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Multiple-stressor effects along gradients of deposited fine sediment and dissolved nutrients in streams

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Abstract

Ecological degradation of streams and rivers as a result of agricultural land-use intensification is a major concern in New Zealand and other parts of the world. Agriculture introduces multiple stressors to streams, presenting a challenge for freshwater managers who must understand the relative strengths of each individual stressor and their combined multiple-stressor effects if they are to implement the most effective management actions and avoid ‘ecological surprises’ that arise from complex interactions between stressors. To investigate patterns of ecological response variables across broad gradients of two major stressors, augmented levels of dissolved inorganic nutrients and deposited fine sediment, I designed a streamside mesocosm experiment with eight levels each of nutrients (36 to 6900 μg/L of dissolved inorganic nitrogen plus 1.4 to 450 μg/L of dissolved reactive phosphorus) and deposited fine sediment (0 to 100 % cover of the streambed), and conducted a field survey in a regional set of 43 streams ranging from 2nd to 6th order. I used multiple linear regression and an information-theoretic approach to select the best predictive models for a series of ecological response variables, including algal, invertebrate and ecosystem variables, and tested (1) the subsidy-stress hypothesis for each stressor (where at low stressor levels an ecological variable responds positively until an inflection point beyond which the effect is negative), (2) whether sediment and nutrients operated as single or multiple stressors and whether they interacted with each other, and (3) whether sediment effects were more pervasive than those of augmented nutrient concentrations. In the 21-day long experiment, subsidy-stress patterns across the nutrient gradient were frequently found for algal and invertebrate taxa and communities, but consistently negative response shapes were more prevalent across the sediment gradient. The subsidy-stress hypothesis was not supported by the response of an ecosystem variable (organic matter breakdown, measured using cotton strips and fresh mahoe leaves). Overall, nutrients and fine sediment acted predominantly as multiple stressors and sometimes in complex interactive ways. The relative strengths of fine sediment and nutrient effects were similar for algal response variables but sediment was the more pervasive stressor for invertebrates, a finding that was also supported by the field survey. My field survey further suggested that nutrients and sediment commonly interact in synergistic ways to affect invertebrate variables, with fine sediment overwhelming any subsidy effects that nutrients may have in isolation. The combined experimental and survey results indicate that augmented levels of fine sediment and dissolved inorganic nutrient concentrations need to be managed together because they mostly act as multiple stressors in their effects on algal, invertebrate and ecosystem response variables. While managers should seek to control both nutrient and fine sediment inputs to streams to achieve good ecological stream condition, measures to reduce or avoid further sedimentation are particularly likely to be effective in mitigating ecological impairment and preventing further harm. Finally, in order to best assess the likely causes of decline in stream health, it will be highly desirable for managers to routinely monitor both nutrients (as currently done) and fine sediment in the future.

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CHAPTER 1 General Introduction

1.1 Land use, multiple stressors and freshwater management

Agricultural land-use intensification has been identified as one of the main drivers worldwide of ecological degradation of streams and rivers, threatening freshwater biodiversity and human water resource security, which are intimately interrelated (Malmqvist & Rundle, 2002; Strayer & Dudgeon, 2010; Vörösmarty *et al.*, 2010). A major challenge in freshwater management, addressing both freshwater biodiversity conservation and human water resource security, is to deal with multiple stressors that arise from anthropogenic land-use practices and which are further exacerbated by climate change (Sutherland *et al.*, 2006; Ormerod *et al.*, 2010). Knowledge of the relative strengths of individual stressor effects and the combined effects of multiple stressors are crucial to make effective management decisions and avoid 'ecological surprises' that can arise from multiple-stressor interactions (Paine *et al.*, 1998; Ormerod *et al.*, 2010). The term 'stressor' in the management and policy context is not unambiguous and stressors are sometimes described as 'pressures', 'pollutants' or 'pollution' (Cormier *et al.*, 2000; Friberg, 2010). I define a stressor as an in-stream variable that, as a result of human activity, exceeds its range of normal variation and affects individual taxa, community composition or ecosystem functioning. Effects include increases or, more typically, decreases in biological response variables such as invertebrate densities or taxon richness (Townsend *et al.*, 2008), and the level of stress is the magnitude of change in a stressor load or concentration relative to a reference condition (Johnson *et al.*, 2007).

Complex multiple-stressor effects are, of course, not only of concern in streams but also in other aquatic and terrestrial ecosystems. Nitrogen and sulphur deposition, increased ozone concentrations and rapidly changing climatic environmental drivers are likely to interactively affect forest ecosystems and functioning, possibly in a synergistic or antagonistic manner (Aber *et al.*, 2001). Coral reefs are affected by hurricanes, eutrophication and overfishing (Hughes & Connell, 1999), marine benthic communities of coastal regions face organic enrichment and various toxicants (Lenihan *et al.*, 2003), and destructive blooms of a green macroalga in coastal regions are a consequence of potentially synergistically interacting multiple human influences (Lotze & Worm, 2002). Crain *et al.* (2008) conducted a meta-analysis investigating multiple-stressor effects in marine systems using evidence from experimental studies. Amongst a list of thirteen potential stressors in marine and coastal ecosystems, including nutrients, sedimentation, toxins, disease and variables associated with climate change (CO_2, temperature, UV radiation and sea level rise), pairs of stressors most frequently produced outcomes involving complex interactions. Folke *et al.* (2004) took a different approach, reviewing documented regime shifts in real terrestrial and aquatic ecosystems (lakes, wetlands and marine systems) in order to find evidence of undesirable dramatic ecological outcomes consequent on reduction in resilience due to human actions. They concluded that it is the combined and often synergistic effects of multiple stressors that make ecosystems more vulnerable to change. Equivalent meta-analyses have not been performed for stream ecosystems.

Stressor loads and their consequences for stream ecosystems are dependent on the intensity of land use in the catchment (Allan, 2004). Land-use intensity increases with increasing percent land cover under agricultural land use in the catchment but also with increasing farming intensity per area of land. The broad range of catchment land-use intensities occurring in New Zealand make its streams excellent model ecosystems to test multiple-stressor effects. In the provinces of Otago and Southland on New Zealand's South Island, for example, pastoral development can reach up to 100 % land cover in the catchment (Hamill & McBride, 2003; Niyogi *et al.*, 2007) and farming practices range from extensive pastoral use for sheep or cattle to intensive use for dairy or deer farming (Matthaei *et al.*, 2006). Along this land-use gradient, intensification occurs in typical stocking rates (and therefore animal excrement and trampling), fertilisation, bank erosion and replacement of native tussock grasses with more productive exotic grasses and nitrogen-fixing clover. These changes lead to increasing inputs of nutrients and fine sediment to streams (Dolédec *et al.*, 2006; Matthaei *et al.*, 2006) that mostly enter via surface or subsurface runoff as diffuse non-point source pollution (Carpenter *et al.*, 1998; Cover *et al.*, 2008). These two kinds of perturbations are among the most critical stressors in New Zealand grassland streams resulting from agriculture (Quinn, 2000; Riley *et al.*, 2003), and are of similar concern in streams and rivers worldwide (Allan, 2004; Paulsen *et al.*, 2008; Vörösmarty *et al.*, 2010). Hence, my thesis focuses on these two stressors (augmented dissolved inorganic nutrient concentrations and deposited inorganic fine sediment, i.e. nutrients and fine sediment that exceed their range of normal variation). Other important agriculturally-derived stressors in streams include pesticides, pathogens (Allan, 2004; Magbanua *et al.*, 2010), and decreased water quantity and increased water temperature arising as a result of water abstraction for irrigation (Dewson *et al.*, 2007; Matthaei *et al.*, 2010). Furthermore, removal of riparian vegetation is a land-use stressor through its consequences for light regime, water temperature, terrestrial food resources and riparian microclimate (Collier & Scarsbrook, 2000; Quinn, 2000).

Deposited fine sediment and dissolved inorganic nutrients are physicochemical variables that also occur naturally in streams at varying levels depending mainly on catchment geology (Richards *et al.*, 1996; Holloway *et al.*, 1998). Against this natural background, extensive research has sought to link changes to catchment land use with in-stream physicochemical degradation (stressors) and to changes in ecological endpoints such as the composition of communities of benthic macroinvertebrates (Allan *et al.*, 1997; Roy *et al.*, 2003; Schriever *et al.*, 2007) that are commonly used as indicators of ecological condition or so-called 'stream health' by water resource managers in New Zealand and elsewhere (Boothroyd & Stark, 2000; Bonada *et al.*, 2006). Other organism groups such as benthic algae (Perona *et al.*, 1998; Douterelo *et al.*, 2004; Andren & Jarlman, 2008; Kelly *et al.*, 2008; Delgado *et al.*, 2010), macrophytes and fish (Barbour *et al.*, 1999; Furse *et al.*, 2006; Hering *et al.*, 2006; Hurford *et al.*, 2010) sometimes serve as indicators of stream condition but are less commonly used than macroinvertebrates (Barbour *et al.*, 1999; Dolédec & Statzner, 2010). Bacterial communities also have recently been described as holding promise for land-use impact assessment (Lear & Lewis, 2009). Furthermore, the search for functional indicators for bioassessment has been gaining momentum (Gessner & Chauvet, 2002; Young *et al.*, 2008; Clapcott *et al.*, 2010; Imberger *et al.*, 2010) in response to the recognition that ecosystem function needs to be integrated into biomonitoring programmes to complement the traditional assessment of structural ecosystem components (such as those of biological communities) to fully assess ecosystem integrity (Gessner & Chauvet, 2002; Sandin & Solimini, 2009).

Independent of the type of indicator used for assessment of current stream ecological condition, however, it is not enough to know simply that a stream is in a degraded ecological condition. Managers also need to understand cause-effect relationships between stressors and ecological responses so they can prioritize actions according to the likelihood and speed of achieving positive outcomes. Because multiple stressors typically operate in concert in the land-use context, knowledge of cause-effect relationships between multiple stressors and ecological response variables is crucial. However, cause-effect relationships involving multiple stressors and ecological indicators have rarely been established. Some indicators became established because they have been shown to respond to general pollution but without discrimination of the individual stressor effects in mind. Examples of such indicators widely used around the world are species/taxon richness of diatoms or macroinvertebrates and the EPT indices (EPT richness, EPT density and % EPT; EPT stands for the pollution-sensitive insect orders Ephemeroptera, Plecoptera and Trichoptera). Other indicators were developed to target organic pollution using species-specific sensitivities (Friberg *et al.*, 2010) but have been shown to also be responsive to other types of pollution. For example, the Macroinvertebrate Community Index (MCI), which is a metric specific to New Zealand, was originally developed in the 1980s to assess organic enrichment in stony streams (Boothroyd & Stark, 2000), but is now known to also respond to deposited fine sediment cover on the streambed and inorganic nutrient concentrations in the water (Quinn & Hickey, 1990a; Dolédec *et al.*, 2006). While sensitivity to a variety of anthropogenic stressor gradients may be desirable because it renders a biotic index a useful tool in the assessment of overall stream condition, such indices will not be so helpful in diagnosing causes of impairment. Further indicators have been developed to target a particular type of pollution, based on taxon-specific sensitivities, and may have potential for bio-diagnostic monitoring and have sometimes been successful in discriminating between different types of pollution (Chessman & McEvoy, 1998; Clews & Ormerod, 2009), but this is not always the case probably because many species may be sensitive to several types of stressors (Vinebrooke *et al.*, 2004). Failure to incorporate knowledge of multiple-stressor effects may lead to the unwise implementation of costly and inefficient management actions for mitigation of stream ecological condition. Furthermore, prediction of outcomes for future scenarios with change in land-use intensity based on knowledge of single-stressor effects may lead to over- or underestimates of outcomes in situations where multiple stressors interact (Townsend *et al.*, 2008).

1.2 Theory and test of multiple-stressor interactions

The general conceptual framework of multiple-stressor effects is that they produce three possible outcomes for ecological response variables: a simple outcome where the multiple-stressor effect is additive (i.e. can be predicted based on evidence from single-stressor studies) or complex outcomes where the combined effects are either larger (i.e. worse) or smaller than the additive single-stressor effects because stressors interact synergistically or antagonistically, respectively (Folt *et al.*, 1999; Vinebrooke *et al.*, 2004; Townsend *et al.*, 2008). While this broad framework is widely used in the literature there is no consensus about the experimental designs and models needed to test for and quantify multiple-stressor effects because multiple stressors represent a relatively recent field of research. Furthermore, classification of a multiple-stressor response of two stressors as additive, synergistic or antagonistic is intuitive if the single-stressor responses are both strictly negative or strictly positive, but is less straightforward if the two stressors are opposing (one has negative and the other positive effects) or if one or both single stressors produce a hump-shaped (subsidy-stress) response. Therefore, clear definitions of when a combined stressor response can be called additive, synergistic or antagonistic are important. There is also a need to establish a consensus regarding these definitions to maximise comparability between different studies testing multiple-stressor hypotheses.

Crain *et al.* (2008) assessed additive, synergistic and antagonistic multiple-stressor responses, accounting for both positive and negative single-stressor responses, in a meta-analysis of experimental marine studies that had used factorial designs and ANOVAs. Their database included 171 experiments, each testing two or three stressors. They found in individual studies that 26 % of combined effects were additive, 36 % were synergistic and 38 % were antagonistic. However, their meta-analysis across all studies revealed an overall synergistic interaction effect, and they concluded that combined effects will often be worse than expected on the basis of knowledge of individual stressors (Crain *et al.*, 2008). They also found that the predominant type of interaction was context-specific and depended on the level of biological organisation (community: antagonistic; population: synergistic), trophic level (autotrophs: antagonistic; heterotrophs: synergistic) and the specific stressor pair investigated.

