

# Multiple stressors in agricultural streams: interactions among sediment addition, nutrient enrichment and water abstraction

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## Summary

1. Many ecosystems are influenced simultaneously by multiple stressors, and the consequences of stressors are often unpredictable on the basis of knowledge of single effects. Agriculture affects streams world-wide via nutrient enrichment, elevated fine sediment and water abstraction for irrigation, but the combined impacts of these stressors are unknown.

2. We manipulated all three stressors simultaneously in an 18-day experiment and determined their individual and pair-wise combined effects on benthic invertebrates, algal biomass and leaf decay. We added nutrients (phosphorus plus nitrogen) and/or fine sediment (grain size 0.2 mm) to 18 experimental stream channels (dimensions 250 × 15 × 15 cm) supplied with water from a nearby stream. Three sediment and three nutrient treatments (high, intermediate, natural) were applied to each of six channels while flow was reduced by 80% in half the channels. Invertebrates (composition, abundance) and algae (chlorophyll a) were assayed using ceramic tile substrata and leaf decay was assayed using bundled leaves of a native shrub. Invertebrates colonizing leaf packs were also sampled.

3. Effects of sediment addition and flow reduction on biological response parameters were twice as common as nutrient enrichment effects. Nutrient enrichment increased total invertebrate abundance on tiles, algal biomass accrual and leaf decay rates, whereas both sediment addition (at the highest level) and flow reduction had mostly negative effects (e.g. reduced algal biomass, invertebrate abundance and/or taxonomic richness).

4. Stressors interacted often, and interactions between sediment and flow were particularly common. The negative impact of added sediment on aquatic biota was stronger at reduced flow, especially on tile substrata that were more exposed to the current than leaf-pack substrata.

5. *Synthesis and applications.* Our key findings imply that abstracting water from a stream already subjected to high fine sediment inputs may have far worse effects on the invertebrate fauna than abstraction from a similar stream with lower sediment levels. Aquatic resource managers should be aware of this important interaction between multiple stressors.

**Key-words:** agriculture, decomposition, flow, land use, nitrate, phosphate, species richness

## Introduction

A stressor can be defined as a variable that, as a result of human activity, exceeds its range of normal variation and adversely affects individual taxa, community composition or ecosystem functioning (Townsend, Uhlmann & Matthaei 2008). Many ecosystems are influenced simultaneously by multiple stressors (Munns 2006; Niyogi *et al.* 2007; Couillard *et al.* 2008), and the consequences of stressors are often unpredictable on the basis of knowledge of single effects (Preston 2002;

Townsend *et al.* 2008). Therefore, if managers of natural resources only consider the effects of individual stressors, their assessment of risk may be higher or lower than reality. Consequently, calls to move beyond single-stressor studies to a more holistic, multiple-stressor approach have come from areas including wildlife (Munns 2006), river (Allan 2004), coastal and marine management (Couillard *et al.* 2008).

River water quality world-wide has been reduced by inputs of nutrients, fine sediment (generally defined as inorganic particles < 2 mm in diameter; Zweig & Rabeni 2001), pesticides and pathogens associated with intensive agriculture (Allan 2004), deforestation (Naymik & Pan 2005), urbanization

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(Taylor *et al.* 2004) and mining (Bruns 2005). In New Zealand and other developed countries, agriculture has been implicated as the single largest cause of water pollution (Soranno *et al.* 1996; Davies-Colley *et al.* 2004), mainly via non-point sources (Monaghan *et al.* 2007). Large areas of native grassland in southern New Zealand have been converted to agricultural pasture, a conversion that increases inputs of nutrients (mainly nitrogen and phosphorus) and fine sediment to streams (Townsend *et al.* 2008). Further, the degree of enrichment or sediment addition differs among pastoral practices, with deer farming resulting in high levels of sediment and intermediate nutrient enrichment while dairying causes high enrichment and intermediate sediment levels (Matthaei *et al.* 2006). Another increasingly important stressor of streams and rivers world-wide is water abstraction for irrigation (Malmqvist & Rundle 2002; Dewson, James & Death 2007). Abstraction is especially relevant in drier parts of the world, including most of the western USA, southern Europe, northern Africa, central Asia and Australia, and in, New Zealand, the regions in the eastern rain shadow of the Southern Alps.

Because anthropogenic stressors usually operate in concert (see references above), resource managers need to understand how multiple stressors interact. Nevertheless, such studies are rare in streams, possibly due to the challenges involved in manipulating more than one stressor in a realistic experimental setting. In our previous research, we used surveys to reveal that nutrients, sediment (Townsend *et al.* 2008) and flow reduction (Leprieur *et al.* 2006) are key stressors in grassland streams. We have also used a reach-scale experiment to study the joint effects of nutrients and sediment in a set of real streams (Townsend *et al.* 2008). However, reach-scale experiments will usually be inadequate for simultaneous studies of three or more stressors. Consequently, we used experimental stream channels supplied with water from a nearby stream to unravel the interactions between nutrient enrichment, sediment augmentation and flow reduction in the present experiment.

We focus on the responses of key components of stream ecosystems (aquatic invertebrates, algae and organic matter decomposition) to nutrient enrichment, addition of fine sediment and flow reduction (simulated water abstraction). To our knowledge, this is the first time these stressors have been manipulated simultaneously. Based on three recent reviews (Dewson *et al.* 2007; Gruner *et al.* 2008; Young, Matthaei & Townsend 2008), we predicted that individual effects of nutrient enrichment on biological response parameters would generally be positive (e.g. increased invertebrate density, richness or algal biomass), but sediment addition and flow reduction would have generally negative consequences. Of more interest here are possible interactions between stressors, which are largely unknown (Dewson *et al.* 2007). Nevertheless, we predicted that

1. negative effects of sediment addition would be stronger at reduced flow (because more sediment gets deposited on the stream bed instead of being washed away),
2. interactions between flow reduction and sediment addition would be more common on exposed substrata (tiles rather than leaf packs) because normal flow should remove more sediment from exposed substrata, and

3. based on earlier results from real streams (Townsend *et al.* 2008), the effects of nutrient enrichment should change from positive to negative at high sediment levels whereas the effects of sediment addition should not be affected by nutrient levels, because sediment addition should be the main driver of this interaction.

