47	0.23	0.38
Phormidium spp. *(log)*	2.8	0.49		0.41		0.30		0.89	0.76	**<0.001** (0.16)	0.39
Filamentous green spp. *(log)*	2.6	0.13		**<0.001** (0.38)	-	0.15		0.17	0.44	0.56	0.63
Rossithidium spp. *(log)*	2.1	**<0.001** (0.13)	+	**<0.001** (0.36)	-	0.15		0.07	0.49	0.20	0.34
Gomphon. clavatum (log)	1.4	0.54		0.13		0.54		0.68	0.60	0.27	0.43
Scenedesmus spp. *(log)*	1.3	**<0.001** (0.40)	+	**<0.001** (0.10)	-	0.79		0.22	0.48	**<0.001** (0.18)	0.38
Gomphoneis minuta var. *cass. (log)*	1.2	**0.02** (0.05)	+	**0.02** (0.04)	-	0.57		0.60	0.13	0.07	0.53

Table S5. Experiment 2 – Invertebrate responses to DCD additions, nutrient additions, flow reductions, and sediment additions. *P*-values for single-stressor effects (stressor main effects) plus interactions among stressors are shown for community metrics and the 8 most common taxa. Effect sizes (partial eta squared values, range 0.0 – 1.0) are given in brackets for all significant (*P* < 0.05) results. Response variables were log(x)- or log(x+1) transformed where necessary (as indicated after each variable name) to improve normality and homoscedasticity. For each common taxon, "%" indicates its contribution to the total number of individuals counted in all 128 channels.

Dependent variable	%	DCD	Dir	Nutrient addition	Dir	Flow reduction	Dir	Sediment addition	Dir	DCD × Nut	DCD × Flow	Nut × Flow	DCD × Sed	Nut × Sed	Flow × Sed
Community metrics															
Invertebrate abundance		**0.002 (0.14)**	+	**0.047 (0.04)**	+	0.72		**<0.001 (0.14)**	-	0.32	0.60	0.19	0.98	0.63	0.12
Invertebrate taxon richness		0.090		0.29		**0.011 (0.07)**	+	**<0.001 (0.24)**	-	0.23	0.49	0.77	0.90	0.90	0.33
Invertebrate taxon evenness (Simpson)		**0.031 (0.09)**	(-)	0.14		**<0.001 (0.19)**	+	**<0.001 (0.20)**	-	0.81	0.10	0.50	0.17	0.79	0.35
Macroinvertebrate Community Index (MCI)		0.46		0.70		0.074		**0.032 (0.05)**	-	0.61	0.48	0.15	0.93	0.11	0.44
EPT abundance		0.46		**<0.001 (0.14)**	-	**0.014 (0.06)**	-	**<0.001 (0.79)**	-	**0.001 (0.11)**	**0.009 (0.11)**	0.077	0.45	0.39	0.092
EPT taxon richness		0.44		0.87		0.42		**<0.001 (0.39)**	-	0.086	0.62	0.20	0.66	0.15	0.42
Dominant taxa															
Potam. antipodarum	35.7	**0.002 (0.14)**	(+)	0.089		**<0.001 (0.65)**	-	**0.002 (0.09)**	+	0.38	0.40	0.72	0.77	0.27	0.060
Oliogochaeta spp.	27.0	**0.006 (0.12)**	(+)	0.11		**<0.001 (0.37)**	+	0.46		0.89	0.87	0.089	0.79	0.78	0.59
Copepoda spp.	12.0	0.24		**0.039 (0.04)**	+	**<0.001 (0.60)**	+	**<0.001 (0.36)**	-	0.089	0.77	0.49	0.69	0.64	**0.002 (0.09)**
Chironomidae spp.	10.4	**0.043 (0.08)**	(+)	**0.009 (0.07)**	+	**<0.001 (0.30)**	-	**<0.001 (0.33)**	-	0.64	0.58	0.14	0.97	0.37	0.47
Psilochorema spp.	3.2	0.38		0.39		**<0.001 (0.18)**	-	**<0.001 (0.41)**	-	0.052	0.87	**0.028 (0.05)**	0.74	0.74	0.78
Deleatidium spp.	2.6	0.25		**<0.001 (0.16)**	-	**0.006 (0.08)**	+	**<0.001 (0.56)**	-	0.98	**0.026 (0.09)**	0.79	0.068	0.36	0.59
Ostracoda spp.	2.3	0.15		0.91		**0.017 (0.06)**	+	0.72		0.36	0.47	0.17	0.34	0.66	0.72
Cladocera spp.	1.5	0.39		0.17		**<0.001 (0.35)**	+	**<0.001 (0.17)**	-	0.55	0.44	0.18	0.39	0.48	**0.032 (0.05)**

Table S5 continued.