While an experimental approach using a factorial ANOVA design can provide the relative strengths of all single-stressor effects (main effects) together with their interactive effect and also allows the type of interaction to be classified, this approach does not evaluate the precise response shape across stressor gradients, including potential nonlinearities that are common in nature (Allan, 2004; Wang *et al.*, 2007; Dodds *et al.*, 2010). Such gradient patterns, however, are needed to inform managers about the likely benefit of reductions in a stressor load and about potential stressor thresholds beyond which ecological variables decline drastically or beyond which a decline is no longer acceptable (Dodds *et al.*, 2010; Friberg, 2010). Despite the need to define thresholds of harm and the knowledge that multiple stressors operate in most stressed ecosystems, definition of ecological response shapes across multiple gradients that account for both potential nonlinearities and multiple-stressor interactions has, to my knowledge, never been attempted. Typically, stressor-response relationships have been defined from observational data across single stressor gradients using parametric or non-parametric statistical approaches (Yuan & Norton, 2003; Friberg, 2010; Yuan, 2010) and multiple-stressor effects have been tested with factorial experimental designs using ANOVA (Townsend *et al.*, 2008; Matthaei *et al.*, 2010; Ferreira & Chauvet, 2011), which does not allow for precise definition of response shapes. While Townsend *et al.* (2008) used multiple regression analysis to define response surfaces across two stressor gradients they did not attempt to account for nonlinearities.

When defining stressor response surfaces across two stressor gradients, the concept of the three possible outcomes (additive, synergistic, antagonistic) can be applied but is not always straightforward because the two stressors may interact differentially across the gradients depending on stressor levels. Furthermore, it should be noted that the detection of an interactive effect will also depend on the statistical model or type of analysis used (Cottingham *et al.*, 2005). Hence, while the broad framework of additive, synergistic and antagonistic outcomes is useful to summarise and communicate interactive effects, precise definition of stressor-response relationships is crucial if managers need to know the relative importance of each stressor at various stressor combinations in order to prioritise actions. Note also that definition of stressor-response relationships or types of interaction becomes extremely complex when more than two stressors are operating and a third stressor modifies the interaction between the first two, which is a common occurrence (Crain *et al.*, 2008), or when the interaction is context-specific or time-specific (depending on the frequency and timing of effects) (Crain *et al.*, 2008).

Classification and quantification of multiple-stressor effects is one important step in multiple-stressor research. Another is to understand the underlying mechanisms of such response patterns and to test multiple-stressor hypotheses that link an expected outcome to the mode of action. Application of ecological theory to predict and help understand ecosystem responses might be extremely useful. Thus, Vinebrooke *et al.* (2004) argued that, in scenarios with two stressors operating, the impact of the second stressor on biodiversity is determined by whether species are co-tolerant to the two types of stressors, something that is linked to species traits. Linking stressors to biological invertebrate traits where *a priori* mechanistic predictions can be made also forms the basis for the potentially promising use of invertebrate traits as indicators of certain stressors in freshwater ecosystems (Statzner & Bêche, 2010). In the context of multiple-stressor research, Statzner & Bêche (2010) investigated the potential of invertebrate traits to unravel the simultaneous action of the two focal stressors in my thesis, augmented nutrient concentrations and deposited fine sediment associated with land use. They noted that although definite conclusions cannot be drawn based on current knowledge, the indirect manner in which nutrient pollution affects macroinvertebrates (via several different mechanisms) might preclude the use of macroinvertebrate traits to disentangle multiple-stressor effects involving nutrients. The complex nature of these stressors might also preclude prediction of their multiple-stressor outcomes based on theory that originated from pharmacology and has been adopted by ecotoxicologists. Thus, simple additive outcomes (no interaction) might be explained by two quite different concepts: ‘concentration addition’ when different chemicals have equivalent modes of action, or ‘independent action’ when the chemicals differ in their mode of action (Greco *et al.*, 1995; Altenburger *et al.*, 2003). Departure from an additive outcome (synergism or antagonism) is hence likely to be a consequence of different modes of action that influence each other. Further complexity may arise if different combinations of stressors each produce individual multiple-stressor outcomes (Crain *et al.*, 2008) and if anthropogenic stressors interact with natural stressors (Relyea & Hoverman, 2006), such as physical disturbance, acidity and species interactions.

1.3 Theory of subsidy-stress responses

The perturbation theory of Odum *et al.* (1979) presents two principal response shapes of ecological variables across a human perturbation gradient: a strictly negative shape that is expected for toxic inputs and a unimodal shape expected for 'usable' inputs. A unimodal shape describes the response across a subsidy-stress gradient, where at low levels of a perturbation an ecological variable receives a subsidy until the point of perturbation at which the maximum response is reached and the subsidy turns into a stress effect. According to my stressor definition, a stressor-response relationship can show subsidy-stress patterns but both the subsidy (positive) and stress (negative) effects are considered outcomes of a stressor effect because they result in a deviation from the reference condition. The point of inflection might be indicative of an ecosystem's reduced stability (Odum *et al.*, 1979) and hence is a natural breakpoint that could be defined as a threshold of harm beyond which we should not allow an ecosystem to move. Other hypothetical nonlinear response shapes that could potentially also provide definitions of a threshold of harm were proposed by Allan (2004), distinguishing between those responses that show a sudden decline in biological condition at the lower and at the higher end of the perturbation gradient.

Stream ecosystems are subject to different types of perturbations from agricultural land-use activities such as pasture development, and Quinn (2000) applied Odum *et al.*'s (1979) conceptual models to the shapes of benthic macroinvertebrate variables in response to these different types of stressors. He expected pesticides and sedimentation to have only negative effects while usable resources such as nutrients and light were expected to conform to subsidy-stress responses. Fine sediment, however, can also be defined as a usable resource because this is, like nutrients and light, a natural feature in streams and it provides habitat for certain taxa. Thus, some stream invertebrates have been shown to positively respond to increasing amounts of deposited fine sediment (Angradi, 1999; Matthaei *et al.*, 2006; Townsend *et al.*, 2008). Nevertheless, I am not aware of any studies that have specifically tested the subsidy-stress hypothesis in regard to fine sediment. The majority of studies have reported sedimentation to negatively affect most stream invertebrate variables (Wood & Armitage, 1997; Rabeni *et al.*, 2005; Matthaei *et al.*, 2006; Larsen *et al.*, 2009), in accord with Quinn's (2000) expectation. In contrast, subsidy-stress or strictly positive responses of macroinvertebrates to elevated nutrient concentrations in streams have been reported more frequently (Riley *et al.*, 2003; Heino *et al.*, 2007; Niyogi *et al.*, 2007).

Theory suggests that elevated nutrients predominantly affect macroinvertebrates via indirect pathways (Quinn, 2000), with a subsidy effect of increased algal productivity (Feminella & Hawkins, 1995; Hillebrand, 2002) shifting to a stress effect when excessive algal growth reduces habitat availability and causes further problems associated with eutrophication such as extreme fluctuations in diel dissolved oxygen concentrations, availability of toxic inorganic compounds and toxins released by cyanobacteria (Camargo & Alonso, 2006). Each of these consequences could theoretically be considered as a stressor in itself, with the potential to interact with each other. This complexity may help to explain why predictions of invertebrate responses to a primary cause of increased inorganic nutrients may fail (Miltner, 2010) and why the mechanistic pathways that produce observable consequences (positive and negative) for stream invertebrate response variables are still far from being completely understood (Evans-White *et al.*, 2009; Yuan, 2010).

The mechanistic pathways underlying the observed effects of deposited fine sediment on macroinvertebrates are also not fully understood (Larsen & Ormerod, 2010) although sediment is generally thought to have a more direct mode of action, relating to reduced habitat quality, impaired respiration due to clogging of gills or reduced oxygen levels, and impaired feeding due to reduced food quality or physical harm to feeding apparatus (Wood & Armitage, 1997). However, the lack of empirical support for Odum *et al.*'s (1979) subsidy-stress hypothesis in regard to fine sediment, and other resource stressors in general, may not only be a result of the complexity of ecological responses to stressors but also arise because typically multiple stressors are operating (Tockner *et al.*, 2010). Most studies aimed at quantifying stressor-response relationships across a single stressor gradient have used field survey approaches and failed to account in their analysis for potential interactions between multiple stressors or between stressors and natural environmental gradients (Heino *et al.*, 2007; Niyogi *et al.*, 2007; Friberg *et al.*, 2010). Furthermore, it should be noted that fine sediment effects may potentially be confounded with nutrient effects in a field survey approach when nutrients, especially phosphorus, are bound to sediment particles and hence not dissolved in the stream water which but may be released and become available to the periphyton community.

The subsidy-stress hypothesis has rarely been specifically tested on other structural components of stream ecosystems such as periphyton biomass or community composition although theory suggests that, for example, periphyton diversity should follow a unimodal response shape across nutrient and disturbance gradients with maximum diversity at low to intermediate levels (Biggs *et al.*, 1998). Also, few studies have investigated subsidy-stress responses of functional variables. Net ecosystem metabolism has been shown to follow a unimodal shape across a land-use stress gradient (Young & Collier, 2009) and breakdown of tussock grass in streams to be positively correlated with increased nutrient concentrations (Niyogi *et al.*, 2003). However, because both these studies surveyed streams across a gradient of land-use intensity, the observed effects are probably a product of multiple stressors operating, which prevents predictions regarding potential cause-effect relationships.

1.4 Establishing cause-effect relationships in freshwater ecosystems subject to multiple stressors

Diffuse pollution from agricultural land-use activities, such as inorganic nutrient pollution and augmented levels of deposited fine sediment, are major issues but are particularly difficult to control. Knowledge of cause-effect relationships between multiple stressors and degradation of stream condition is crucial for effective resource management and prevention of further harm in a changing world. Research needs to provide management with (1) scientifically-defensible and ecologically-based thresholds of harm for individual stressors that can be translated into water quality and sediment standards or criteria, (2) knowledge of how these individual stressors act in combination with other stressors and natural environmental drivers, which may affect thresholds of harm and necessitate the mitigation of more than one type of stressor to reach good ecological outcomes, and (3) a set of well-performing ecological indicators, including structural and functional ones that have diagnostic value to discriminate between a suite of multiple potential stressors operating and evaluate their relative contributions to ecological degradation.

Establishing causality between multiple stressors and stream ecological responses needs a variety of research strategies to satisfy the call for multiple lines of evidence (Culp & Baird, 2006), for which a set of criteria has been formulated by Adams (2003). In particular, the integration of field surveys and experiments (in streamside mesocosms or ‘artificial streams’) has been suggested because surveys and experiments have different strengths and limitations (Cash *et al.*, 2003; Culp & Baird, 2006; Townsend *et al.*, 2008). Field surveys provide a study environment with high realism but co-occurrence of, and potential interactions with, other influential anthropogenic and natural environmental gradients precludes the establishment of cause-effect relationships using this approach alone. Stream mesocosm experiments, on the other hand, provide a controlled environment but lack elements of realism, particularly in regard to their spatial and temporal scale. Furthermore, because multiple-stressor gradients can affect ecological response variables differently according to level of biological organisation it is important to incorporate population, community and ecosystem-level variables (Odum *et al.*, 1979; Culp *et al.*, 2000; Crain *et al.*, 2008; Townsend *et al.*, 2008; Sandin & Solimini, 2009) as well as multiple organism groups or trophic levels (Biggs *et al.*, 2000) to capture a full picture of multiple-stressor impacts on stream condition and to identify indicator taxa or other useful ecological indicators.

1.5 Thesis aim and outline

My thesis aims to investigate the individual and combined effects of multiple stressors on ecological response variables in streams and rivers in order to inform resource management about potentially complex (synergistic or antagonistic) multiple-stressor interactions, the ecological response shapes to individual stressor gradients, the relative strengths of the individual stressors when both are operating, and potential thresholds of harm beyond which ecosystems should not move.

The two focal stressors of my thesis are augmented levels of deposited fine sediment and dissolved inorganic nutrients, both of which are major issues in streams and rivers worldwide that drain intensively-used agricultural catchments. I have used multiple linear regression models and an information-theoretic approach to test the following main hypotheses: (1) augmented sediment and nutrients can act as a subsidy to ecological response variables (macroinvertebrates, periphyton and ecosystem function) at low levels but as a stressor at high levels, (2) ecological variables show a range of relationships with sediment and nutrients including no effect, single stressor effect, simple multiple-stressor effect (no interaction) or complex multiple-stressor effect (interaction) and, based on earlier results of Townsend *et al.* (2008) and Matthaei *et al.* (2010), that (3) fine sediment augmentation is associated with stronger stressor patterns and therefore has more detrimental consequences on macroinvertebrate variables than nutrients.

I used both a field survey and an experimental approach to draw conclusions about multiple-stressor effects. In my field survey (Chapter 2), I investigated mostly macroinvertebrate response variables but a few algal variables were also considered. In streamside mesocosms, I experimentally tested broad gradients of both fine sediment and nutrients and determined patterns of a series of macroinvertebrate (Chapter 3) and periphyton response variables (Chapter 4). Finally, and using the same mesocosms, I also investigated an ecosystem-functional response variable, organic matter breakdown (Chapter 5). A general discussion (Chapter 6) integrates the results from Chapters 2 to 5 and reviews evidence of subsidy-stress and multiple-stressor outcomes from both survey and experimental mesocosm approaches. This discussion also includes comparisons of multiple-stressor effects, response shapes of the two individual stressors and their relative strengths among the different levels of biological organisation (population, community, ecosystem function) and among different organism groups and trophic levels (benthic macroinvertebrates and algae). My thesis concludes with several recommendations for resource managers.

CHAPTER 2 Multiple-stressor effects in a regional set of streams and rivers

2.1 Summary

Stream managers need to understand relationships between multiple stressors and ecological responses. I examined responses of benthic invertebrates and algae along two land-use related stressor gradients of concern in running waters. My correlative study of the consequences of augmented deposited fine sediment and nutrient concentrations was conducted in a regional set of 43 streams and rivers monitored by a water management authority in New Zealand and incorporated a wide range of catchment geologies and stream orders. I used multiple linear regression analysis and an information-theoretic approach to select the best predictive models for my biological response variables by testing multiple competing hypotheses that include nonlinear subsidy-stress relationships and interactive effects between the two stressors. Patterns consistent with a subsidy-stress response to increasing dissolved inorganic nitrogen concentration were found for the relative abundances of the common invertebrate genera *Pycnocentrodes* and *Deleatidium* and for the relative abundance of total individuals in the EPT orders (Ephemeroptera, Plecoptera, Trichoptera). Fine sediment seemed the more pervasive stressor, apparently counteracting and overwhelming any initial subsidy effect of increased nutrients, and accounting for more of the variance in biological response variables. Relationships with high nutrient concentrations were weaker and modelled with less certainty, probably reflecting the indirect modes of action of nutrients compared to those underlying sediment effects. Nevertheless, in several cases the models indicated that nutrients interacted synergistically with fine sediment, lending further weight to the conclusion that managers need to address both stressors to achieve the best outcomes.