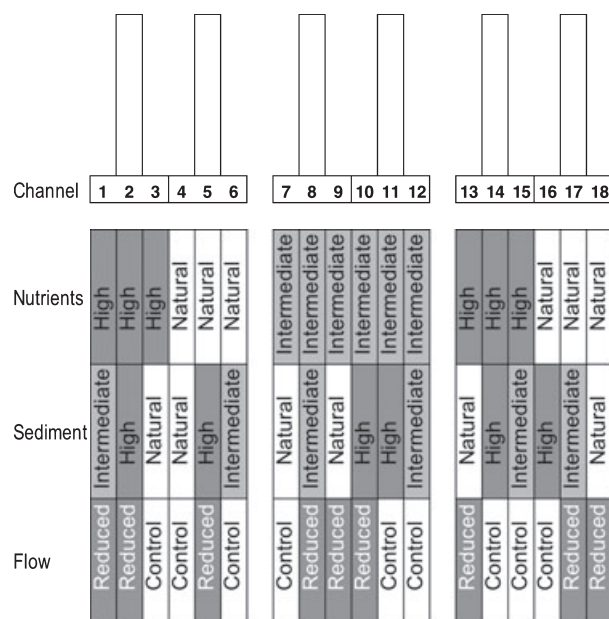
## Materials and methods

### STUDY SITE

Our study was conducted during austral spring from 26 September to 30 October 2006 in experimental stream channels installed on the floodplain of the Kauru River, a third-order river in the Otago province of New Zealand (170°44'6" East, 45°6'5" South, 98 m a. s. l.). The Kauru catchment (124 km<sup>2</sup>) lies in the rain shadow of the Southern Alps, ranges from 55 to 1273 m a. s. l. and receives an annual rainfall of 817 mm [Otago Regional Council (ORC)]. The vegetation in the catchment consists predominantly of native tussock grass and exotic pasture. Land use is mainly sheep grazing at low stock densities (0–3 animals per hectare). The river water is relatively nutrient-poor (see non-enriched nutrient values below) but contains diverse and abundant algal (Liess *et al.* 2009) and invertebrate communities (Herrmann 2009). Mean annual discharge 300 m upstream of our site is 1.29 m<sup>3</sup> s<sup>-1</sup> (ORC).

### EXPERIMENTAL DESIGN

We manipulated sediment, nutrients and flow in 18 stream channels using 3 nutrient levels × 3 sediment levels × 2 flow treatments in a factorial design (Fig. 1). Six sets of three channels (dimensions 250 × 15 × 15 cm) made of steel sheet were installed on a flat gravel area between two arms of the river. River water was supplied through PVC drainage pipes (Humes Pipeline Systems, Dunedin, New Zealand; diameter 15 cm) and adjustable inflow and outflow weirs



**Fig. 1.** Schematic drawing of the stream-channel setup and experimental levels of the factors nutrients, sediment and flow in each of the 18 channels. River water was supplied to channel triplets via six pipes from a run about 50 m upstream. For more details see text.

equalized flow and water depth across the nine channels in each flow treatment. To prevent clogging, a 50-mm mesh fence was erected upstream of the intake and pipe inflows were cleaned every 3 days.

Channels were filled with 3 cm of mixed gravels (mainly 16–64 mm width) taken from a nearby dry riverside channel. The size range of these gravels was similar to the substratum composition in second- and third-order pasture streams in the Otago region (Townsend *et al.* 2008). Flow began on 26 September, and flow rates and water depths were standardized across all channels, which were left to be colonized by drifting algae and invertebrates for 14 days. This period sufficed to cover the substratum surfaces in the channels with visible biofilms that were similar to those in the nearby river.

On 9 October, natural colonization in each channel was assisted by adding one standard load of invertebrates, obtained from the nearby riverbed by kick-net sampling for 3 min (frame 60 × 40 cm; mesh size 200 µm) from a bed patch of about 0.36 m<sup>2</sup> (comparable with the 0.38 m<sup>2</sup> channel surface area). Samples were collected sequentially from downstream to upstream in a uniform area of riverbed and assigned randomly to channels.

On 10 October (day 0), the three nutrient treatments were assigned randomly within two blocks of three pipes each, with each pipe feeding three channels (Fig. 1). The two nutrient blocks were included as a block factor in the statistical analysis. Nutrients (nitrate and phosphate as NaNO<sub>3</sub> and KH<sub>2</sub>PO<sub>4</sub>) were enriched continuously for 18 days using battery-driven fluid-metering pumps (model QBG; Fluid Metering Inc., Syosset, NY, USA). Six channels each were enriched to levels normally found in Otago in deer farming streams [intermediate; means achieved, measured on days 5, 10 and 15, of 101 ± 8 (SE; *n* = 36) µg L<sup>-1</sup> for nitrate-N and 52 ± 4 µg L<sup>-1</sup> for phosphate-P] or dairy farming streams (high; 563 ± 44 µg L<sup>-1</sup> for N and 263 ± 21 µg L<sup>-1</sup> for P; Townsend *et al.* 2008). The remaining six channels were not enriched (natural; means of 14 ± 0.3 µg L<sup>-1</sup> for N and 3 ± 0.3 µg L<sup>-1</sup> for P).

Fine sediment (mean grain size 0.2 mm, similar to the size of naturally occurring fine sediment in pasture and dairy farming streams in Otago; see Matthaei *et al.* 2006) was added on day 0, resulting in sediment values occurring in Otago dairy (intermediate; six channels: means achieved of 66 ± 3% cover of stream bed and 5.0 ± 0.4 mm sediment depth; *n* = 54) or deer farming streams (high; six channels: means 80 ± 4% cover and 13.5 ± 1.2 mm depth; Townsend *et al.* 2008). Six channels had no sediment added (natural: 7 ± 1% cover, 0.3 ± 0.04 mm depth). The added sediment settled out quickly on the channel bed surfaces and water turbidity was similarly low in all channels during the whole manipulative period.

Stream flow was reduced in half the channels (mean flows achieved, measured on days 0, 5, 10 and 15, of 2.10 ± 0.12 L s<sup>-1</sup> for normal flow and 0.43 ± 0.02 L s<sup>-1</sup> for reduced flow; *n* = 36). This reduction was based on recent water abstraction studies in New Zealand (Leprieur *et al.* 2006; Dewson *et al.* 2007). Nutrient, sediment and flow measures were all significantly different among the corresponding experimental treatments (nested repeated-measures ANOVAS: *P* ≤ 0.002, Tukey HSD tests: *P* ≤ 0.01). The reduced-flow treatment was associated with significant reductions to average mid-channel flow velocity (from 15.5 ± 0.9 to 5.3 ± 0.5 cm s<sup>-1</sup>) and channel water depth (from 7.6 ± 0.3 to 4.8 ± 0.2 cm), and increases to sediment cover (from 40 ± 5% to 62 ± 5% in the high sediment treatment) and sediment depth (from 4.8 ± 0.9 to 7.7 ± 1.1 mm).

#### BIOLOGICAL RESPONSE PARAMETERS

On day -1, we introduced three terracotta tiles (10 × 10 × 1.4 cm) and three leaf packs per channel, one each in the upstream, middle

and downstream thirds. As a bioassay for organic matter decay, we used 10 g (wet weight; 10–15 fresh leaves bolted together) of the fast-decomposing, native shrub mahoe *Meliclytus ramiflorus* Forst & Forst, held in place by surface stones.