Dependent variable	%	DCD × Nut × Flow	DCD × Nut × Sed	DCD × Flow × Sed	Nut × Flow × Sed	DCD × Nut × Sed × Flow
Community metrics						
Invertebrate abundance		0.63	0.68	0.074	0.84	0.84
Invertebrate taxon richness		0.76	0.098	0.85	0.97	0.25
Invertebrate taxon evenness (Simpson)		0.90	0.051	0.60	0.62	0.96
Macroinvertebrate Community Index (MCI)		0.85	0.76	0.25	0.45	0.47
EPT abundance		0.085	0.97	0.13	0.60	0.14
EPT taxon richness		0.98	0.39	0.86	**0.026 (0.05)**	0.59
Dominant taxa						
Potam. antipodarum	35.7	0.18	**0.040 (0.08)**	0.066	0.77	0.63
Oliogochaeta spp.	27.0	0.35	0.70	0.17	0.47	1.00
Copepoda spp.	12.0	0.73	0.63	0.20	0.38	0.57
Chironomidae spp.	10.4	0.55	0.88	0.42	0.17	0.28
Psilochorema spp.	3.2	**0.008 (0.12)**	0.33	0.47	0.62	0.37
Deleatidium spp.	2.6	0.57	0.43	**0.008 (0.12)**	0.14	0.17
Ostracoda spp.	2.3	0.78	0.18	0.72	0.84	0.60
Cladocera spp.	1.5	0.61	0.85	0.15	0.93	0.98

Table S6. Experiment 2 – Algal responses to DCD additions, nutrient additions, flow reductions, and sediment additions. *P*-values for single-stressor effects (stressor main effects) plus interactions among stressors are shown for community metrics and the 8 most common taxa. Effect sizes (partial eta squared values, range 0.0 – 1.0) are given in brackets for all significant ($P < 0.05$) results. Response variables were log(x)- or log(x+1) transformed where necessary (as indicated after each variable name) to improve normality and homoscedasticity. For each common taxon, "%" indicates its contribution to the total number of individuals counted in all 128 channels.

Dependent variable	%	DCD	Dir	Nutrient addition	Dir	Flow reduction	Dir	Sediment addition	Dir	DCD × Nut	DCD × Flow	Nut × Flow	DCD × Sed	Nut × Sed	Flow × Sed
Community metrics															
Algal biomass (Chl-*a*, log)		0.11		**<0.001 (0.17)**	+	**<0.001 (0.52)**	-	**<0.001 (0.51)**	-	0.71	0.73	0.49	0.073	0.44	0.55
Algal taxa richness		0.76		0.76		**<0.001 (0.16)**	+	0.55		0.80	0.75	0.98	0.43	0.19	0.54
Algal taxa evenness (Simpson, log)		0.41		0.36		0.22		0.56		0.39	**0.027 (0.09)**	0.66	0.54	0.55	**0.010 (0.07)**
Dominant taxa (cell densities)															
Encyonema minuta (log)	19.1	0.32		0.88		**<0.001 (0.69)**	-	**<0.001 (0.60)**	-	0.75	0.76	0.11	0.88	0.34	0.89
Gomphonema minatum (log)	16.1	0.38		0.95		0.078		**<0.001 (0.65)**	-	0.077	0.10	0.10	0.43	0.69	0.11
Nitzschia palea (log)	16.0	0.65		0.31		**<0.001 (0.20)**	-	0.62		0.46	0.47	0.17	0.52	0.37	**0.014 (0.06)**
Melosira varians (log)	8.0	0.17		**<0.001 (0.16)**	+	**<0.001 (0.37)**	-	**<0.001 (0.24)**	-	0.86	0.21	0.25	0.18	0.72	0.065
Cymbella kappii (log)	8.0	**0.019 (0.10)**	(-)	0.14		**<0.001 (0.41)**	-	**<0.001 (0.56)**	-	0.98	0.39	0.38	0.98	0.84	0.77
Fragilaria vaucheriae (log)	4.8	**0.047 (0.08)**	(+)	0.80		**<0.001 (0.14)**	-	**<0.001 (0.27)**	-	0.94	0.61	0.48	1.00	0.89	0.51
Achnanthidium minutissimum (log)	4.2	0.36		0.25		0.65		**<0.001 (0.29)**	-	0.30	0.11	0.24	0.40	0.83	0.28
Cocconeis placentula (log)	2.6	0.30		0.33		0.18		**<0.001 (0.66)**	-	0.94	0.98	1.00	0.84	0.44	**0.011 (0.07)**
Rossithidium spp. *(log)*	2.2	0.76		0.87		0.56		**<0.001 (0.30)**	-	0.30	0.71	0.78	0.75	0.43	0.99
Gomphoneis minuta (log)	1.8	0.051		0.42		**0.002 (0.10)**	-	**<0.001 (0.29)**	-	0.39	0.53	0.11	0.12	0.96	0.70
Gomphonema angustum (log)	1.6	0.46		0.67		0.16		**0.020 (0.06)**	-	0.070	0.25	0.39	0.87	0.60	0.98
Navicula cryptocephala (log)	1.1	0.72		**0.031 (0.05)**	+	0.46		0.12		0.26	0.78	0.36	0.22	0.92	0.053
Mougeotia spp. *(log)*	1.0	0.094		0.38		0.15		0.29		0.087	0.76	0.45	0.84	0.43	0.68