2.2 Introduction

The intensification of agriculture is one of the major threats to streams and rivers, affecting water quality, flow patterns and habitat for stream biota (Malmqvist & Rundle, 2002). Extensive research has linked catchment land use to in-stream physicochemical degradation and to changes in ecological endpoints such as the composition of communities of macroinvertebrates (Allan *et al.*, 1997; Roy *et al.*, 2003; Schriever *et al.*, 2007), which are commonly used as indicators of ecological condition by water resource managers (Friberg *et al.*, 2010). However, it is not enough to know that a river is in a degraded ecological condition. Managers also need to understand cause-effect relationships between stressors and ecological responses so they can prioritize actions according to the likelihood and speed of achieving positive outcomes. I define a stressor as an in-stream variable that, as a result of human activity, exceeds its range of normal variation and affects individual taxa, community composition or ecosystem functioning. Effects include increases or, more typically, decreases in biological response variables (Townsend *et al.*, 2008) and the level of stress is the magnitude of change in a stressor load or concentration relative to a reference condition (Johnson *et al.*, 2007).

Stressor-response relationships are not invariably linear (or log linear) but often follow unimodal or threshold response shapes (Allan, 2004) and, moreover, may be complicated by interactions among multiple stressors. Few studies have addressed the issue of nonlinearity and multiple-stressor interactions in combination. Previous studies using an experimental approach (Folt *et al.*, 1999; Townsend *et al.*, 2008; Matthaei *et al.*, 2010) have focused on unravelling interactions among stressors rather than investigating individual stressor-response relationships in detail. On the other hand, studies focusing on the shapes of stressor-response relationships by incorporating 2nd-order polynomial terms in regression models have usually involved field observations (surveys) and tested only for the effects of one stressor at a time, including nutrient concentration (Heino *et al.*, 2007), biological oxygen demand (Friberg *et al.*, 2010) and deposited fine sediment (Niyogi *et al.*, 2007). Others have used nonlinear parametric methods (2007) or nonparametric methods, such as generalized additive models (Yuan & Norton, 2003; Yuan, 2010), to identify nonlinear stressor-response relationships. Only rarely has survey data been used to identify the shape of multiple stressor-response relationships (Townsend *et al.*, 2008). Some researchers have resorted to a single synthetic multiple-stressor gradient using multivariate methods such as principal component analysis (Sanchez-Montoya *et al.*, 2010), while others have regressed ecological responses on several predictor variables to quantify the relative importance of each stressor but without including interaction terms (Roy *et al.*, 2003; Hutchens *et al.*, 2009). Because multiple stressors can be considered the rule rather than the exception (Townsend *et al.*, 2008), the diagnosis of ecological impairment requires the simultaneous quantification of biological response variables across multiple-stressor gradients to account for both potential nonlinearity and interactions among the stressors.

Here I examine biological response variables along gradients of two stressors of concern in streams, namely deposited fine sediment (inorganic particles less than 2 mm in diameter; Zweig & Rabeni, 2001) and dissolved nutrients (dissolved inorganic nitrogen and dissolved reactive phosphorus) (Paulsen *et al.*, 2008). Both are naturally present in streams and can be referred to as ‘usable inputs’ as defined by Odum *et al.* (1979). Therefore, they may be expected to conform to unimodal response shapes, acting on biological response variables as a subsidy at low levels but as a stressor at levels augmented by anthropogenic activity. This contrasts with inputs of toxic substances (e.g. pesticides, heavy metals) that are likely to have adverse effects at any level. Subsidy-stress responses of macroinvertebrates to elevated nutrient concentrations in streams have been reported frequently (Heino *et al.*, 2007; Niyogi *et al.*, 2007), most likely reflecting a subsidy effect of increased algal productivity (Feminella & Hawkins, 1995; Hillebrand, 2002) that shifts to a stress effect when excessive algal growth reduces habitat availability and causes further problems associated with eutrophication. However, I am not aware of studies investigating potential subsidy-stress responses to gradients of deposited fine sediment. Rather, deposited fine sediment has been hypothesized to act only as a stressor (Quinn, 2000; Niyogi *et al.*, 2007) and many studies have confirmed an overall negative effect on most biological response variables (Wood & Armitage, 1997; Rabeni *et al.*, 2005; Matthaei *et al.*, 2006; Larsen *et al.*, 2009). On the other hand, some studies have reported an overall positive (subsidy) effect of fine sediment for total invertebrate density and individual taxa such as Oligochaeta (Matthaei *et al.*, 2006; Townsend *et al.*, 2008) and Nematoda (Angradi, 1999) that are known to make use of habitat provided by sediment. Most of these studies only used models to test for positive or negative effects and could not comment on subsidy-stress responses.

Stressor-response relationships are further complicated if the response along one stressor gradient is influenced by another stressor gradient due to complex multiple-stressor interactions. In contrast to simple multiple-stressor effects (additive, no interaction) where response shapes along the first gradient are consistent across levels of the second gradient, complex multiple-stressor effects (with interaction; synergistic or antagonistic) lead to response shapes that vary across the second gradient. Slopes may become steeper, shallower or even change direction. Complex interactions have been reported in two stream studies (Townsend *et al.*, 2008; Matthaei *et al.*, 2010), which both concluded that augmented sediment was generally more influential than nutrients, but I am unaware of simultaneous investigations of interactions among multiple stressors and subsidy-stress responses along gradients of more than one stressor.

Although deposited fine sediment and nutrient concentrations are influenced by land-use intensity, both can also vary naturally with catchment geology, and sediment can vary with factors such as reach slope or the prevalence of slow-flowing habitat (Johnson *et al.*, 1997; Anlauf & Moffitt, 2010). It is possible, therefore, that the effects of anthropogenic stressors will sometimes be obscured because of natural variation in the stressors. The aim of this study was to investigate individual and combined effects of the two stressors in a wide range of stream sites varying in terms of land use, stream order and geology, using ecological data that are typically collected by water management authorities in New Zealand and elsewhere. I tested the following hypotheses: (1) augmentation of deposited fine sediment and nutrient concentrations reflect human land-use intensity, (2) human-induced augmentations of sediment and nutrients are so pervasive in a regional set of streams and rivers that they are not obscured by local geology or stream order, (3) augmented sediment and nutrients can act as a subsidy to invertebrate and algal response variables at low levels but as a stressor at high levels, (4) invertebrate and algal variables show a range of relationships with sediment and nutrients including no effect, single-stressor effect, simple multiple-stressor effect (no interaction) or complex multiple-stressor effect (interaction), and (5) fine sediment augmentation is associated with stronger stressor patterns and therefore has more detrimental consequences for ecological variables than nutrients.

2.3 Methods

2.3.1 Field sites

Forty-three study sites were chosen to incorporate the full range of agricultural intensities in the province of Southland, New Zealand. They are a subset of 85 sites in Southland Regional Council's long-term State-of-the-Environment stream health monitoring programme involving riffle/run segments of wadeable streams (Table 2.1). I ignored 1st and 7th-order streams, those that were lake-fed or in wetland or exotic pine plantation settings, and, for logistic reasons, those that were particularly remote. According to New Zealand's River Environment Classification (Snelder & Biggs, 2002), the study sites fall into one of three surficial geological categories: (1) hard sedimentary spatially dominant (15 sites), (2) alluvium spatially dominant (10 sites) or (3) soft sedimentary greater than 25 % (18 sites). Stream order ranged from 2nd to 6th order, wetted width from 2 to 45 m, and sampled stream reach length from 5 to 56 m. Each site was visited once during a two-week period in January 2008 (Austral summer) at baseflow conditions to take water quality, deposited fine sediment and biological samples. As supplementary information I also measured water depth (from two random locations along each of five equidistant transects along the site) and surface water velocity (at the same ten locations by timing, twice, the passage of an apple over a distance of a metre).

Stream sites differed in the proportions of their catchments devoted to pastoral land use, ranging from near-pristine to close to 100 % pasture. My index of land-use intensity for each stream site was the runoff-weighted percentage cover of land in the catchment that falls into the pasture land-cover category (according to the Freshwater Ecosystems of New Zealand [FENZ] river classification database, Environment Waikato, Hamilton, New Zealand). Runoff-weighted values for each site were derived by first multiplying percent pastoral land cover by the mean annual runoff ([Rainfall – Actual Evapotranspiration] expressed in mm/yr) and summing for each of the sub-catchment units in the FENZ database that were located upstream of the site in question, before dividing by the total mean annual runoff in the site's catchment as a whole (Wild *et al.*, 2005).

Table 2.1 Summary statistics of abiotic and biotic characteristics of 43 sites in the Southland Province of New Zealand (DRP = dissolved reactive phosphorus, DIN = dissolved inorganic nitrogen, SIS = suspendable inorganic sediment).

Abiotic variables	Mean	Min	Max
Elevation (m a.s.l.)	119	9	393
Stream order	4.6	2	6
Stream width at site (m)	12	2	45
Water depth (cm)	20	6	41
Total catchment area (km^2)	316	6	2143
Mean annual low flow (L/s)	965	9	5676
% annual runoff from pasture	57	0	100
DRP (µg/L)	24	2.5	250
DIN (µg/L)	327	10	1928
% cover of fine sediment	14	0	100
Sediment depth (mm)	5	0	79
SIS (g/m^2)	827	23	10870
Biological variables	**Mean**	**Min**	**Max**
Taxon relative abundances (%):			
Deleatidium	27	0	80
Oligochaeta	12	0	53
Elmidae	12	0	49
Potamopyrgus antipodarum	9	0	55
Aoteapsyche	5	0	29
Orthocladiinae	7	0	41
Pycnocentrodes	4	0	33
Relative trait representations (%):			
> 2 reprod. cycles per individual	29	0	86
Single individual reproduction	18	0	62
Lays eggs at water surface	33	0	82
Burrower	10	0	32
Low body flexibility	24	2	48
Grazer	80	52	96
Shredder	2	0	12
Filter-feeder	2	0	13
Respires using gills	55	15	72
Community-level responses:			
Invertebrate taxon richness	18	8	28
EPT taxon richness	8.5	2	18
% EPT	46	3	89
MCI	100	64	130
Chlorophyll a (mg/m^2)	71	0.4	277
Algal taxon richness	13.3	2	25

2.3.2 Nutrients and fine sediment

The nutrient status at each site was determined, according to Southland Regional Council protocols, from a single water sample using standard methods (APHA, 1999) to determine NO_2^--N, NO_3^--N and NH_4^+-N (summed and reported as dissolved inorganic nitrogen, DIN in μg/L), and dissolved reactive phosphorus (DRP in μg/L). Nutrient concentrations determined at the same sites in a second survey, performed for a different purpose about one year later, were highly correlated with my spot readings (R^2 for DIN and DRP 0.74 and 0.77, respectively; A. Liess, unpublished data).

Samples of fine sediment that was resuspendable by physical disturbance were collected from the uppermost layer of the bed substratum at one random location along each of five equidistant transects distributed down the length of the site using a ‘Quorer’ (Quinn *et al.*, 1997a). At each location, a PVC cylinder (inner diameter 24 cm, height 40 cm) was sealed tightly onto the streambed and five water-depth measurements were taken within it. Then substratum was disturbed to a depth of 5 cm with a metal rod for 30 seconds and a 120-ml subsample of the slurry collected. Two water samples were also taken outside the Quorer to correct for background suspended solids. In the laboratory, sediment samples were dried, weighed, ashed at 550°C and weighed again to determine the mass of suspendable inorganic sediment per stream area sampled (SIS in g/m^2).

Deposited fine sediment was determined in two further ways to provide a more complete understanding of the effects of sediment on streambed habitat. Mean percent cover of fine sediment (grain size < 2 mm) on the bed was estimated visually within a standardized gridded quadrat observed through a viewing box at two random locations along each of the five transects (Niyogi *et al.*, 2007). Finally, mean sediment depth (mm) was measured at three random points (where fine sediment < 2 mm was present) in each of the same ten quadrats by inserting a ruler into soft, permeable fine sediment with gentle pressure until the underlying, coarser streambed substrate was reached. If fine sediment was less than 1 mm deep but greater than zero it was recorded as 0.5 mm.

2.3.3 Biological sampling

A single semi-quantitative macroinvertebrate sample was taken from each site with a D-shaped hand net (0.5 mm mesh size) following the standard protocol described in Stark *et al.* (2001). In hard-bottomed streams, macroinvertebrates were collected using the foot-kicking method and sampling effort and area were standardized by disturbing bed substrata in ten locations of varying velocity regimes in the study reach and pooling the collected animals. In soft-bottomed streams, different habitat units such as bank margins, macrophytes and woody debris were sampled in proportion to their frequency of occurrence and pooled. From bank margins and macrophyte beds, animals were dislodged with the hand net and collected from the water column by net sweeps while animals on woody debris were washed or picked off into the net. Each semi-quantitative macroinvertebrate sample represented a standard sampling effort in terms of net sweeps. Samples were preserved in ethanol.

Individuals from a fixed count of 200 macroinvertebrates per sample, and an additional scan for rare taxa, were identified in the laboratory to the lowest practical taxonomic level for determination of relative abundances of the most abundant taxa, relative representation of biological traits and community composition variables. Stark *et al*. (2001) have established that 200 individuals are sufficient to provide accurate assessments of most biomonitoring indices in New Zealand streams. Of the 68 recorded taxa, 16 comprised more than 95 % of the total individuals, each making up at least 1 % of the total. Of these, the relative abundances of seven widespread taxa (present at more than 66 % of the sites) were retained for analysis: *Deleatidium* spp., Oligochaeta, Elmidae, *Potamopyrgus antipodarum* Gray, *Aoteapsyche* spp., Orthocladiinae and *Pycnocentrodes* spp. Information on biological traits was available for all taxa found (Dolédec *et al.*, 2006). I selected nine traits relating to life history, reproduction, attachment to substratum, body flexibility and feeding habits and shown to discriminate among land uses by Dolédec *et al.* (2006). These were ‘more than two reproductive cycles per individual’, ‘single individual reproduction’ (can reproduce in isolation), ‘lays eggs at water surface’, ‘burrower’, ‘low body flexibility’, ‘grazer’, ‘shredder’, ‘filter-feeder’ and ‘respires using gills’. The community composition variables that I analysed were invertebrate taxon richness, EPT taxon richness (organisms in the orders Ephemeroptera, Plecoptera, and Trichoptera), % EPT, and MCI (New Zealand’s Macroinvertebrate Community Index, Stark & Maxted, 2007), a widely used stream condition index that weights species according to their tolerance of pollution.