On day 18, tiles and leaf packs were sampled by lifting quickly into a hand net (frame 20 × 15 cm; mesh size 250 µm) and transferring the contents into sealable plastic bags. In channels that contained fine sediment, where tiles and leaf packs were beneath the sediment, the associated sediment was also taken. Samples were placed on ice in the dark, frozen in the laboratory the same day and stored at -18 °C until processing.

#### LABORATORY WORK

Sediment and debris were washed gently with ultrapure water from each tile after thawing. Invertebrates were retrieved in a sieve (mesh size 200 µm) and stored in 70% ethanol. Epilithic algae were scrubbed from the entire tile using a toothbrush and rinsed with ultrapure water into a measuring cylinder, topped up to 50 mL and processed to determine algal biomass as chlorophyll *a* using standard methods (APHA 1998; Biggs & Kilroy 2000).

Leaf packs were defrosted and rinsed, and associated invertebrates were preserved. Remaining leaf biomass in each pack on day 18 was determined as ash-free dry mass using standard methods (APHA 1998) and expressed as a percentage of the average of 10 leaf packs dried and ashed after initial weighing on day -2. Leaf strength was determined as the weight required to force a blunt metal pin through the leaf (Young 1992). One measure was made per leaf (randomly but avoiding veins) for five randomly selected leaves per pack. Leaf strength on day 18 was expressed as percentage of the averaged strength of 25 fresh leaves determined directly after leaf collection on day -2. Benthic invertebrates from tile and leaf-pack samples were identified to the lowest practical taxonomic level, usually genus or family, using a dissecting microscope (Olympus SZ51, 8–40×, Japan).

#### DATA ANALYSIS

All ANOVA analyses were conducted in SPSS version 15.0 (SPSS Inc., IL, Chicago, USA), and data were log-transformed where necessary to improve normality and homoscedasticity. Nested ANOVAS were used to analyse algal biomass, leaf mass and strength loss, and invertebrate community parameters [total abundance, taxon richness and mayfly, caddisfly and stonefly (EPT) taxon richness] in tile and leaf-pack samples (*n* = 3 per channel). Nutrients, sediment and flow were the fixed main factors in the ANOVA, and block (the two nutrient blocks) was a fixed factor (see Quinn & Keough 2002) without interaction terms. Sample (positions 1–3 in each channel) was a fixed (rather than a random) nested factor (Quinn & Keough 2002) because, based on the findings of previous sediment additions to streams (see 'Introduction'), we expected the proportion of sediment retained to increase with distance down the channel since current velocity was fastest at the channel inflows (position 1).

The model of this ANOVA was intercept (d.f. 1) + nutrients (2) + sediment (2) + flow (1) + nutrients × sediment (4) + nutrients × flow (2) + sediment × flow (2) + sample(nutrient) (6) + sample (sediment) (4) + sample(flow) (2) + block (1) + error (27; *n* = 54). We selected the Type I (sequential) sums of squares, the appropriate method for analysing this type of nested design in SPSS (Field 2005; Garson 2009). Note that this model does not include a term testing three-way interactions because there was just one channel replicate of each three-factor treatment combination, so this interaction term could not be separated from the residual error (Quinn & Keough

2002). This limitation of our model implies that our analysis is likely to somewhat underestimate the actual frequency of significant single-factor effects and two-way interactions in our data (Garson 2009).

To assess effects on invertebrates, we first performed exploratory non-metric multidimensional scaling (NMDS) based on Bray–Curtis distances using the software PRIMER<sup>®</sup> version 5.2.9 (PRIMER-E Ltd, Ivybridge, UK) on log-transformed densities of 12 taxa representing 99% of all individuals in both tile and leaf-pack samples. We then used the same data to carry out five-way, nested MANOVAs (with the multivariate equivalent of the model used for the invertebrate community parameters). Finally, we examined the between-subjects effects in the MANOVA for the eight most consistently common taxa in each sample type (see below).

If between-subjects effects were significant, pairwise comparisons were performed for the factors nutrients and sediment using *post hoc* tests (Tukey's HSD or, in cases of persisting heteroscedasticity, Games–Howell). For the sake of brevity, the results for the nested factor sample are not presented in tabular form. Including this factor enabled us to quantify within-channel variation and improve overall predictive power, but significant sample effects merely indicate that response variable values differed between channel thirds across the 18 channels. This is irrelevant to our research objectives. For the same reason, the results for the block factor are not presented either.

Significance level for all tests was  $P < 0.05$ . We present standardized effect sizes for all significant findings (partial  $\eta^2$  values, range 0–1; Garson 2009) to allow readers to evaluate the biological importance of each individual result (see Tables 1 and 2) and the overall findings (Table 4; Nakagawa 2004).

## Results

### ALGAL ACCRUAL AND LEAF DECOMPOSITION

Algal biomass accrual (determined as chlorophyll *a*) on tile substrata was significantly lower in channels without nutrient enrichment than in highly enriched channels (Table 1, Fig. 2) and declined with sediment addition and reduced flow. The effects of sediment and flow on algal biomass interacted, with the reduction in biomass with increasing sediment levels more marked at reduced than at normal flow (Fig. 2).

Leaf packs lost more biomass in intermediately or highly enriched channels and in channels with high sediment addition or reduced flow (Table 2, Fig. 2). The effects of nutrients and sediment interacted, with the positive effect of nutrient enrichment on mass loss being strongest and most consistent at high sediment levels. Leaf strength loss showed the same patterns as leaf mass loss for sediment and flow but was unaffected by nutrient enrichment and showed no interaction between nutrients and sediment (Table 2).

### INVERTEBRATES

On tiles, total invertebrate abundance was lower in channels without nutrient enrichment than in highly enriched channels (Table 1, Fig. 2) and in channels with reduced flow. Sediment addition affected total abundance via two-way interactions with both flow and nutrients ( $P = 0.05$  for the latter interaction). Thus, total abundance increased with rising sediment levels at normal flow whereas the opposite pattern occurred at

**Table 1.** Summary ( $P$ -values) of (M)ANOVAS comparing algal and invertebrate response parameters in tile samples between nutrient, sediment and flow treatments