Table S6 continued.

Dependent variable	DCD × Nut × Flow	DCD × Nut × Sed	DCD × Flow × Sed	Nut × Flow × Sed	DCD × Nut × Sed × Flow
Community metrics					
Algal biomass (Chl-*a*, log)	0.80	0.74	0.90	0.82	1.00
Algal taxa richness	0.31	0.62	0.93	0.79	0.78
Algal taxa evenness (Simpson, log)	0.39	**0.007 (0.12)**	0.84	0.11	0.21
Dominant taxa (cell densities)					
Encyonema minuta (log)	0.93	0.92	0.68	0.82	0.34
Gomphonema minatum (log)	0.39	0.61	0.88	0.089	0.65
Nitzschia palea (log)	0.58	0.65	0.89	0.092	0.53
Melosira varians (log)	0.99	0.90	0.88	0.45	0.97
Cymbella kappii (log)	0.93	1.00	0.45	0.66	0.87
Fragilaria vaucheriae (log)	0.23	0.52	0.37	0.58	0.35
Achnanthidium minutissimum (log)	0.80	0.60	0.41	0.42	0.47
Cocconeis placentula (log)	0.16	0.90	0.92	0.13	0.21
Rossithidium spp. *(log)*	0.89	0.90	0.82	0.50	0.81
Gomphoneis minuta (log)	0.90	0.49	0.59	0.80	0.58
Gomphonema angustum (log)	0.87	0.34	0.36	0.74	0.29
Navicula cryptocephala (log)	0.21	0.79	0.22	0.23	0.73
Mougeotia spp. *(log)*	0.83	0.17	0.31	0.92	0.17

Table S7. Experiment 3 – Invertebrate and fish responses to DCD addition, nutrient enrichment with different N:P-ratios, and flow reduction. *P*-values for single-stressor effects (stressor main effects) and interactions among stressors are shown for invertebrate community metrics, the 10 most common invertebrate taxa, and fish survival and condition. Effect sizes (partial eta squared values, range 0.0 – 1.0) are given in brackets for all significant ($P < 0.05$) results. Response variables were log(x)- or log(x+1) transformed where necessary (as indicated after each variable name) to improve normality and homoscedasticity. For each common taxon, "%" indicates its contribution to the total abundance in all 128 channels.