Periphyton samples for determination of algal biomass (as chlorophyll a in mg/m^2) and algal taxon richness were collected from each site by scraping biofilm from a circular area of 6.5 cm diameter from each of five randomly selected stones representative of patches of different current velocity. Chlorophyll was extracted from the periphyton using ethanol and the amount of chlorophyll a was estimated using a spectrophotometer at 665 nm and 750 nm wavelengths, before and after acidification (Biggs & Kilroy, 2000). Samples for community analysis were preserved in Lugol’s solution and algae were identified in the laboratory at

200x magnification to the lowest practical taxonomic level for the determination of algal taxon richness as described in Biggs & Kilroy (2000).

It should be noted that several of my response variables are not entirely independent of each other (e.g. *Deleatidium* and % EPT). The variables that I subjected to the model-fitting procedure were chosen to include those that are used within New Zealand and globally as measures of stream condition. My applied focus requires that resource managers can see how both the stream community and stream condition indices are related to two important stressors.

2.3.4 Data analysis

Physicochemical variables were transformed to meet the assumptions of the analyses and to reduce the impact of extreme values. SIS, sediment depth, DIN and DRP were log-transformed (natural logarithm) and because zero-values were present for sediment depth, this was multiplied by 10 and a constant of 1 was added before transformation. Percent sediment cover was cube-root transformed.

To test the hypotheses that fine sediment and nutrient concentration can be considered anthropogenic stressors, rather than simply reflecting geological origin, and that any relationship with anthropogenic activity is not obscured by geology or stream order, I used one-way ANCOVAs with a varying-intercept and varying-slope design and log(SIS) and log(DIN) as response variables (see Results for justification). The continuous predictor variable was percent of catchment annual runoff from pasture and the linear relationship (intercept and slope) was allowed to vary with either geological category (Alluvium, Hard Sedimentary, Soft Sedimentary) or with stream order. I obtained the minimal adequate model by AIC in a stepwise algorithm (R function stepAIC) which incorporates both forward and backward modes of selection.

To describe the relationships between log(SIS) and the other fine sediment variables, percent sediment cover and sediment depth (both untransformed), I fitted a two-parameter logistic curve and a two-parameter exponential curve, respectively, using nonlinear regression analysis in statistical package R.

To explore the relationships between biological response variables and the two stressors log(SIS) and log(DIN) and to test the competing hypotheses about different shapes formulated in my hypotheses 3 and 4 described in the Introduction, I used a set of linear regression models for each biological response variable and adopted an information-theoretic model selection approach that allows inferences to be drawn based on the relative support for the models provided by the data (Johnson & Omland, 2004). I followed the protocol of Johnson & Omland (2004) to perform the model selection procedure in five steps.

Step 1 involved the generation of biological hypotheses. My candidate set of competing models included the null model (intercept only), the global model (intercept plus five predictor terms: fine sediment S, nutrients N, interaction SxN, and the 2nd-order polynomial terms SxS and NxN) and simplified 'nested' versions of the global model (with one or more terms removed). Simplified models were obtained by removing terms in a hierarchical fashion but if an interaction or polynomial term was retained then the lower-order terms were retained as well (Quinn & Keough, 2002). The predictor terms in each of the 13 models of the candidate set can be seen in column 2 of Table 2.2. Models 9, 10, 11 and 12 are single-stressor models with only sediment (S, SxS) or nutrients (N, NxN) as predictor terms. Models 1 to 8 are multiple-stressor models, with models 4, 6, 7 and 8 being simple multiple-stressor models (no SxN interaction term), and models 1, 2, 3 and 5 being models where S and N interact (SxN term present). Model 13 is the null model.

Step 2 fitted the models to the data. Transformations of the response variables were performed when needed to meet the assumptions of multiple linear regression analysis. These included log-transformation of chlorophyll a and square-root transformation of all invertebrate taxon and trait variables except for 'low body flexibility', 'grazer' and 'respires using gills'. I only calculated the set of models for biological response variables where the global model provided a reasonable fit to the data (i.e. when a likelihood ratio test [F-test] comparing the fit of the global model with that of the null model was significant at $\alpha = 0.05$). In cases without a reasonable fit, I concluded that the response variable was not affected by either sediment or nutrients.

Step 3 selected the top models from among the candidate set by ranking all models using the small sample unbiased AIC_c (Akaike information criterion) and then selecting those models that were supported best by the data using the cut-off rule $\Delta\ AIC_c \leq 2$ (Burnham & Anderson, 2001).

Step 4 involved the estimation of regression coefficients of the top model set and model averaging if there was more than one top model. Model averaging produced one final model for each biological response variable with regression coefficients (plus 95 % CI) that were weighted averages derived from the top models using the 'zero method' of Burnham &

Anderson (2002); see Nakagawa & Freckleton (2010) for justification. My explanatory input variables log(SIS) and log(DIN) were centred (by subtracting their sample means from each value) and scaled (divided by their sample standard deviations). Centring improves the interpretability of regression coefficients when interaction and polynomial terms are present and deals with collinearity problems that arise when 1st-order terms are highly correlated with their interactions (Quinn & Keough, 2002; Schielzeth, 2010). Scaling makes regression coefficients of predictor variables measured in different units comparable and permits their use as measures of effect size (Schielzeth, 2010). I also centred and scaled all biological response variables, allowing the calculation of scale-independent standardized partial regression coefficients that can be used as measures of effect size when comparing models for different biological response variables (Schielzeth, 2010). I used the R extension package MuMIn (Bartoń, 2009) for calculation of AIC_c values, Akaike weights and model averaging.

Finally, Step 5 comprised the drawing of inferences from the averaged models for each biological response variable to test my stated hypotheses.

2.4 Results

2.4.1 Land use, deposited fine sediment and nutrient concentrations

All three deposited sediment variables were significantly positively related to land use but a simple linear regression model with log(SIS) as the response variable accounted for more variation in percent of catchment annual runoff from pasture ($R^2 = 0.27$; $P < 0.001$) than did percent sediment cover or sediment depth ($R^2 = 0.16$ for both; $P = 0.008$ and $P = 0.007$, respectively). Likewise, both nutrient variables were positively related to my land-use index but log(DIN) accounted for more variation ($R^2 = 0.32$; $P < 0.001$) than log(DRP) ($R^2 = 0.11$; $P = 0.03$). Because they were most strongly related to land use, I used log(SIS) and log(DIN) as measures for deposited fine sediment and nutrients in streams to test my hypotheses about biological response variables.

The relationship between land use and log(SIS) did not depend on geology (Figure 2.1a) and the minimal adequate model was simply the linear regression model (solid line). However, this relationship was influenced by stream order, with the positive correlation between land use and log(SIS) being present in stream orders 4-6 but not in order 3 (Figure 2.1b). The relationship between land use and log(DIN) was dependent on both geology and stream order. Log(DIN) was positively related to land use in each geological category (broken lines), with equal slopes but varying intercepts (Figure 2.1c). There was variation in both slopes and intercepts when log(DIN) was plotted against land use for the different stream orders (Figure 2.1d), but, once again, a positive relationship between land use and log(DIN) occurred in all cases except stream order 6. Stream depth was not significantly related to either log(SIS) or log(DIN) ($R^2 = 0.001$; $P = 0.84$ and $R^2 = 0.07$; $P = 0.09$, respectively) so this potentially confounding variable could not be responsible for any patterns found between biological response variables and my focal stressors. On the other hand, surface water velocity was negatively correlated with log(SIS) ($R^2 = 0.39$; $P < 0.001$) (but not with log(DIN); $R^2 = 0.02$; $P = 0.35$). I used simple linear regressions to test the relationships between three key biological response variables (discussed fully below) and both log(SIS) and current velocity. MCI and relative abundances of *Deleatidium* (both negatively) and *P. antipodarum* (positively) were all significantly related to log(SIS) ($P < 0.001$ and R^2 values of 0.40, 0.32 and 0.35, respectively). The three variables were also correlated with current velocity ($P < 0.05$) but the proportion of variation accounted for was much smaller (R^2 values of 0.10 for MCI, 0.13 for *Deleatidium* (both positive) and 0.21 (negative) for *P. antipodarum*) than was the case with log(SIS). Therefore, I am confident that patterns involving biological response variables and my focal stressors will not be confounded by these potential covariates.

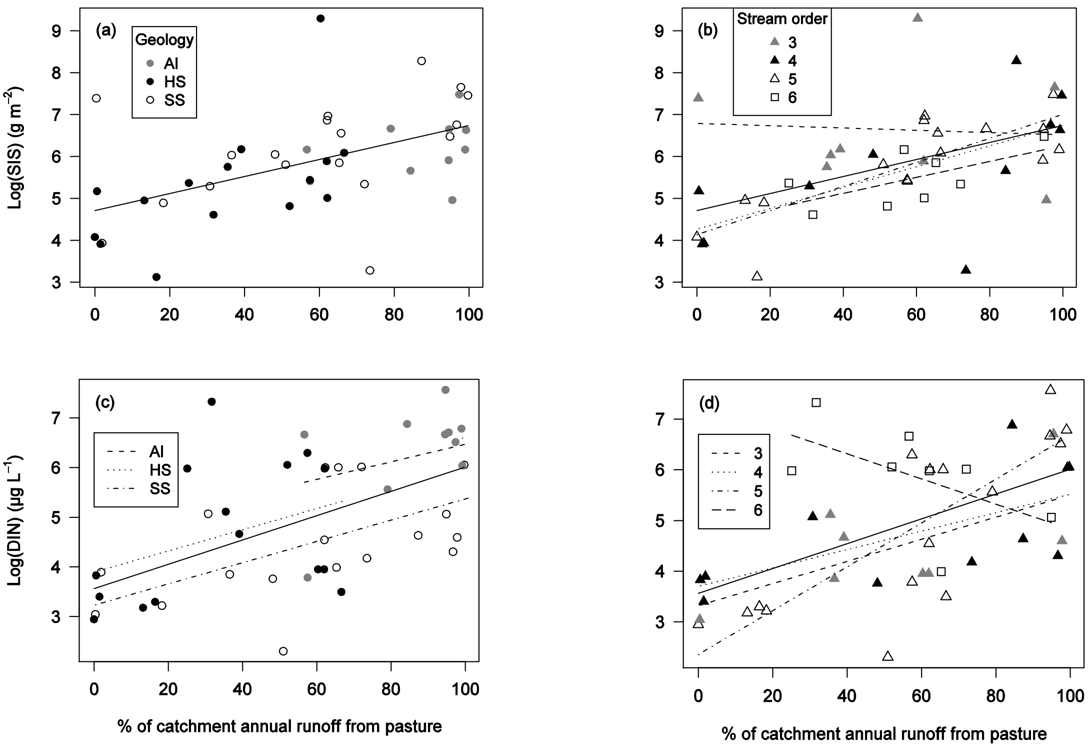


Figure 2.1 Linear regressions of deposited fine sediment and nutrients in relation to the percentage of catchment annual runoff derived from pasture. Data points are coded according to underlying geology (Al = Alluvium, HS = Hard Sedimentary, SS = Soft Sedimentary) in panels (a) and (c) and according to stream order (3 to 6) in panels (b) and (d). A single 2nd-order site has been grouped together with 3rd-order streams. Solid lines are regression lines for all data points (43 sites). Broken lines are regression lines for sites within a particular geological or stream order category. These are shown only if the factor geology or stream order was retained in the minimal adequate model.

The relationship between log(SIS) and percent sediment cover on the streambed was well described by a two-parameter logistic curve fitted by nonlinear regression analysis (Figure 2.2a) while the relationship between log(SIS) and sediment depth was best described by a two-parameter exponential curve (Figure 2.2b).