Dependent variable	Nutrients	Ranking	Sediment	Ranking	Flow	Ranking	Nutrients × sediment	Nutrients × flow	Sediment × flow
Algal biomass	<b>0.04</b> (0.24)	N < H	< <b>0.0001</b> (0.71)	N > I > H	<b>0.002</b> (0.29)	Norm > Red	0.30	0.10	< <b>0.0001</b> (0.44)
Total invertebrate density	<b>0.04</b> (0.22)	N < H	0.28		<b>0.001</b> (0.32)	Norm > Red	<b>0.05</b> (0.29)	0.11	<b>0.004</b> (0.33)
Taxon richness	0.96		0.27	I > H	0.44		0.66	0.12	0.06
EPT richness	0.55		<b>0.03</b> (0.23)		0.08		0.17	0.62	<b>0.001</b> (0.43)
Community composition (MANOVA; 12 taxa)	0.24		<b>0.002</b> (0.67)		<b>0.03</b> (0.67)		0.19	0.13	< <b>0.0001</b> (0.71)
<i>Deleatidium</i> spp.	0.79		<b>0.02</b> [0.25]	I > H	< <b>0.0001</b> (0.37)	Norm > Red	0.80	0.95	<b>0.048</b> (0.20)
Chironomidae	<b>0.008</b> (0.30)	I < H	0.12		0.08		0.41	0.43	< <b>0.0001</b> (0.45)
Tanypodinae	0.54		0.33		0.82		0.78	0.42	<b>0.005</b> (0.32)
<i>Pycnocentroides</i> spp.	0.70		<b>0.001</b> (0.38)	N > H	<b>0.002</b> (0.29)	Norm > Red	0.24	0.63	<b>0.02</b> (0.25)
<i>Oxyethira</i> spp.	0.19		0.30		0.94		0.86	0.12	<b>0.03</b> (0.23)
<i>Austrosimulium</i> spp.	0.16		<b>0.03</b> (0.22)	N > I	< <b>0.0001</b> (0.45)	Norm > Red	<b>0.04</b> (0.31)	0.16	<b>0.006</b> (0.31)
Copepoda	0.78		0.74		< <b>0.0001</b> (0.37)	Norm < Red	0.80	0.51	0.57
Nematoda	0.23		0.34		0.32		0.95	<b>0.02</b> (0.25)	0.39

MANOVA  $P$ -values are for the Pillai's Trace statistic. Rankings for *post hoc* tests in cases with significant between-subjects effects are given in columns 3, 5 and 7. Nutrient and sediment treatments: N, natural; I, intermediate; H, high. Flow: Norm, normal; Red, reduced. Significant  $P$ -values (with effect sizes in parentheses) are indicated in bold print.

**Table 2.** Summary (*P*-values) of (M)ANOVAs comparing leaf decay rates and invertebrate parameters in leaf-pack samples between nutrient, sediment and flow treatments

Dependent variable	Nutrients	Ranking	Sediment	Ranking	Flow	Ranking	Nutrients × sediment	Nutrients × flow	Sediment × flow
Leaf mass loss (%)	< <b>0.0001</b> (0.51)	N < (I = H)	< <b>0.0001</b> (0.74)	(N = D) < H	< <b>0.0001</b> (0.42)	Norm < Red	< <b>0.0001</b> (0.57)	0.66	0.44
Leaf strength loss (%)	0.37		<b>0.003</b> (0.35)	(N = D) < H	<b>0.006</b> (0.25)	Norm < Red	0.08	0.99	0.62
Total invertebrate density	0.43		<b>0.02</b> (0.25)	N > H	< <b>0.0001</b> (0.37)	Norm > Red	0.07	0.38	0.07
Taxon richness	0.27		<b>0.002</b> (0.37)	(N = D) > H	0.47		0.19	0.42	0.45
EPT richness	0.54		< <b>0.0001</b> (0.50)	(N = D) > H	<b>0.02</b> (0.19)	Norm > Red	0.19	<b>0.02</b> (0.25)	0.43
Community composition (MANOVA; 12 taxa)	<b>0.03</b> (0.59)		< <b>0.0001</b> (0.75)		<b>0.001</b> (0.82)		0.23	0.23	0.34
<i>Deleatidium</i> spp.	<b>0.01</b> (0.28)	N > (I = H)	<b>0.001</b> (0.40)	(N = D) > H	< <b>0.0001</b> (0.73)	Norm > Red	< <b>0.0001</b> (0.54)	0.86	0.55
Chironomidae	0.11		0.53		< <b>0.0001</b> (0.38)	Norm > Red	0.19	0.60	0.47
Tanypodinae	<b>0.03</b> (0.22)	N < H	0.16		0.89		0.46	<b>0.047</b> (0.20)	<b>0.001</b> (0.39)
<i>Oxyethira</i> spp.	0.16		0.30		0.52		0.73	0.23	0.35
<i>Potamopyrgus antipodarum</i>	<b>0.005</b> (0.33)	N > (I = H)	<b>0.05</b> (0.20)	I > H	0.17		0.17	0.63	0.10
<i>Austrosimulium</i> spp.	0.10		<b>0.003</b> (0.34)	N > (I = H)	< <b>0.0001</b> (0.40)	Norm > Red	0.67	0.55	<b>0.03</b> (0.22)
Hydrobiosidae	0.84		0.14		< <b>0.0001</b> (0.54)	Norm > Red	0.11	0.34	0.23
Copepoda	0.08		<b>0.03</b> (0.24)	I > H	0.14		0.07	<b>0.01</b> (0.27)	<b>0.001</b> (0.42)

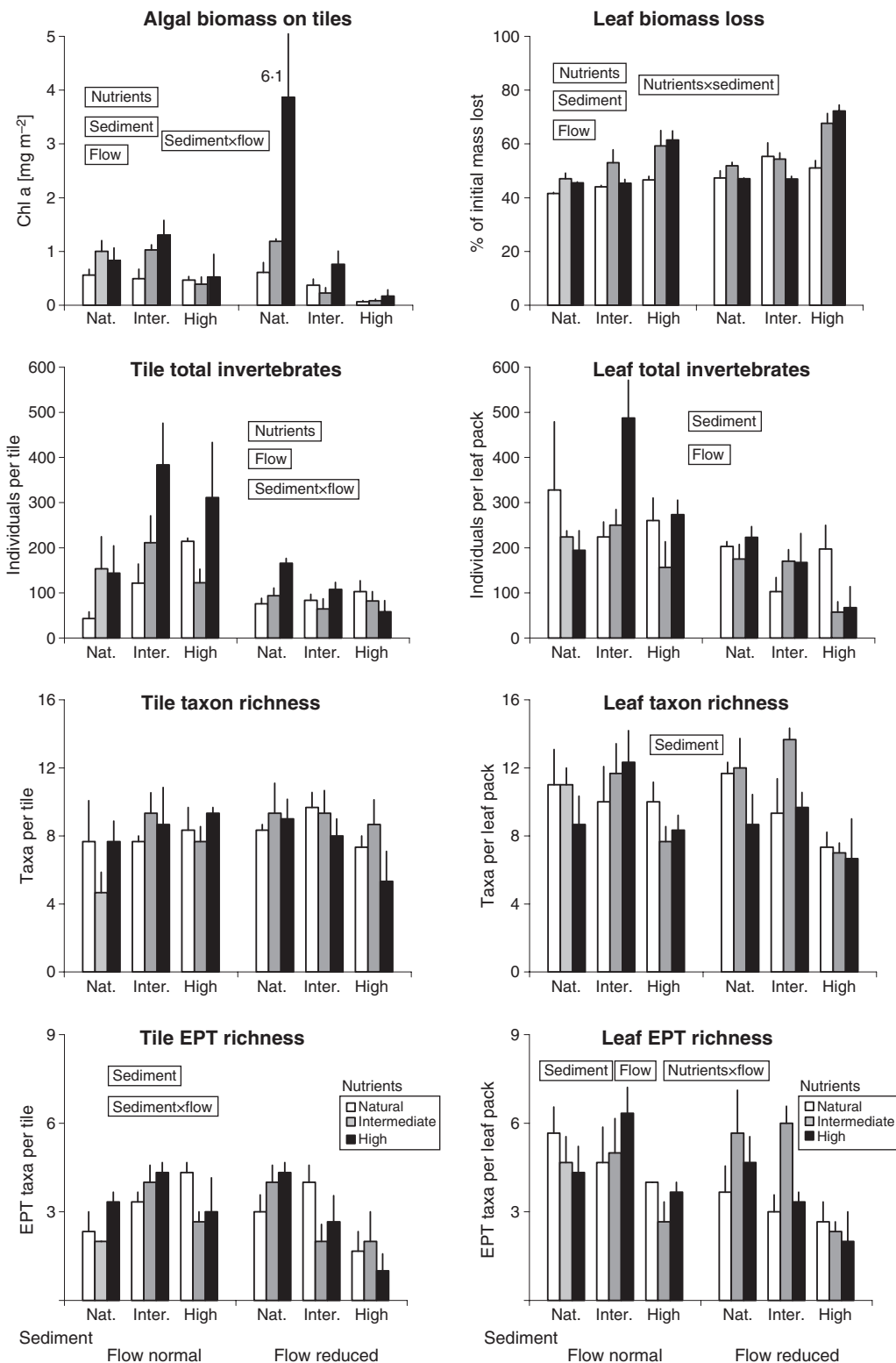
Rankings for *post hoc* tests in cases with significant between-subjects effects are given in columns 3, 5 and 7. For abbreviations and further details, see Table 1. Significant *P*-values (with effect sizes in parentheses) are indicated in bold print.