Dependent variable	%	DCD addition	Dir	Nutrient enrichment	Dir	Flow reduction	Dir	DCD × Nutrients	DCD × Flow	Nutrients × Flow	DCD × Nutrients × Flow
Invert. Community Metrics											
Total invertebrate abundance		0.21		**<0.001 (0.16)**	+	**<0.001 (0.40)**	-	0.76	0.33	0.49	**0.031 (0.17)**
Invertebrate taxon richness		0.38		0.30		**0.003 (0.09)**	-	0.17	**0.023 (0.10)**	**0.012 (0.11)**	0.61
Invertebrate diversity (Simpson)		0.40		0.84		**<0.001 (0.12)**	-	0.81	0.06	0.31	0.15
Invertebrate evenness (Simpson)		0.10		0.94		0.10		0.55	0.28	0.26	**0.030 (0.17)**
Macroinvertebrate Community Index (MCI)		0.48		0.53		**<0.001 (0.23)**	-	0.80	0.59	0.10	0.40
EPT abundance		**0.049 (0.08)**	-	0.07		**<0.001 (0.77)**	-	0.68	**0.049 (0.08)**	0.84	0.76
EPT taxon richness		0.61		0.48		**<0.001 (0.12)**	-	0.92	0.26	0.38	0.39
Common Invertebrate Taxa											
Tanitarsus spp.	36.1	0.28		**0.025 (0.09)**	+	**0.003 (0.09)**	-	0.79	0.93	1.00	**0.005 (0.21)**
Orthocladiinae	33.3	0.08		**0.002 (0.14)**	+	**<0.001 (0.43)**	-	0.54	0.15	0.10	0.05
Deleatidium spp. *(log)*	8.5	0.06		0.30		**<0.001 (0.75)**	-	0.84	0.39	0.39	0.94
Tanypodinae	7.4	0.97		**0.045 (0.08)**	+	**<0.001 (0.16)**	+	0.44	0.16	0.79	**0.046 (0.16)**
Olinga spp.	2.7	0.31		**0.005 (0.13)**	+	**0.048 (0.04)**	-	0.40	0.32	0.17	0.51
Pycnocentrodes spp.	2.4	0.12		0.65		**<0.001 (0.31)**	-	0.57	0.30	0.56	0.34
Copepoda spp.	2.1	0.44		0.10		**<0.001 (0.68)**	+	0.64	0.49	0.20	0.56
Ostracoda spp.	1.6	0.37		0.82		0.51		0.44	0.98	0.20	0.10
Psilochorema spp.	1.4	0.79		0.10		**<0.001 (0.51)**	-	0.85	0.87	0.47	0.84
Corynoneura spp.	1.2	0.22		**0.019 (0.10)**	+	**<0.001 (0.48)**	-	0.27	0.25	**0.043 (0.08)**	0.66
Fish											
Trout survival *(log)*		0.47		0.37		**0.034 (0.05)**	-	0.96	0.85	0.63	0.39
Trout condition		0.46		0.52		**0.014 (0.06)**	-	0.94	0.75	0.29	0.82

Table S8. Experiment 3 – Algal responses to DCD additions, nutrient enrichment with different N:P-ratios, and flow reductions. See Table S7 for details.