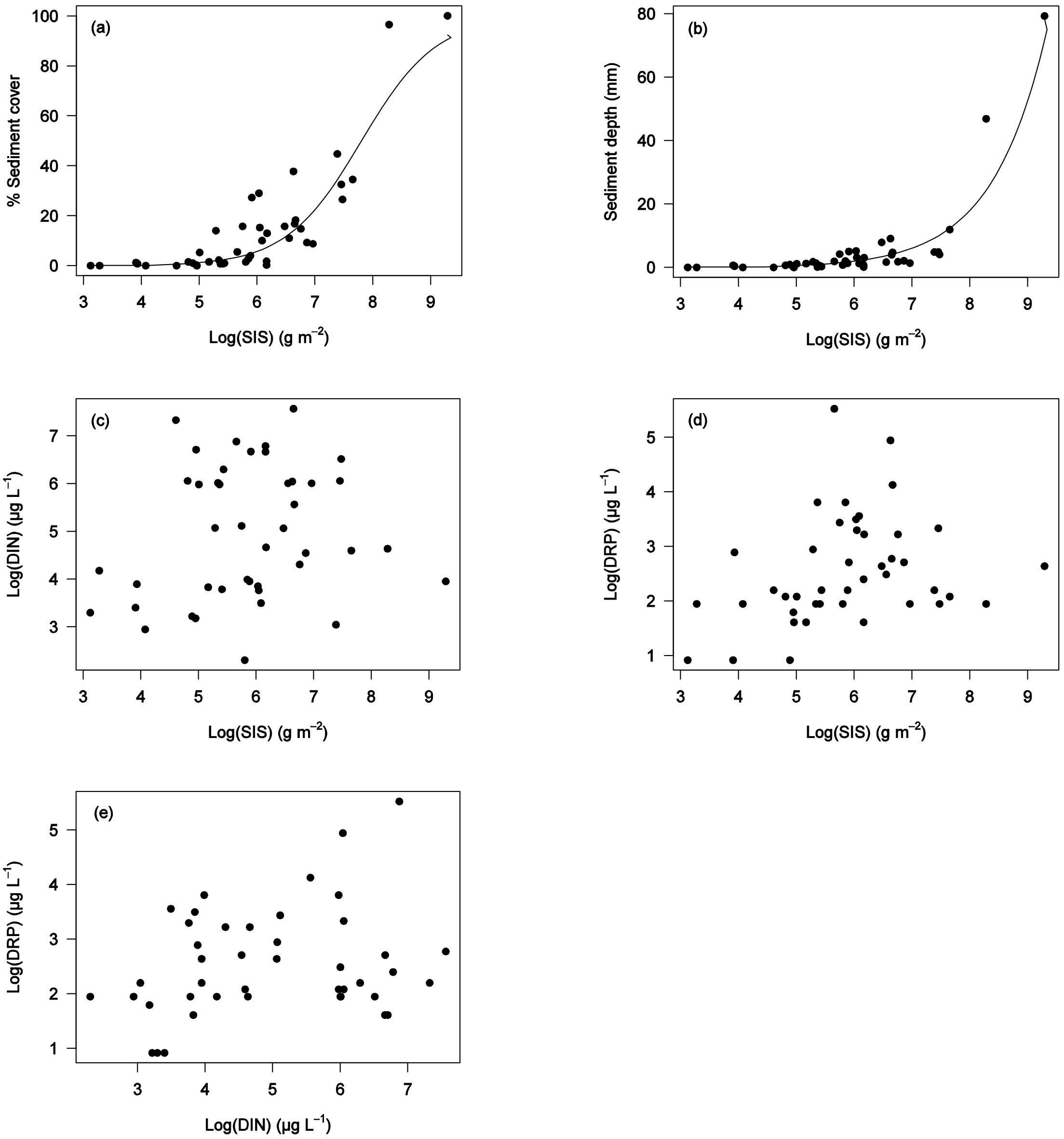


Figure 2.2 (a) Relationship between log(SIS) and percent sediment cover fitted as a two-parameter logistic curve $y = 100e^{a+bx}(1+ e^{a+bx})^{-1}$ with parameter estimates $a = -12 \pm 1.5$ and $b = 1.6 \pm 0.21$ (mean ± 1SD, $P < 0.001$). (b) Relationship between log(SIS) and sediment depth fitted as a two-parameter exponential curve $y = ae^{bx}$ with $a = 0.003 \pm 0.0019$ and $b = 1.10 \pm 0.064$ ($P = 0.09$ and $P < 0.001$, respectively). (c) Relationships between log(SIS) and log(DIN), (d) log(SIS) and log(DRP), and (e) log(DIN) and log(DRP) (all non-significant as shown by simple linear regression, see text).

A broad spread of values was found for both anthropogenic stressors across my study sites (Figure 2.2c). Deposited fine sediment measured as SIS ranged from 23 to 10,870 g/m^2 with a mean of 827 g/m^2 (log-transformed range 3.13 to 9.29 with a mean of 5.87). Nutrient concentration measured as DIN ranged from 10 to 1928 µg/L with a mean of 24 µg/L (log-transformed range 2.30 to 7.56 with a mean of 4.96). There was no relationship between log(SIS) and log(DIN) ($R^2 = 0.03$; $P = 0.28$; Figure 2.2c), ruling out multicollinearity as a problem in subsequent analyses. Deposited fine sediment recorded as percent sediment cover and sediment depth ranged from 0 to 100 % and from 0 to 79 mm, respectively, while DRP concentrations ranged from 2.5 to 250 µg/L. There was a marginally statistically significant relationship between log(SIS) and log(DRP) ($R^2 = 0.09$; $P = 0.05$; Figure 2.2d) and no significant relationship between log(DIN) and log(DRP) ($R^2 = 0.06$; $P = 0.10$; Figure 2.2e).

2.4.2 Anthropogenic stressors and biological response variables

For ten of the 22 biological response variables, the global model (1) provided no statistically significantly better fit than the null model (13; Table 2.2). In these cases (*Aoteapsyche*, Orthocladiinae, burrowers, low body flexibility, grazer, shredder, filter-feeder, gills, invertebrate taxon richness and algal taxon richness) I conclude that neither SIS nor DIN was an important determinant of patterns of occurrence. For the remaining response variables, Table 2.2 presents the results of steps 2 and 3 of the model fitting procedure. Only a single top model was retained for *Deleatidium*, Elmidae, *Potamopyrgus antipodarum* and *Pycnocentrodes*, with two to seven top models retained for the others. The Akaike weights in Table 2.2, ranging from 0.08 to 0.92, can be interpreted as the probability that a model is the best, given the observed data and the candidate set of models (Johnson & Omland, 2004). A low weight of 0.08 was calculated for two of the top models for ‘surface egg laying’ and the weight of the best model for this trait was only 0.20; using the protocol described in the Methods, I conclude here that none of the six top models was significantly more likely than the others. In contrast, a very high weight of 0.92 provided convincing evidence for the only top model for Elmidae. R^2 values were not used for model selection, because these cannot be compared amongst models with different numbers of predictors, but I present R^2 values for each regression model to provide a general measure of model fit (Table 2.2). R^2 values of the best models ranged from 0.17 (single individual reproduction) to 0.64 (Elmidae).

Table 2.2 AICc values, Akaike weights and R^2 values of models in the candidate set of 13 linear regression models calculated for each of 12 biological response variables where the global model (model 1) provided a statistically significantly better fit to the data than the null model (model 13). The predictor variables in the models are S = deposited fine sediment (log(SIS g/m^2), centred and scaled), N = nutrients (log(DIN µg/L), centred and scaled), SxN = interaction term, SxS and NxN = 2nd-order polynomial terms. The 12 biological response variables (also centred and scaled) include five relative abundances of taxa (square-root transformed), three relative trait representations (square-root transformed) and four community-level responses (chlorophyll a was log-transformed, others untransformed). AIC_c values and Akaike weights are results of step 2 of the model selection procedure (see Methods). Bold values are those models that were in the top model set selected by the cut-off rule Δ $AIC_c \leq$ 2 in step 3 of the model selection procedure. The underlined model is the best top model. Sample size is n = 43.

		Biological response variables											
		Deleatidium	Oligochaeta	Elmidae	*Potamopyrgus antipodarum*	*Pycnocentrodes*	> 2 reprod. cycles/individual	Single individual reproduction	Surface eggs	EPT richness	% EPT	MCI	Chlorophyll a
Model	Predictor terms	AIC$_c$ of models in candidate model set											
1	S+ N+ SxN+ SxS+ NxN	112.9	119.2	**94.2**	117.9	121.2	119.4	123.6	123.6	121.2	**100.4**	107.2	109.1
2	S+ N+ SxN+ SxS	110.0	**117.6**	103.4	115.9	126.9	**116.6**	**120.7**	**120.8**	118.5	**100.8**	105.4	106.8
3	S+ N+ SxN+ NxN	110.2	**117.1**	99.3	115.3	128.6	118.9	122.1	122.8	119.9	**100.8**	104.3	111.1
4	S+ N+ SxS+ NxN	114.6	128.4	109.6	115.5	**118.3**	122.1	124.7	121.9	120.0	**101.2**	106.1	106.8
5	S+ N+ SxN	**107.5**	**115.8**	107.5	113.4	132.2	**116.2**	**119.4**	**120.1**	**117.4**	**100.9**	**102.7**	108.7
6	S+ N+ SxS	111.9	126.4	114.3	113.6	124.2	119.5	122.0	**119.2**	**117.6**	**101.8**	104.6	**104.6**
7	S+ N+ NxN	112.3	127.0	114.3	113.1	125.9	122.3	123.8	121.5	119.3	**102.3**	**103.4**	108.4
8	S+ N	109.8	125.2	118.6	111.3	129.7	119.8	**121.3**	**119.0**	**116.9**	102.6	**102.0**	106.2
9	S+ SxS	113.3	124.3	113.8	111.4	123.9	118.4	**120.7**	**120.8**	**117.7**	110.3	107.4	**103.7**
10	N+ NxN	124.9	128.0	119.1	126.5	123.7	128.6	128.6	126.2	127.2	121.0	123.4	118.0
11	S	110.9	122.9	120.6	**109.1**	127.6	**117.9**	**119.4**	**119.2**	**116.1**	108.4	105.4	106.3
12	N	122.9	127.1	125.5	126.6	127.2	126.4	126.4	123.9	124.8	118.8	120.9	116.7
13	Null model	125.3	125.3	125.3	125.3	125.3	125.3	125.3	125.3	125.3	125.3	125.3	119.7
Model	Predictor terms	Akaike weights of models in candidate model set											
1	S+ N+ SxN+ SxS+ NxN	0.03	0.08	**0.92**	0.01	0.16	0.06	0.03	0.02	0.02	**0.19**	0.02	0.02
2	S+ N+ SxN+ SxS	0.12	**0.18**	0.01	0.02	0.01	**0.23**	**0.12**	**0.08**	0.07	**0.15**	0.05	0.07
3	S+ N+ SxN+ NxN	0.11	**0.24**	0.07	0.02	0.00	0.07	0.06	0.03	0.04	**0.15**	0.09	0.01
4	S+ N+ SxS+ NxN	0.01	0.00	0.00	0.02	**0.65**	0.01	0.02	0.05	0.03	**0.13**	0.04	0.07
5	S+ N+ SxN	**0.42**	**0.46**	0.00	0.05	0.00	**0.28**	**0.23**	**0.11**	**0.13**	**0.14**	**0.21**	0.03
6	S+ N+ SxS	0.05	0.00	0.00	0.05	0.04	0.05	0.06	**0.17**	**0.12**	**0.09**	0.08	**0.22**
7	S+ N+ NxN	0.04	0.00	0.00	0.06	0.01	0.01	0.02	0.06	0.05	**0.07**	**0.15**	0.03
8	S+ N	0.13	0.00	0.00	0.15	0.00	0.05	**0.09**	**0.20**	**0.17**	0.06	**0.29**	0.10
9	S+ SxS	0.02	0.01	0.00	0.15	0.04	0.10	**0.12**	**0.08**	**0.11**	0.00	0.02	**0.34**
10	N+ NxN	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.01	0.00	0.00	0.00	0.00
11	S	0.08	0.01	0.00	**0.47**	0.01	**0.12**	**0.23**	**0.18**	**0.25**	0.00	0.05	0.09
12	N	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.02	0.00	0.00	0.00	0.00
13	Null model	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Model	Predictor terms	R^2 of models in candidate model set											
1	S+ N+ SxN+ SxS+ NxN	0.45	0.36	**0.64**	0.38	0.33	0.35	0.29	0.29	0.33	**0.59**	0.51	0.44
2	S+ N+ SxN+ SxS	0.45	**0.34**	0.52	0.36	0.18	**0.35**	**0.29**	**0.29**	0.32	**0.55**	0.50	0.43
3	S+ N+ SxN+ NxN	0.44	**0.35**	0.57	0.37	0.15	0.32	0.26	0.25	0.30	**0.55**	0.51	0.37
4	S+ N+ SxS+ NxN	0.38	0.15	0.45	0.37	**0.33**	0.26	0.22	0.27	0.30	**0.55**	0.49	0.43
5	S+ N+ SxN	**0.44**	**0.32**	0.44	0.36	0.01	**0.32**	**0.26**	**0.25**	**0.30**	**0.52**	**0.50**	0.36
6	S+ N+ SxS	0.38	0.14	0.35	0.36	0.18	0.26	0.22	**0.27**	**0.30**	**0.51**	0.48	**0.42**
7	S+ N+ NxN	0.38	0.12	0.35	0.37	0.15	0.21	0.18	0.23	0.27	**0.51**	**0.49**	0.37
8	S+ N	0.38	0.11	0.23	0.35	0.01	0.21	**0.18**	**0.23**	**0.26**	0.47	**0.48**	0.36
9	S+ SxS	0.32	0.13	0.31	0.35	0.13	0.24	**0.20**	**0.19**	**0.25**	0.37	0.41	**0.40**
10	N+ NxN	0.11	0.05	0.23	0.08	0.14	0.03	0.03	0.09	0.06	0.19	0.14	0.15
11	S	0.32	0.10	0.15	**0.35**	0.00	**0.20**	**0.17**	**0.18**	**0.24**	0.36	0.40	0.32
12	N	0.11	0.01	0.05	0.02	0.01	0.03	0.03	0.08	0.06	0.19	0.14	0.12
13	Null model	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Step 4 in the model fitting procedure involved the determination of model-averaged coefficients to provide a final model for each biological response variable, which can be used to determine the average value of the response for any sediment and nutrient level of interest (see Table 2.3), and from which three-dimensional response surfaces could be generated. In their first column, Figures 2.3, 2.4 and 2.5 present the standardized partial regression coefficients (plus 95 % CIs) of the final model for 12 response variables. Note that these coefficients are also standardized effect sizes of the predictors (for example, the effect size of sediment (S) is the reduction or increase in the response in unit standard deviations due to the main effect of sediment at the mean level of nutrients when sediment increases from the mean by one standard deviation). Furthermore, the 95 % CI is a precision estimate of the effect size and an inclusion of zero means that the effect is not statistically significant (at $\alpha = 0.05$) under the conventional null hypothesis significance testing framework (Nakagawa & Cuthill, 2007). In their second column, Figures 2.3, 2.4 and 2.5 present the three-dimensional response surfaces that the final models describe. For ease of comprehension, the axes for these response surfaces are shown without centring or scaling (the general shapes of the surfaces were not affected by this form of presentation). Several of the final models included a term whose effect size was very small. If the partial regression coefficient of a predictor was less than 0.1, I considered it to be of no ecological significance. Note that the effect size of the main-effect term nutrients (N) for ‘single individual reproduction’ was less than 0.1, but this effect could not be neglected in the corresponding model because the interaction term was present and larger than 0.1.

Table 2.3 Intercept and regression coefficients for the final model (the top model or averaged for the top model set) for each of the twelve biological response variables. Transformations of response variables are shown in parentheses. The predictor variables are S = deposited fine sediment (log(SIS) g/m^2), N = nutrients (log(DIN) µg/L), SxN = interaction term, SxS and NxN = 2nd-order polynomial terms. All response and predictor variables were also centred and scaled. The means and standard deviations of the predictor variables are the following: log(SIS): mean = 5.87, SD = 1.26, log(DIN): mean = 4.96, SD = 1.39. Sample size is n = 43.