reduced flow. Further, abundance increased with rising nutrient levels at natural and intermediate sediment levels, but not at high sediment levels. In leaf packs, total abundance showed the same pattern for flow (higher at normal flow) as in tile samples (Table 2, Fig. 2), but was affected neither by nutrient enrichment nor by interactions between experimental factors. Leaf packs in channels without added sediment contained more invertebrates than those in channels with high addition.

Total taxon richness on tiles was not influenced by the manipulations (Table 1), but richness in leaf packs was lower in channels with high sediment addition (Table 2, Fig. 2). EPT richness on tiles was greater at intermediate sediment levels than at high levels. Further, flow affected EPT richness via an interaction with sediment addition. At normal flow, EPT richness increased with sediment addition until intermediate levels and declined somewhat at high levels. At reduced flow, in contrast, EPT richness declined steadily with rising sediment levels. In leaf packs, EPT richness was affected in the same way by sediment addition as total taxon richness, was greater at normal flow and was affected by nutrient enrichment via an interaction with flow: at reduced flow, EPT richness was highest at intermediate nutrient levels, whereas no consistent effect of enrichment on EPT richness was apparent at normal flow (Table 2, Fig. 2).

Non-metric multidimensional scaling plots with acceptable stress values indicated that invertebrate community compositions were distinct from each other in the sediment treatments and in the flow treatments but were similar across the nutrient treatments (Fig. 3). This result corresponded quite well with the more complex MANOVA that included interactions between the three factors plus the block factor and the nested term sample. For the tile data, the MANOVA showed community composition was unaffected by nutrient enrichment but differed across sediment and flow treatments, and that the sediment and flow interacted (Table 1). Leaf-pack communities were affected by sediment and flow (here without interaction; Table 2), and by nutrients (in contrast to the NMDS plots).

To identify which invertebrate taxa were driving the overall multivariate effects, we examined the between-subjects effects in the MANOVA for the eight taxa that occurred in at least 50% of all tile or leaf-pack samples (Table 3). On tiles (Table 1), nutrient enrichment influenced only Chironomidae (excluding Tanypodinae), which were less common in intermediately enriched than in highly enriched channels. Sediment addition affected the mayfly *Deleatidium* spp. (rarer at high than at intermediate sediment levels), the caddis fly *Pycnocentroides* spp. (rarer at high than at natural levels), and the black fly *Austrosimulium* spp. (larvae plus pupae; rarer at intermediate than at natural levels). These three taxa were also influenced by flow manipulation, all with lower densities at reduced than at normal flow. Copepoda were also affected by flow but showed the opposite pattern. In leaf packs (Table 2), nutrient enrichment influenced *Deleatidium*, the snail *Potamopyrgus antipodarum* Gray (both most abundant at natural nutrient levels) and the chironomid subfamily Tanypodinae (rarer at natural than at high levels). In addition, *Deleatidium* and *Potamopyrgus* (*P* = 0.05 for the latter) were affected by sediment

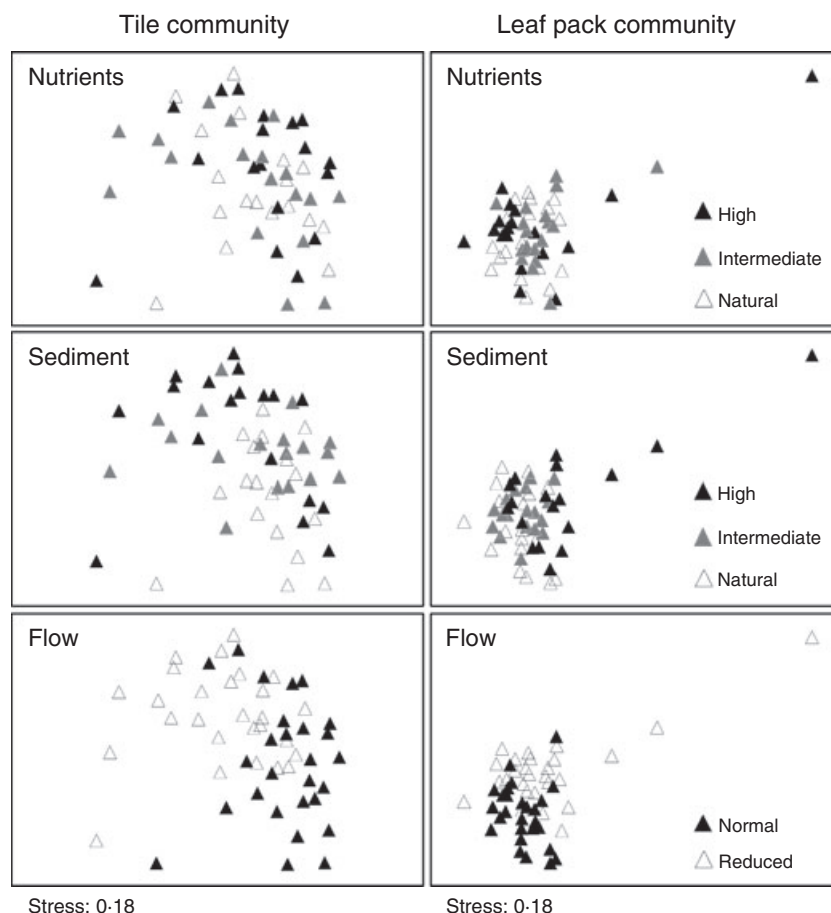


**Fig. 2.** Averages of (left) algal biomass and invertebrate community parameters in tile samples and (right) leaf mass loss and invertebrate community parameters in leaf-pack samples across the experimental treatments on day 18. Error bars (SEs) show the within-channel variation ( $n = 3$ ) that is represented by the factor 'sample' in the statistical analysis (see text). Text in rectangles indicates significant single-factor effects or interactions. Some errors are too small to be visible.