Dependent variable	%	DCD addition	Dir	Nutrient enrichment	Dir	Flow reduction	Dir	DCD × Nutrients	DCD × Flow	Nutrients × Flow	DCD × Nutrients × Flow
Algal Community Metrics											
Algal biomass (Chl-*a*)		0.08		**<0.001 (0.56)**	+	**0.047 (0.04)**	+	0.63	0.57	0.37	0.56
Algal taxon richness		**0.020 (0.10)**	+	**<0.001 (0.21)**	-	**<0.001 (0.11)**	+	0.39	0.15	**0.006 (0.12)**	0.50
Algal diversity (Simpson, *log*)		0.51		**<0.001 (0.21)**	-	**<0.001 (0.14)**	+	0.13	0.84	**<0.001 (0.31)**	0.96
Algal evenness (Simpson)		0.51		**0.006 (0.12)**	-	**0.014 (0.06)**	+	0.22	0.14	**<0.001 (0.24)**	0.92
Algal Taxanomic Groups (cell densities)											
Diatoms *(log)*	85.7	0.64		**<0.001 (0.36)**	+	**<0.001 (0.19)**	+	0.32	0.40	0.10	0.77
Non-filamentous greens *(log)*	11.1	0.17		**<0.001 (0.60)**	+	**<0.001 (0.13)**	+	0.40	0.13	**0.027 (0.09)**	0.84
Filamentous greens *(log)*	1.8	0.96		0.71		**<0.001 (0.12)**	-	**0.004 (0.22)**	0.11	**0.030 (0.09)**	0.52
Blue-green (cyanobacteria) *(log)*	1.5	0.93		0.35		0.30		0.60	0.27	**0.026 (0.09)**	0.43
Common Algal Taxa (cell densities)											
Encyonema minuta (log)	25.4	0.20		**<0.001 (0.70)**	-	**<0.001 (0.54)**	+	0.72	0.35	0.08	0.30
Gomphonema minutum (log)	24.9	0.10		0.10		**<0.001 (0.13)**	-	0.31	0.79	0.28	0.67
Scenedesmus spp. *(log)*	10.1	0.07		**<0.001 (0.62)**	+	**<0.001 (0.18)**	+	0.37	**0.020 (0.10)**	**0.005 (0.13)**	0.48
Cocconeis placentula (log)	8.5	0.83		**<0.001 (0.29)**	+	**<0.001 (0.50)**	-	0.18	0.24	0.07	0.79
Rossithidium spp. *(log)*	7.1	0.87		**<0.001 (0.29)**	+	0.36		0.34	0.14	0.60	0.85
Cymbella kappii (log)	5.1	0.26		**<0.001 (0.37)**	-	**<0.001 (0.48)**	+	0.31	0.73	0.66	0.94
Achnanthidium min. (log)	4.5	0.32		**<0.001 (0.48)**		**<0.001 (0.32)**	+	0.27	0.46	0.75	0.33
Nitzschia palea (log)	4.2	0.42		**<0.001 (0.21)**	-	**<0.001 (0.64)**	+	0.24	0.71	**0.016 (0.10)**	0.46
Fragilaria vaucheriae (log)	1.6	0.30		**0.005 (0.13)**		0.75		0.41	0.80	**0.040 (0.08)**	0.75
Aphanocapsa (log)	1.3	0.83		0.61		0.08		0.25	0.32	**0.018 (0.10)**	0.84
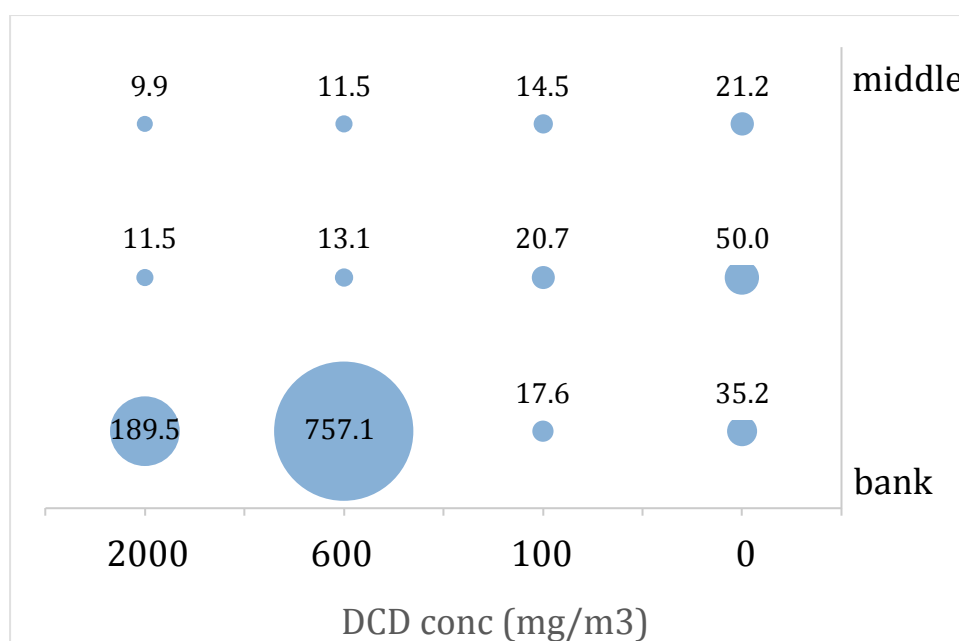
Figure S1. Nitrous oxide concentrations in chamber headspace after 24 hours (numbers are relative values; units are not defined here). Figure represents a bird's eye view of the mesocosm array, with replicates on the vertical axis and DCD concentrations (treatments) on the horizontal axis. "Bubble" sizes are proportional to nitrous oxide concentrations.