			Standardized partial regression coefficients					
Biological response variable	Mean	SD	Intercept	S	N	SxN	SxS	NxN
Taxon relative abundances:								
Deleatidium (sqrt)	0.446	0.262	0.053	-0.656	-0.271	-0.320		
Oligochaeta (sqrt)	0.284	0.211	-0.067	0.532	0.130	0.579	0.017	-0.046
Elmidae (sqrt)	0.299	0.180	-0.113	-0.537	0.106	-0.547	-0.188	0.397
Potamopyrgus antipodarum (sqrt)	0.211	0.211	0.000	0.592				
Pycnocentrodes (sqrt)	0.138	0.139	0.722	-0.029	-0.183		-0.294	-0.445
Relative trait representations:								
> 2 reprod. cycles/individual (sqrt)	0.457	0.284	-0.098	0.552	0.135	0.314	0.048	
Single individual reproduction (sqrt)	0.374	0.214	-0.055	0.463	0.080	0.150	0.031	
Surface eggs (sqrt)	0.515	0.257	0.055	-0.404	-0.177	-0.043	-0.049	
Community-level responses:								
EPT richness	8.47	3.53	0.035	-0.479	-0.107	-0.039	-0.030	
% EPT	45.6	26.8	0.226	-0.621	-0.382	-0.177	-0.079	-0.123
MCI	100.3	18.0	0.040	-0.617	-0.284	-0.059		-0.031
Chlorophyll a (log)	3.44	1.60	0.264	0.461	0.064		-0.282	

The final model for *Potamopyrgus antipodarum* was a single-stressor model, showing a linear increase in relative abundance with increasing sediment but no relationship with nutrients (nor any interaction or polynomial term in the model) (Figure 2.3). The model for algal biomass as chlorophyll a was also essentially a single-stressor model, including a strong relationship with sediment and a barely perceptible response to nutrients (partial regression coefficient less than 0.1). In the case of chlorophyll a, however, the polynomial term for SxS indicated a nonlinear, subsidy-stress response. In four simple multiple-stressor models (Figure 2.4), the response variable changed in relation to both stressors but in a straightforward manner (no interaction term in the final model or with a partial regression coefficient for the interaction term of less than 0.1). EPT richness, MCI and representation of the surface egg-laying trait all declined both with increasing deposited sediment and increasing nutrient concentration. *Pycnocentrodes* also followed a simple multiple-stressor pattern (no interaction term), but polynomial terms SxS and NxN produced nonlinear, subsidy-stress relationships along both sediment and nutrient axes.

Single-stressor responses

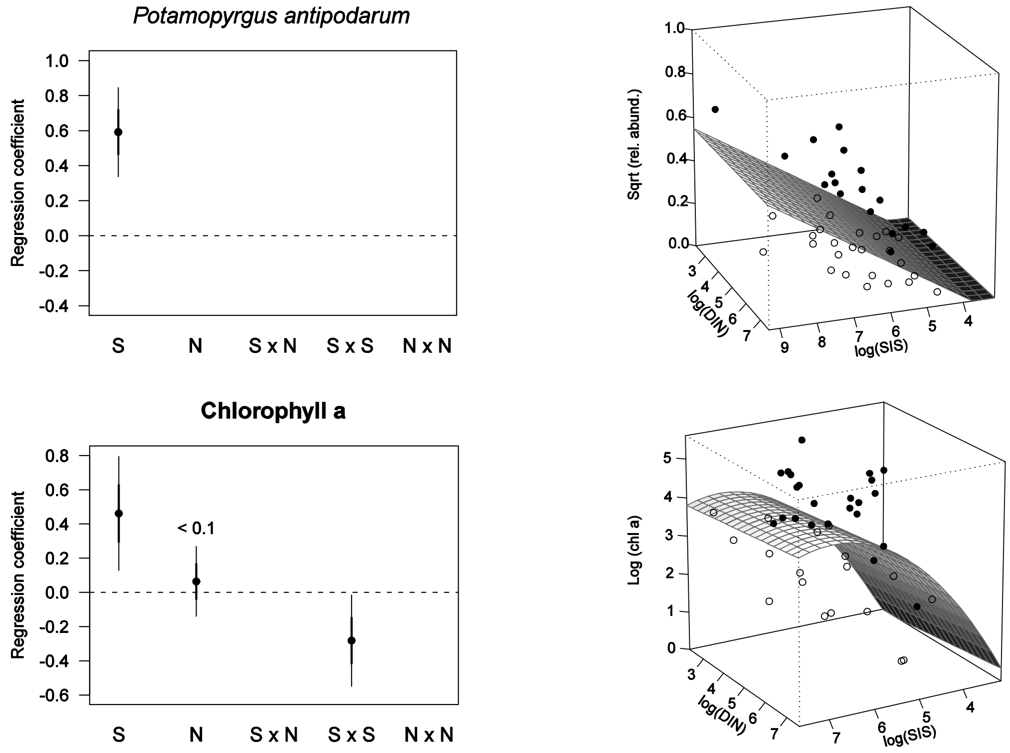


Figure 2.3 Relationships between anthropogenic stressors and biological response variables where the model selection procedure revealed a single-stressor response. Standardized partial regression coefficients (plus 95% CIs) of the terms in the final model are shown on the left and three-dimensional response surfaces on the right. Note that these coefficients are also standardized effect sizes of the predictors (for example, a negative or positive effect size of sediment (S) is the reduction or increase in the response in unit standard deviations due to the main effect of sediment at the mean level of nutrients when sediment increases from the mean by one standard deviation). See Table 2.3 for details of transformations used for predictor and response variables, all of which were centred and scaled. The predictor variables are S = deposited fine sediment (log(SIS) g/m^2), N = nutrients (log(DIN) µg/L) , SxN = interaction term, SxS and NxN = 2nd-order polynomial terms. The response surfaces are plotted for the range of observed stressor values but ecologically meaningless values (<0 or >1 for relative abundance or trait representation) were set to 0 or 1, respectively. For ease of comprehension, all three axes of the three-dimensional response surfaces have been plotted with non-centred and non-scaled values. Please note that the highest nutrient and sediment levels are in the front corner of each 3D plot. The data points are the observed values at the 43 field sites with data points that lie above the surface shown as solid circles and those below it as open circles.

Simple multiple-stressor responses

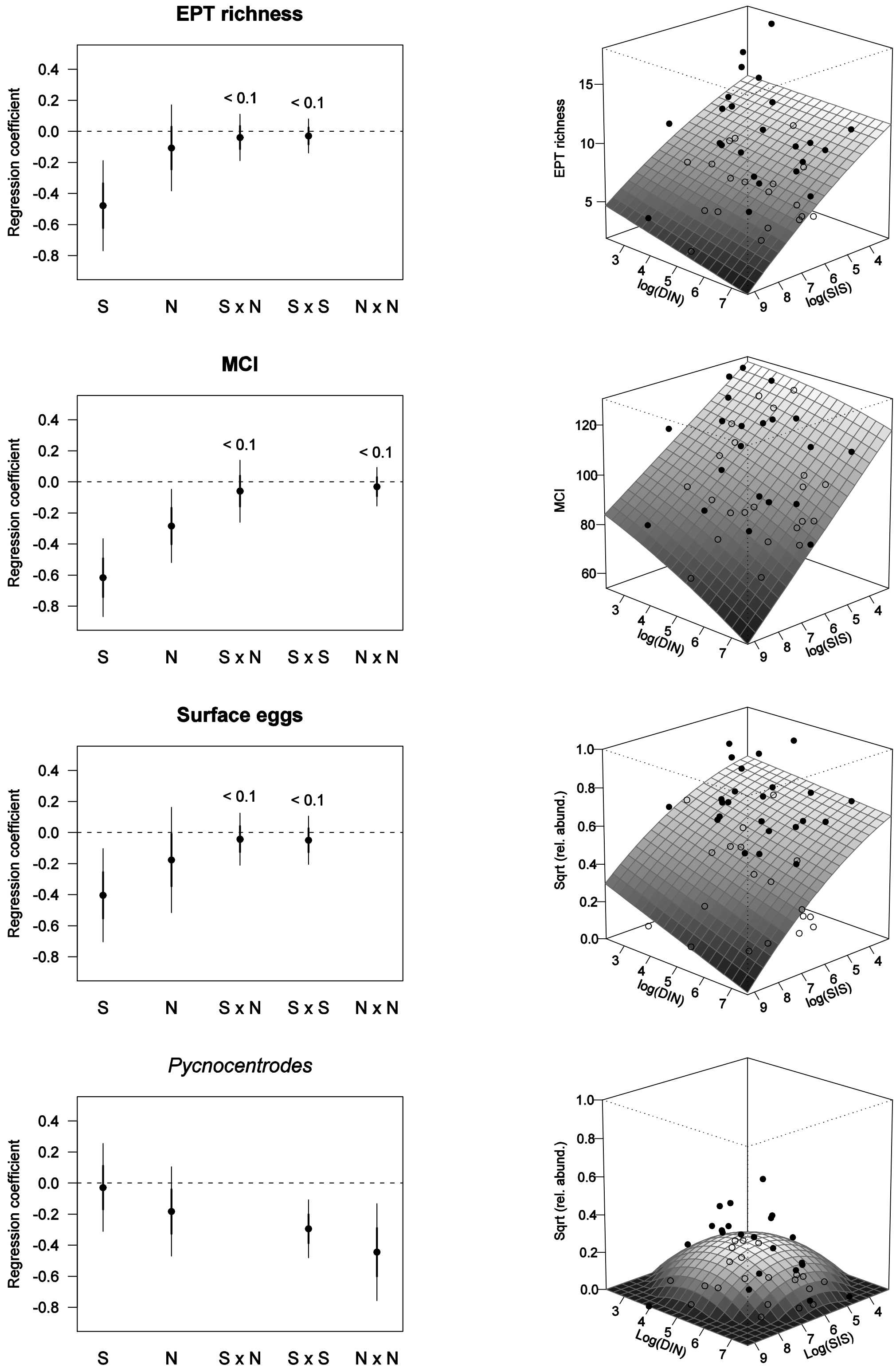
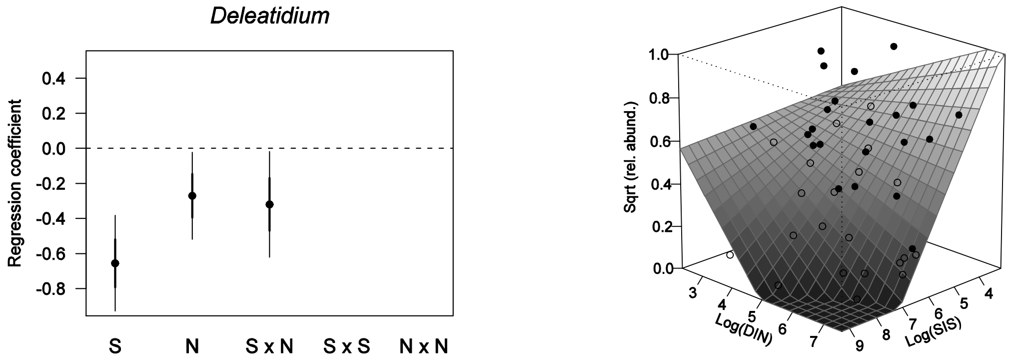
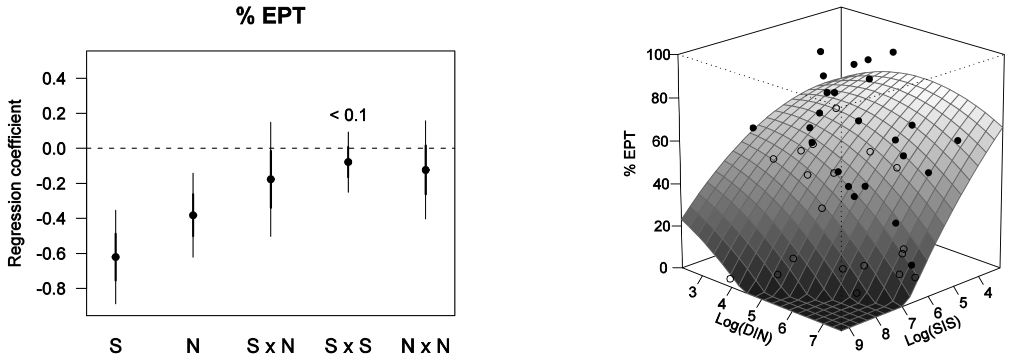


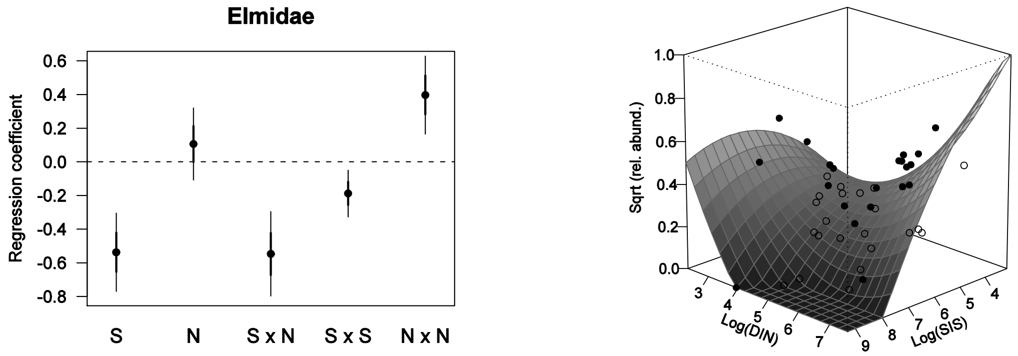
Figure 2.4 Relationships between anthropogenic stressors and biological response variables where the model selection procedure revealed a simple multiple-stressor response (no interaction term in the final model or partial regression coefficient of interaction term less than 0.1). Standardized effect sizes (plus 95% CIs) of the terms in the final model are shown on the left and three-dimensional response surfaces on the right. See Table 2.3 and Figure 2.3 for more details.