addition (the former rarest at high sediment, and the latter rarer at high than intermediate sediment). Sediment addition also influenced *Austrosimulium* (most common at natural lev-

els) and Copepoda (rarer at high than at intermediate levels). Flow manipulation affected four leaf-pack taxa, all showing lower densities at reduced than at normal flow.

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**Fig. 3.** Invertebrate community compositions determined by non-metric multidimensional scaling for (left) tile samples and (right) leaf-pack samples on day 18. The top plots show the patterns across the three nutrient levels, the middle plots those across the three sediment levels and the bottom plots those across the two flow treatments. For more details see text.

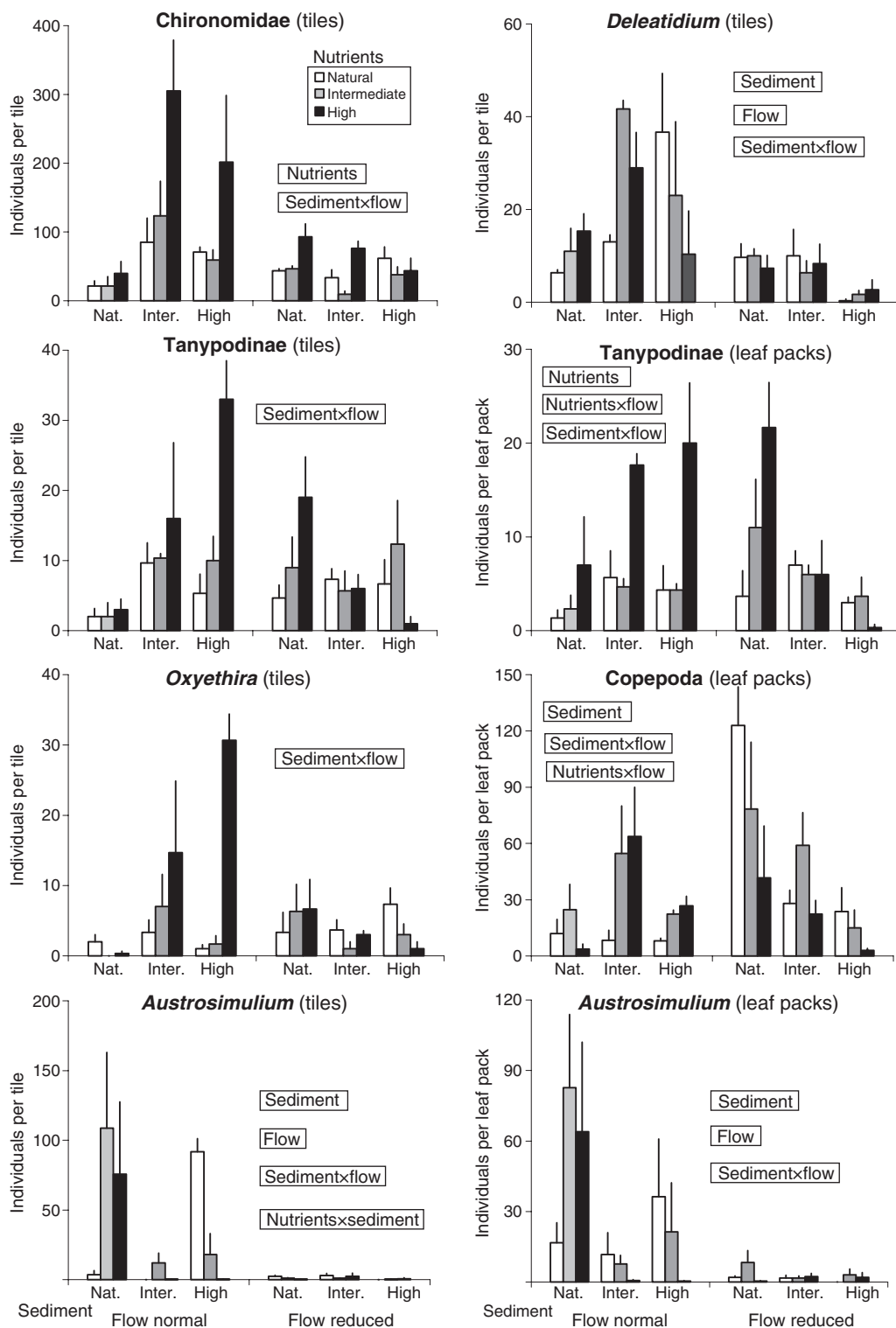
**Table 3.** Invertebrate taxa in tile and leaf-pack samples that were included in the NMDS and MANOVA analyses of invertebrate community composition

Taxon (tile samples)	% of total counted	In % of samples	Taxon (leaf-pack samples)	% of total counted	In % of samples
<b>Chironomidae</b>	<b>54.0</b>	<b>98.1</b>	<b>Chironomidae</b>	<b>54.8</b>	<b>100.0</b>
<b>Austrosimulium spp.</b>	<b>12.6</b>	<b>61.1</b>	<b>Copepoda</b>	<b>16.4</b>	<b>90.7</b>
<b>Deleatidium spp.</b>	<b>9.6</b>	<b>88.9</b>	<b>Deleatidium spp.</b>	<b>9.9</b>	<b>98.1</b>
<b>Tanypodinae</b>	<b>6.4</b>	<b>83.3</b>	<b>Austrosimulium spp.</b>	<b>7.0</b>	<b>57.4</b>
<b>Copepoda</b>	<b>5.4</b>	<b>74.1</b>	<b>Tanypodinae</b>	<b>3.4</b>	<b>83.3</b>
<b>Oxyethira spp.</b>	<b>3.8</b>	<b>72.2</b>	<b>Oxyethira spp.</b>	<b>1.6</b>	<b>74.1</b>
<b>Nematoda</b>	<b>3.3</b>	<b>64.8</b>	<b>Potamopyrgus antipodarum</b>	<b>1.6</b>	<b>50.0</b>
<b>Pycnocentroides spp.</b>	<b>2.2</b>	<b>74.1</b>	Ostracoda	1.1	37.0
Ostracoda	0.6	31.5	<b>Hydrobiosidae</b>	<b>1.0</b>	<b>66.7</b>
<i>Hydora</i> spp.	0.6	27.8	Nematoda	0.9	48.1
<i>Potamopyrgus antipodarum</i>	0.5	29.6	<i>Paraoxyethira</i> spp.	0.8	44.4
Hydrobiosidae	0.3	22.2	<i>Pycnocentroides</i> spp.	0.4	44.4
All 12 taxa combined	99.2			98.9	
<b>Taxa in bold print</b>	<b>97.3</b>			<b>95.7</b>	

For each sample type, taxa printed in bold occurred in at least half of all samples. Abundance patterns of these taxa were examined individually (see text).