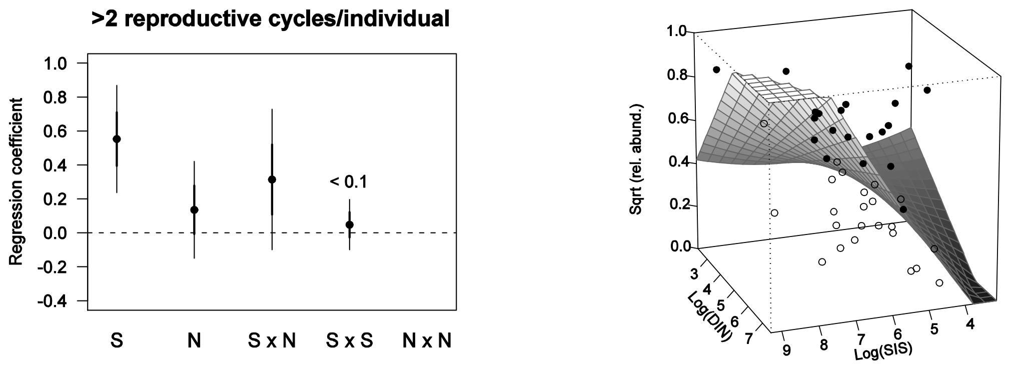
Figure 2.5 presents six response variables that followed complex multiple-stressor patterns, where the relationship along the sediment axis depended on the nutrient level, and vice versa. The first three variables showed no response (*Deleatidium*), a negative response (% EPT) or a subsidy-stress response (Elmidae) to sediment at low nutrient levels, but at high nutrient levels in each case there was a strong decline with increasing sediment, consistent with a synergistic interaction between deposited fine sediment and nutrients. At the same time, these three invertebrate variables showed a positive response (*Deleatidium* and Elmidae) or a subsidy-stress response (% EPT) to nutrients at low sediment levels, but a strong decline at high sediment levels, also consistent with synergistic negative effects. The remaining three invertebrate variables in Figure 2.5 showed weak negative (‘more than two reproductive cycles per individual’), weak positive (‘single individual reproduction’) or strong negative (Oligochaeta) linear responses to increasing sediment at low nutrient levels. Further, they exhibited strong (‘more than two reproductive cycles per individual’ and Oligochaeta) or weak (‘single individual reproduction’) linear declines with increasing nutrients at low sediment levels. However, at high sediment levels the three models indicated a strong (‘more than two reproductive cycles per individual’ and Oligochaeta) or weak (‘single individual reproduction’) linear increase with increasing nutrients. At the same time, these three invertebrate variables showed a strong positive linear response to sediment at high nutrient levels, indicating synergistic positive effects.

Complex multiple-stressor responses









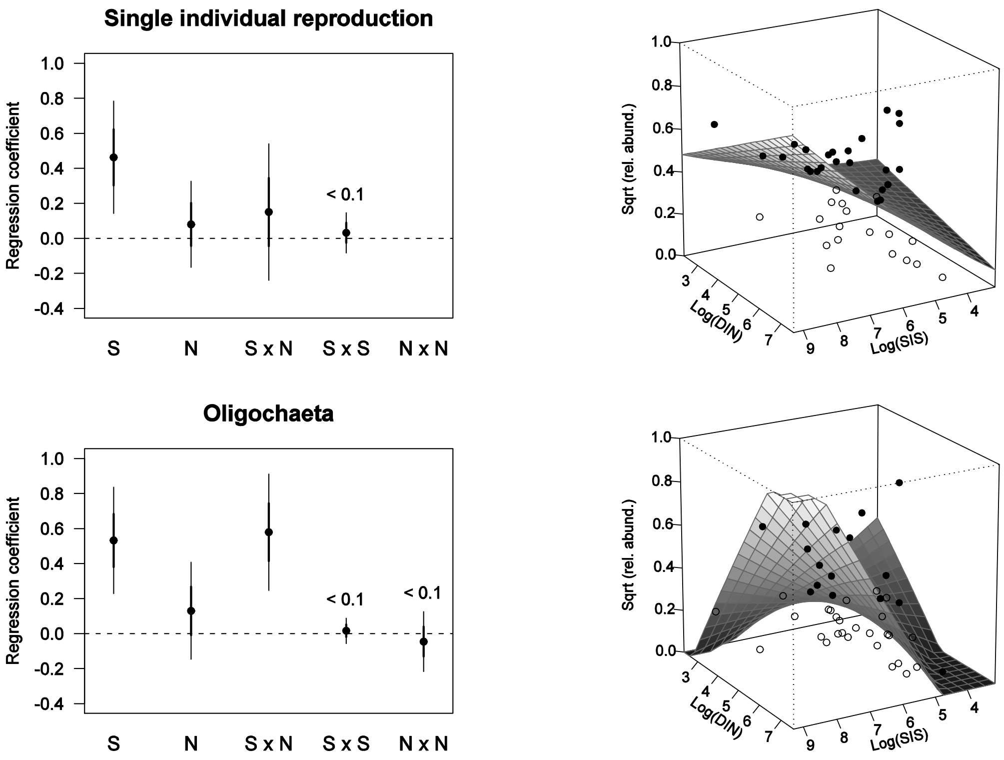


Figure 2.5 Relationships between anthropogenic stressors and biological response variables where the model selection procedure revealed a complex multiple-stressor response (with an interaction term whose partial regression coefficient was 0.1 or greater in the final model). Standardized effect sizes (plus 95% CIs) of the terms in the final model are shown on the left and three-dimensional response surfaces on the right. See Table 2.3 and Figure 2.3 for more details.

2.5 Discussion

2.5.1 Land use, deposited fine sediment and nutrients

I had predicted in my first and second hypotheses that augmentation of deposited fine sediment and nutrient concentrations should reflect human land-use intensity and that human-induced augmentations of sediment and nutrients should be so pervasive in my regional set of streams and rivers that they are not obscured by local geology or stream order. My findings supported both these predictions.

Deposited fine sediment increased significantly with land-use intensity as measured by the percent of catchment runoff derived from pasture, despite the natural variation in bed sediment that might be expected because of disparities in catchment geology. Although stream order was a significant predictor of deposited fine sediment in my analysis of covariance, I found positive relationships of suspendable inorganic sediment (SIS) with agricultural land use for all stream orders except the lowest (3rd order). These results contrast with a study in Michigan where surficial geology, catchment area and mean catchment slope accounted for more variance in fine sediment than did land use (Richards *et al.*, 1996). Contrary to expectation, I did not find more deposited fine sediment where the upstream catchment was strongly represented by soft sedimentary geology or at downstream sites (higher order) where shear stresses near the bed are likely to be lower. However, I cannot rule out some role for these variables, amongst others, given that my land-use index accounted for only 27 % of the variation in SIS (a subsurface measure) and just 16 % of the variation in both percent sediment cover and depth. By contrast, percent upstream catchment in agriculture in a Canadian river accounted for 67 % of variation in percent fine sediment in bed cores (another subsurface measure) and 50 % of variation in visually estimated percent fine sediment (Sutherland *et al.*, 2010). These higher values may reflect the narrower range of stream orders (2nd to 4th) and more uniform surficial geologies in the Canadian case. Sutherland *et al.* (2010) found, like us, that land-use intensity was reflected better in the subsurface measure. Nevertheless, my visual estimates of sediment (cover and depth) were also positively related to land-use intensity and to the subsurface measure, as reported by Larsen *et al.* (2009) and Sutherland *et al.* (2010). I found logistic and exponential relationships with log(SIS) of sediment cover and sediment depth, respectively. Up to a threshold, suspendable inorganic sediment accumulates in interstitial spaces while sediment cover and sediment depth on the bed remain near zero. Beyond this, further deposits accumulate on the surface, increasing sediment cover logistically to the maximum of 100 % and sediment depth exponentially and without a theoretical maximum. It can be noted that particles on the surface are more prone to be transported in high discharge events than those in interstitial space (Sutherland *et al.*, 2010), helping to explain the stronger relationship between land-use intensity and log(SIS).

Dissolved inorganic nitrogen (DIN) was also positively related to land-use intensity but this time geology and stream order were also significant predictors. Average DIN concentrations were highest at sites whose catchments included alluvium, intermediate where hard sedimentary geology was dominant, and lowest where soft sedimentary geology extended over more than 25 % of the catchment. Higher nitrogen concentrations in New Zealand rivers associated with alluvium have been explained by their suitability for agricultural land use rather than geology per se (Unwin *et al.*, 2010) and Johnson *et al.* (1997) also found agricultural land use in streams in the USA accounted for more variation in water chemistry than geology or catchment area. I found positive relationships of DIN with agricultural land use for all stream orders except the largest (6th order).

2.5.2 Subsidy-stress responses

Amongst the twelve models I fitted to my ecological response variables, three showed patterns consistent with unimodal subsidy-stress responses to fine sediment (chlorophyll a, *Pycnocentrodes* and Elmidae) and two to nutrients (*Pycnocentrodes* and % EPT), supporting my third hypothesis that such subsidy-stress responses should occur. I found one further unimodal relationship with nutrients (Elmidae at intermediate levels of sediment), but this was U-shaped and does not fall into the subsidy-stress response category. Tockner *et al.* (2010), noting that previously few empirical data in the literature had clearly supported Odum *et al.*'s (1979) subsidy-stress hypothesis, argued this was because ecological responses to 'usable inputs' (Odum *et al.*, 1979) are rarely a consequence of a single mechanism. Stressors may affect biota directly or indirectly via changes in habitat or food quality or quantity, which in turn may modify interspecific interactions. The situation is further complicated because the subsidy effect is most likely due to a mechanism very different from that underlying the stressor effect. While I agree with Tockner *et al.*'s (2010) assertions, my results provide some of the most convincing evidence for subsidy-stress responses to date.

A frequently proposed mechanism for an initial subsidy effect of increasing nutrients is the direct stimulation of algal productivity, which in turn stimulates macroinvertebrate responses via enhanced food resources (Feminella & Hawkins, 1995; Hillebrand, 2002). I found no relationship between DIN and standing stocks of chlorophyll a, possibly because this is not a reliable surrogate for algal productivity, with any nutrient-enhanced productivity perhaps obscured by increased sloughing rates or differential grazing pressure (Biggs, 1996). In contrast, I found patterns consistent with indirect subsidy effects of nutrients on relative abundances of the algal grazers *Pycnocentrodes* and *Deleatidium* and on % EPT (for the latter two only at low sediment levels). However, the aggregate measure of relative representation of the 'grazer' trait was not significantly related to DIN, perhaps partly because of assignment errors to functional feeding groups (Yuan, 2010). A measure of absolute abundance might have revealed a clearer pattern because densities of total invertebrates, most of which were grazers, increased with nutrient enrichment in a previous study in New Zealand (Biggs *et al.*, 2000). On the other hand, Cross *et al.* (2006) reported a positive nutrient enrichment effect on total invertebrate abundance driven mainly by increases in shredders and gatherers. In my study, no functional feeding group showed a relationship with nutrients or sediment, as reported also by Wang *et al*. (2007). *Deleatidium* was related positively to nutrients along the entire gradient at low sediment levels while the apparent subsidy effects of nutrients peaked at relatively low values for *Pycnocentrodes* (107 μg DIN /L) and % EPT (144 μg DIN /L).

Further increases were associated with negative (stress) effects (see review by Camargo & Alonso, 2006 for examples of such effects), and these were much more frequently observed than subsidy effects. Stress patterns are more likely related to changes in algal community composition or productivity than direct toxic effects, because the highest DIN concentration in my study was below the limit of 2000 µg NO_3-N /L proposed for the protection of sensitive invertebrates (Camargo *et al.*, 2005).

To my knowledge, a subsidy-stress hypothesis related to deposited fine sediment has not been explicitly tested before. Algal biomass (chlorophyll a) increased with increasing amounts of deposited fine sediment, peaking towards the higher end of the sediment gradient (991 g SIS/m^2) and decreasing thereafter. It is unlikely that sediment stimulated algal production (unless a nutrient such as ammonium was adsorbed to it), but it could conceivably have reduced grazing pressure allowing more algal biomass to accumulate. Alternatively, the algal pattern may have been confounded by an unmeasured variable such as shading. The relative abundance of grazers was unrelated to sediment levels in my study but Rabeni *et al.* (2005), who had data on both relative and absolute grazer densities, found a much stronger negative correlation of fine sediment cover with absolute than with relative grazer density. A reduction in grazing seems a reasonable consequence of increased amounts of fine sediment, considering the negative impact of fine sediment on the relative abundance of key grazers such as *Deleatidium* and other EPT taxa. While a decrease in grazing intensity may be the driving mechanism for the initial increase in algal biomass, I suggest that the unstable substrate associated with further deposited sediment probably limits biomass accrual.

The cased caddisfly *Pycnocentrodes* was the only invertebrate showing a subsidy-stress relationship with fine sediment across all levels of nutrients. It is possible that this is related to a need for some fine sediment for case building (Pollard & Yuan, 2010). Overall, deposited fine sediment seems to have acted as a stressor in my study, with negative effects on most invertebrate taxa and hence also for biotic indices based on species sensitivities such as MCI and EPT taxon richness. Likely mechanisms for such patterns are reduced habitat quality, impaired respiration due to clogging of gills or reduced oxygen levels, and impaired feeding due to reduced food quality or physical harm to feeding apparatus (Wood & Armitage, 1997).

2.5.3 Single-stressor and multiple-stressor responses

A limitation inherent to a survey approach is that patterns related to sediment and nutrients are the result of correlation and that other variables (measured and unmeasured) were not controlled and hence may exert an influence. Nevertheless, single-stressor patterns were found only for chlorophyll a (subsidy-stress response to sediment) and *P. antipodarum* (positively related to sediment) while multiple-stressor patterns (both simple and complex) occurred in ten cases, supporting my fourth hypothesis that multiple-stressor effects should be common. Patterns in relation to sediment were generally robustly modelled (except for *Pycnocentrodes*), whereas nutrient models were uncertain in several cases (95 % CI of the regression coefficient included zero). This is probably because the mechanistic effects of fine sediment are more direct compared to those of augmented nutrient concentrations, which affect invertebrates mainly indirectly via stimulation of algal production, a variable that is also influenced by many other factors (Dolédec *et al.*, 2006; Statzner & Bêche, 2010) and which can itself produce both subsidy and stress responses. Such complexity may help explain why inconsistent nutrient effects have also been reported in previous studies (Friberg *et al.*, 2010; Yuan, 2010).