Interactions between sediment and flow influenced six of the eight common tile taxa (Table 1) and three of the leaf-pack taxa (Table 2). In six of these nine cases, taxa were most abundant at intermediate sediment levels when flow was normal but

decreased steadily with rising sediment levels at reduced flow (Fig. 4). *Austrosimulium* in tile and leaf-pack samples was most common in channels without added sediment at normal flow but uniformly rare across sediment treatments at reduced flow



**Fig. 4.** Density patterns (averages + SEs) for common invertebrate taxa on day 18 in tile or leaf-pack samples that showed significant interactions between flow reduction and sediment addition. Note that the shape of these interactions is similar in the first six plots (see text). For more details see Fig. 2.

(Fig. 4). *Pycnocentroides* numbers on tiles at normal flow decreased only at the highest sediment level, whereas this decrease occurred already at the intermediate sediment level when flow was reduced. Nutrients and flow had interactive effects on just one tile taxon and two leaf-pack taxa while the

effects of nutrients and sediment interacted in one case each in tile and leaf-pack samples (Tables 1 and 2; Figs 4 and 5). Abundance patterns of a single common taxon, the caddis fly *Oxyethira* spp., were not affected at all by the experimental manipulations in either tile or leaf-pack samples.



## Discussion

### SINGLE-STRESSOR EFFECTS

Our predictions about single-stressor effects were mainly supported by our findings. As expected, nutrient enrichment increased total invertebrate abundance (on tiles), algal biomass accrual and leaf decay rates, in agreement with previous studies of nutrient effects (see reviews by Gruner *et al.* 2008 for invertebrates, Biggs 1996 for algae and Young *et al.* 2008 for leaf decay). Sediment addition at the highest level had a negative impact on most of the affected response parameters (algal biomass, invertebrate community parameters and abundances of several common individual taxa), again in agreement with previous work (Matthaei *et al.* 2006; Niyogi *et al.* 2007; Townsend *et al.* 2008). Our final single-stressor hypothesis was also supported, with flow reduction causing decreases in several invertebrate community parameters and changing invertebrate community composition (as indicated by the MANOVAS) by decreasing the abundances of several common invertebrate taxa in both tile and leaf-pack habitats.

A notable exception to our predicted response to sediment, and running counter to most of the published literature (see Young *et al.* 2008), was that sediment addition actually increased leaf decay rates. This puzzling result has been repeated in other reach-scale and stream-channel experiments (C. D. Matthaei, J. J. Piggott, A. Wagenhoff, C. R. Townsend, unpublished data) and deserves further study.

Further notable exceptions to the predicted responses occurred in relation to flow reduction. In contrast to most previous research (see Dewson *et al.* 2007), algal biomass accrual (overall) decreased at reduced flow. At lower flow velocities, we expected an increase in algal biomass because fast currents can limit periphyton accrual via high shear stress at the substratum surface (Biggs 1996). However, in comparison with several of the studies reviewed by Dewson *et al.* (2007), current velocities were already relatively moderate in our normal-flow channels (16 cm s<sup>-1</sup>), reducing further in low-flow channels (5 cm s<sup>-1</sup>). We expected that leaf decay rate would be minimally affected by reduced flow (Young *et al.* 2008) but, in fact, decay rate increased substantially. This reflects the stimulatory

effect of sediment on decay and the fact that flow reduction resulted in greater sediment retention.

Our finding that sediment effects were twice as common as nutrient effects adds to accumulating evidence that sediment input is generally more deleterious to the health of grassland streams than augmented nutrient concentrations (Niyogi *et al.* 2007; Townsend *et al.* 2008). A more novel finding is that flow reduction affected biological response parameters as often as sediment addition (Table 4), indicating that this factor may also strongly influence stream ecosystems.

### MULTIPLE-STRESSOR EFFECTS: INTERACTIONS BETWEEN FLOW AND SEDIMENT

The interaction of flow reduction with fine sediment addition was the most pervasive multiple-stressor interaction in our experiment. We aware of no published experiments that manipulated these two stressors and could therefore be compared with our study. We predicted that negative effects of sediment addition (e.g. reduced algal biomass, invertebrate density or taxonomic richness) would be stronger at reduced flow, and 11 of the 13 significant interactions between sediment and flow showed this synergistic pattern. The exception was *Austrosimulium* (on tiles and leaf packs), which was commonest in channels without sediment addition at normal flow but uniformly rare at reduced flow. However, this taxon, with its preference for fast current (Mackay 1992), was so rare in the reduced-flow channels that marked differences across sediment treatments were impossible.

It is notable that at normal flow, sediment augmentation to intermediate levels had positive or neutral effects on 9 of the 11 invertebrate parameters showing interactive effects of flow and sediment. It seems that added fine sediment provided additional habitat for many taxa, including those known to prefer fine sediment such as Chironomidae (Angradi 1999; Kreutzweiser, Capell & Good 2005) as well as several used as bioindicators of organic pollution in New Zealand, such as *Deleatidium* and *Pycnocentroides* (Stark 1998). Nevertheless, all positive or neutral effects of sediment addition were offset by strong negative effects on invertebrate abundance or richness at reduced flow, resulting in an overall negative (four cases) or neutral (five cases) effect of sediment addition on

**Table 4.** Number of significant effects (plus mean effect sizes  $\pm$  SE) of single factors and interactions between factors (including two cases with  $P = 0.05$ ) on the biological response parameters

	Nutrients	Sediment	Flow	Sediment $\times$ flow	Nutrients $\times$ flow	Nutrients $\times$ sediment
Tile invertebrates (12 parameters)	2	5	6	9	1	2 ( $1 \times P = 0.05$ )
Leaf invertebrates (12 parameters)	4	8 ( $1 \times P = 0.05$ )	7	3	3	1
Algal biomass (1 parameter)	1	1	1	1	0	0
Leaf decay (2 parameters)	1	2	2	0	0	1
Overall (27 cases)	8 (29.6%)	16 (59.3%)	16 (59.3%)	13 (48.1%)	4 (14.8%)	4 (14.8%)
Mean effect size (significant effects)	0.34 $\pm$ 0.05	0.41 $\pm$ 0.05	0.43 $\pm$ 0.04	0.36 $\pm$ 0.04	0.24 $\pm$ 0.01	0.43 $\pm$ 0.07

these invertebrate parameters. Thus, negative effects of increased fine sediment levels, such as clogging of interstices, reduced oxygen levels in the hyporheic zone and smothering of algal food for grazers (Wood & Armitage 1997), outweighed any potential positive effects in the reduced-flow channels.

Our prediction that interactions between sediment and flow would be more pronounced for biota exposed to the current on tiles (simulating non-embedded surface stones) compared with leaf packs (representing less exposed organic matter habitats) was supported. For tile substrata, 10 of a possible 13 interactions were significant, including algal biomass and most of the invertebrate community parameters (Table 4). By comparison, only 3 of 14 interactions were significant for leaf-pack substrata. Because most of these interactions indicated a weaker impact of sediment addition at normal flow (see above), these findings imply that, at normal flow, surface stone communities are likely to suffer fewer negative effects of sedimentation than stream bed habitats less exposed to the current.

#### INTERACTIONS BETWEEN FLOW AND NUTRIENTS

We enriched nutrient concentrations to levels approximating those in deer and dairy farms in Southern New Zealand, but it should be borne in mind that higher levels occur in agricultural streams in other parts of the world (e.g. Royer, Tank & David 2004). Flow and nutrient effects interacted only in four of all analysed cases, three in leaf-pack substrata and one on tiles. The abundances of Tanypodinae and Copepoda in leaf packs showed an antagonistic effect, increasing at the highest nutrient level when flow was normal but remaining unchanged or reducing when flow was reduced. EPT richness in leaf packs showed no response to nutrients at normal flow, but peaked at intermediate nutrient levels when flow was reduced. Finally, Nematoda on tiles achieved greatest abundance at the highest nutrient level in normal flow but at the intermediate nutrient level in reduced flow. Augmented nutrient concentrations probably affect invertebrates via increased productivity of algae, bacteria and fungi or changes to the taxonomic composition of these primary resources. Our findings emphasize that such responses can be moderated in quite subtle ways by other stressors acting simultaneously. Similarly complex multiple-stressor responses

have been observed in other aquatic studies (Chen, Hathaway & Folt 2004; Christensen *et al.* 2006; Leprieur *et al.* 2006).

#### INTERACTIONS BETWEEN NUTRIENTS AND SEDIMENT

Our previous research, using surveys and experiments in real streams, showed that an anthropogenic increase in sediment is generally more deleterious in grassland streams than augmented nutrient concentrations (see above). Based on these findings, we tested two related hypotheses in the present experiment. First, we predicted that effects of nutrient enrichment would change from positive to negative at high sediment levels. This expectation was supported in three of the four cases where significant nutrient by sediment interactions occurred [total invertebrate abundance ( $P = 0.05$ ) and *Austrosimulium* on tiles, *Deleatidium* in leaf packs]. In the fourth case, leaf mass loss increased with enrichment (instead of decreasing as predicted, see earlier 'Discussion'), but the positive effect of nutrient enrichment was also strongest at high sediment levels.

We further predicted that effects of sediment addition should not be affected by nutrient levels, and this was not supported. In all four cases, the effects of sediment addition (negative or positive) were strengthened at high nutrient levels, providing no evidence that sediment addition was the main driver of these interactive patterns and indicating a 'balanced' interaction between the two stressors. These findings imply that raised levels of either fine sediment or nutrients have similar potential to augment other single-stressor effects on benthic biota in grassland streams.

#### MANAGEMENT IMPLICATIONS

Our most important finding is that flow reduction was a key stressor for the stream community, causing reductions in density and taxonomic richness of invertebrate species known to be sensitive bioindicators. These deleterious effects on stream health, according to bioindicator species used by stream managers world-wide, occurred not only as single-stressor effects of flow reduction but via an augmentation of negative effects of fine sediment addition (simulating increased catchment erosion). Consequently, abstracting water from a stream

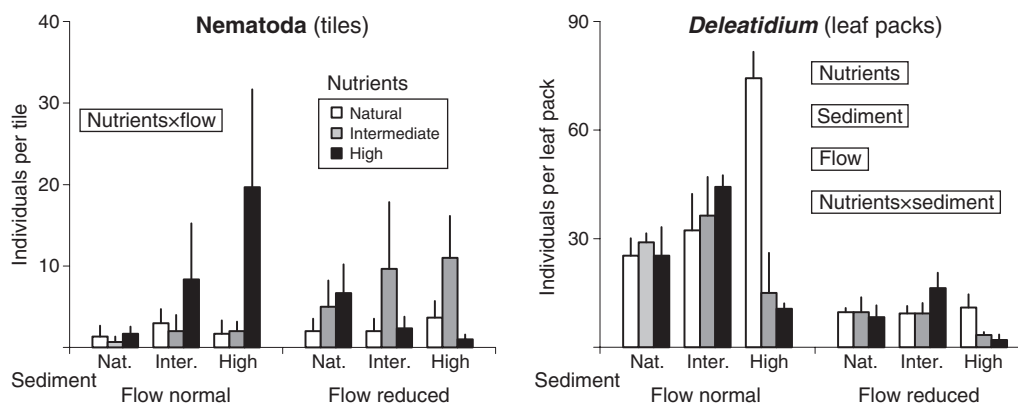


Fig. 5. Density patterns (averages + SEs) for common invertebrate taxa on day 18 in tile or leaf-pack samples that showed significant interactions between nutrient enrichment and flow reduction or sediment addition. For more details see Fig. 2.

already subjected to high fine sediment inputs may have far worse effects on the invertebrate fauna than abstraction from a similar stream with lower sediment levels. Anthropogenic effects on water quality can thus be confounded by changes to water quantity, as more water is removed for irrigation or other purposes. Our combined findings from surveys and manipulative experiments across a wide variety of scales show clearly that complex interactions among multiple stressors occur commonly in grassland streams impacted by agricultural land-use practises, lending more weight to our conclusion in Townsend *et al.* (2008) that the consequences of stressors will often be unpredictable on the basis of knowledge of single effects. The integrated management of catchment land use needs to be informed by knowledge of the combined effects of multiple stressors.

Our understanding of the interacting effects of multiple stressors in grassland streams is still far from complete. For example, the short-term nature of our manipulations may miss some consequences of anthropogenic change to nutrients, sediment and water abstraction that can occur over months or years. Further, our understanding of invertebrate responses would be enhanced by more detailed studies of the algal component of the food web. Finally, there are other potential stressors, such as increased water temperature due to removal of the riparian vegetation and climate change, or pesticides leaching from agricultural catchments, that need to be investigated in concert with the stressors we addressed.

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