Macroinvertebrate traits can be expected to have the closest mechanistic link between stressor and response and may therefore provide a powerful tool for understanding multiple-stressor effects in streams (Statzner & Bêche, 2010). Three of nine traits tested showed significant relationships with my stressors, and one showed a simple multiple-stressor pattern. ‘Surface egg laying’ declined with both stressors (without interaction), confirming previous studies (Dolédec *et al.*, 2006; Magbanua *et al.*, 2010). Dolédec *et al.* (2006) reasoned that this reflects the unsuitability for oviposition of habitat smothered by fine sediment or overgrown with excessive algae. Given that both stressors make this oviposition strategy less favourable via essentially the same smothering mode of action, it is not surprising that their combined effect is simply additive, a pattern also reported by Townsend *et al.* (2008). Surface egg laying is a feature of most EPT taxa, helping to explain why their taxon richness followed the same simple multiple-stressor pattern. The New Zealand Macroinvertebrate Community Index also exhibited a simple multiple-stressor pattern. The decline of this index with increasing nutrients was expected given that it was developed in relation to organic pollution with its associated nutrient enrichment (Boothroyd & Stark, 2000). However, and more interestingly, it also declined with increasing sediment levels.

Complex multiple-stressor patterns occurred in six cases, although the interactive effect was uncertain in three of these. Across the entire range of each stressor, synergistic positive relationships were observed for the traits ‘more than two reproductive cycles per individual’ and ‘single individual reproduction’, confirming earlier findings from New Zealand streams (Townsend *et al.*, 2008). Although these two traits were correlated across my invertebrate taxa (r = 0.65), probably due to phylogenetic constraints (Menezes *et al.*, 2010), the correlation between the traits across my stream sites was considerably stronger (r = 0.96), implying a real relationship with the stressors and not just a pattern arising from their interdependence across taxa (Statzner & Bêche, 2010). An overall increase in the relative representation of each trait with increasing stressor levels probably reflects the importance of population resilience (through rapid reproduction and population growth) in the face of environmental disturbance (Dolédec *et al.*, 2006). Oligochaetes showed a similar synergistic positive pattern, and their ability to reproduce quickly probably contributes to their success, relative to other invertebrates, under high stress of fine sediment and nutrients (Wood & Armitage, 1997; Matthaei *et al.*, 2006; Larsen *et al.*, 2009; Friberg *et al.*, 2010).

Overall synergistic negative patterns were found for *Deleatidium* and % EPT (not entirely independent because *Deleatidium* made up 52 % of all EPT individuals). For these two variables, increasing nutrient concentrations seemed to provide a subsidy either initially or along the entire gradient but only when deposited fine sediment levels were low. This pattern confirms previous findings (Townsend *et al.*, 2008) and adds weight to the suggestion that fine sediment is the more pervasive stressor, able to overwhelm subsidy effects of nutrients. Broekhuizen *et al.* (2001) studied the grazing behaviour of *Deleatidium* and found that even low levels of contamination of algae with inorganic particles resulted in less energy being ingested.

2.5.4 Stressors compared: deposited fine sediment vs. nutrients

My fifth and last hypothesis that fine sediment augmentation would have a more detrimental relationship with ecological variables than nutrients was clearly supported and confirms findings of earlier observational (Niyogi *et al.*, 2007; Townsend *et al.*, 2008) and experimental studies (Matthaei *et al.*, 2010). Whenever ecological variables were related only to a single stressor (*P. antipodarum* and chlorophyll a), this was fine sediment. Moreover, in the multiple-stressor responses the relative strength of the relationship with sediment was generally much larger than that of nutrients (average size of main effect 0.50 for sediment and 0.17 for nutrients). The highest sediment effect size of 0.66 (negative direction) was recorded for *Deleatidium*, while the highest nutrient effect size of 0.38 (negative direction) was recorded for % EPT. Finally, there was generally less uncertainty about fine sediment in contrast to nutrient effects (95 % CIs rarely included zero).

2.5.5 Management implications

When deciding upon measures to prevent degradation or to mitigate adverse land-use effects in streams, it is important to know not just the state of biological indicators of stream condition, but also the current state of potential stressors and the relationships between stressors and indicators. I have shown that the relationships between sediment, nutrients and macroinvertebrate response variables are not always straightforward, and I report several cases of apparent complex multiple-stressor responses. Thus, it is important for resource managers to consider both stressors when making decisions. Streams where erosion and consequent sedimentation are not an issue and where nutrient concentrations are not excessive will not be a priority for management action. On the other hand, where sedimentation is appreciable, even a small increase in nutrient concentrations may lead to markedly lower stream condition and require management intervention.

Following from the pioneering work of Waters (1995), my results add to the growing evidence about the adverse effects in streams of deposited fine sediment on and in the streambed (Townsend *et al.*, 2008; Larsen & Ormerod, 2010; Matthaei *et al.*, 2010). This new knowledge needs to be taken into account by resource managers, who generally do not include sediment in their current routine monitoring of stream condition. Advantages of gathering this information in the future include (i) knowledge of the state of fine sediment and nutrients will allow managers to identify the most effective mitigation measures to improve stream condition, (ii) long-term datasets collected by water management authorities could be used to develop national and international sediment and nutrient guidelines, and (iii) the reporting of sediment levels in streams will raise public awareness of the issue.

CHAPTER 3 Multiple-stressor effects on macroinvertebrate communities: A mesocosm experiment

3.1 Summary

To gain further evidence for causal relationships between multiple stressors and invertebrate response variables in stream ecosystems, I adopted an experimental approach where individual and combined effects of the two focal stressors, augmented deposited fine sediment and nutrient concentrations, could be isolated from other environmental variables and disentangled. Using a replicated regression design, I manipulated eight levels each of fine sediment and nutrients along broad stressor gradients in a total of 128 stream mesocosms fed by water and organisms from a nearby river to determine the shapes of macroinvertebrate responses across both stressor gradients after 21 days of exposure. I tested (1) the subsidy-stress hypothesis for each stressor, (2) whether sediment and nutrients operated as single or multiple stressors and whether they interacted with each other, and (3) whether sediment effects were more pervasive than those of augmented nutrient concentrations. Subsidy-stress patterns occurred frequently for densities of common invertebrate taxa and community-level variables along both stressor gradients. Sediment addition provided an initial subsidy only for some pollution-tolerant taxa and total invertebrate density, whereas it negatively affected all other taxa, total taxon richness and density and richness of pollution-sensitive EPT taxa (mayflies, caddisflies and stoneflies). By contrast, nutrient enrichment provided an initial subsidy for the densities of two common EPT taxa (*Deleatidium* and *Psilochorema*), total EPT density, invertebrate and EPT taxon richness. Grazers and filter-feeders decreased proportionally while deposit-feeders and predators increased with rising sediment levels, whereas only filter-feeders were negatively affected by increasing nutrient concentrations. Burrowers increased proportionally and species with a spherical body shape or low body flexibility became rarer with increasing sediment levels, probably reflecting a shift in habitat quality. Few invertebrate variables were affected by only a single stressor (always fine sediment), whereas the majority of variables showed multiple-stressor patterns, which were in all but two instances simply additive. Only EPT richness and the density of Chironomidae followed complex patterns due to synergistic interactions producing stronger negative effects of each individual stressor across the gradient of the second stressor. Overall, fine sediment was a more pervasive stressor than augmented nutrient concentrations, showing stronger negative effects on the density of individual taxa, functional feeding groups and widely used stream condition metrics. Overall, the results of my experiment imply that managing both fine sediment and nutrient inputs is crucial to reach or maintain good stream condition but priority for management action should be given to streams receiving excessive inputs of fine sediment.

3.2 Introduction

Ecological degradation of streams and loss of freshwater biodiversity are major concerns (Strayer & Dudgeon, 2010), in particular due to ongoing agricultural land-use intensification, identified as the main driver of these processes in New Zealand and other parts of the world (Malmqvist & Rundle, 2002). A major challenge in freshwater management today is to deal with multiple stressors that arise from human activities and are further exacerbated by climate change (Paine *et al.*, 1998; Sutherland *et al.*, 2006; Ormerod *et al.*, 2010). An understanding of the relative strengths of each individual stressor and their combined multiple-stressor effects in stream ecosystems is crucial for managers to implement the most effective actions to produce positive outcomes and avoid 'ecological surprises' that arise from complex interactions between stressors (Paine *et al.*, 1998).

Two major stressors contributing to declines in stream condition in agricultural landscapes, augmented deposited fine sediment and nutrient concentrations, have sometimes produced complex multiple-stressor outcomes in population-level and community-level response variables (Townsend *et al.*, 2008; Chapter 2), highlighting the need to take both stressors into account. The mechanistic pathways for observed effects on ecological responses are still far from being completely understood (Evans-White *et al.*, 2009; Larsen & Ormerod, 2010; Yuan, 2010), and an important step forward for protection and restoration of stream ecosystems would be to quantify stressor-response relationships and identify thresholds of harm (Dodds *et al.*, 2010; Friberg, 2010). These relationships are often nonlinear (Wang *et al.*, 2007; Dodds *et al.*, 2010) and, because fine sediment and nutrients have the potential to interact, their effects need to be quantified simultaneously along both gradients. For example, using a survey approach, I have shown for stream invertebrate communities in Chapter 2 that augmented nutrient concentrations may produce a subsidy-stress response at low levels of fine sediment but at high sediment levels the effects of increasing nutrient concentrations may be negative along the entire gradient.

Despite the common co-occurrence of multiple stressors in running waters, most observational studies have only quantified stressor-response relationships for single stressors (Heino *et al.*, 2007; Niyogi *et al.*, 2007; Friberg *et al.*, 2010). The failure to take into account potential interactions amongst multiple stressors might help explain why researchers have sometimes reported conflicting results, especially in regard to the effects of increasing nutrient concentrations (Yuan, 2010). Inconsistencies might also arise in comparisons of studies that investigated gradients of different lengths, in particular given the knowledge that augmented nutrients can produce subsidy-stress responses. Furthermore, a common drawback of field survey approaches is that variation in natural environmental factors might confound the effects of focal anthropogenic stressors so that causal inference has to be done with extreme care. This is an issue even if multiple-stressors gradients are under investigation, first because the stressors of interest might co-vary making it impossible to disentangle their individual effects (Niyogi *et al.*, 2007), and second because one of the focal stressors might co-vary with an unmeasured concomitant variable, which might have caused the effect (Miltner, 2010). Thus, nutrient concentrations may in fact be a proxy for a suite of other pollutants (Miltner & Rankin, 1998). It is possible to minimize these shortcomings by careful design of field surveys and measurement of a suite of multiple environmental variables. However, to establish causal links between anthropogenic stressors and ecological consequences, experimental work also needs to be carried out (Adams, 2003; Friberg, 2010). Experimental approaches can isolate effects of the focal stressors by minimizing confounding factors that contribute to the determination of invertebrate community structure and hence help to disentangle individual from combined effects (Townsend *et al.*, 2008). But it has to be admitted that experiments also have their limitations, typically related to the small temporal and spatial scales over which stress effects are investigated, and the extent to which results can be extrapolated to the real world.

The aim of this study was to investigate individual and combined effects of augmented nutrient and surface sediment levels on macroinvertebrate response variables in a mesocosm experiment. I used a broad range of stressor levels along both gradients to permit precise definitions of stressor response patterns, with a view to providing resource managers with information about potential stressor thresholds of harm beyond which ecosystems should not move. In addition, I tested the following hypotheses which were based on the findings of previous multiple-stressor research in New Zealand streams (Townsend *et al.*, 2008; Matthaei *et al.*, 2010; Chapter 2): (1) augmented sediment and nutrients can act as a subsidy to invertebrate response variables at low levels but as a stressor at high levels, (2) invertebrate variables show a range of responses to sediment and nutrients including no effect, single-stressor effect, simple multiple-stressor effect (no interaction) or complex multiple-stressor effect (interaction), and (3) fine sediment augmentation produces stronger stressor effects and therefore has a more detrimental impact on response variables than nutrient augmentation.

3.3 Methods

3.3.1 Study site

The mesocosm experiment was conducted from 5 March to 9 April 2008 (Austral late summer/early autumn) with stream water supplied from the Kauru River in North Otago on New Zealand's South Island (170°44.6 ′ E, 45°6.5 ′ S, 98 m a.s.l.). The Kauru is a flood-prone 3rd-order stream with a mean annual low flow of 0.117 m^3/s and a catchment area of 124 km^2, recorded at a monitoring site (Otago Regional Council) 300 m upstream of the experimental set-up. The vegetation in the upstream catchment is predominantly native tussock grass and exotic pasture used for low-intensity sheep and beef farming. The river was chosen for its relatively nutrient-poor status (see nutrient concentrations in non-enriched channels below) and its diverse macroinvertebrate and algal communities (Herrmann, 2009; Liess *et al.*, 2009).

3.3.2 Experimental design

I used a replicated regression design (Gotelli & Ellison, 2004; Cottingham *et al.*, 2005) with a total of 128 experimental units (circular flow-through stream mesocosms). Eight levels of deposited fine sediment (inorganic particles less than 2 mm in diameter; Zweig & Rabeni, 2001) were crossed with eight nutrient concentrations (both N + P enriched) and two replicates of each treatment combination (Figure 3.1). The nutrient treatments were arranged in eight blocks (each consisting of 16 mesocosms) and nutrient levels randomly assigned. Within each nutrient block, sediment levels (8) and replicates (2) were randomly assigned to the 16 mesocosms. The experiment ran for five weeks, with a two-week precolonization period and a three-week manipulative period during which stressor treatments were in place. Both stressors were introduced on the same day (day 0). Fine sediment was added once (and remained in all sediment-added channels until day 21) whereas nutrients were added continuously at constant concentrations for 21 days. Treatment levels of each stressor were chosen to produce gradients from low to extremely high values, simulating increasing levels of anthropogenic stress from low-impact, near-pristine conditions to extremely high-impact levels.

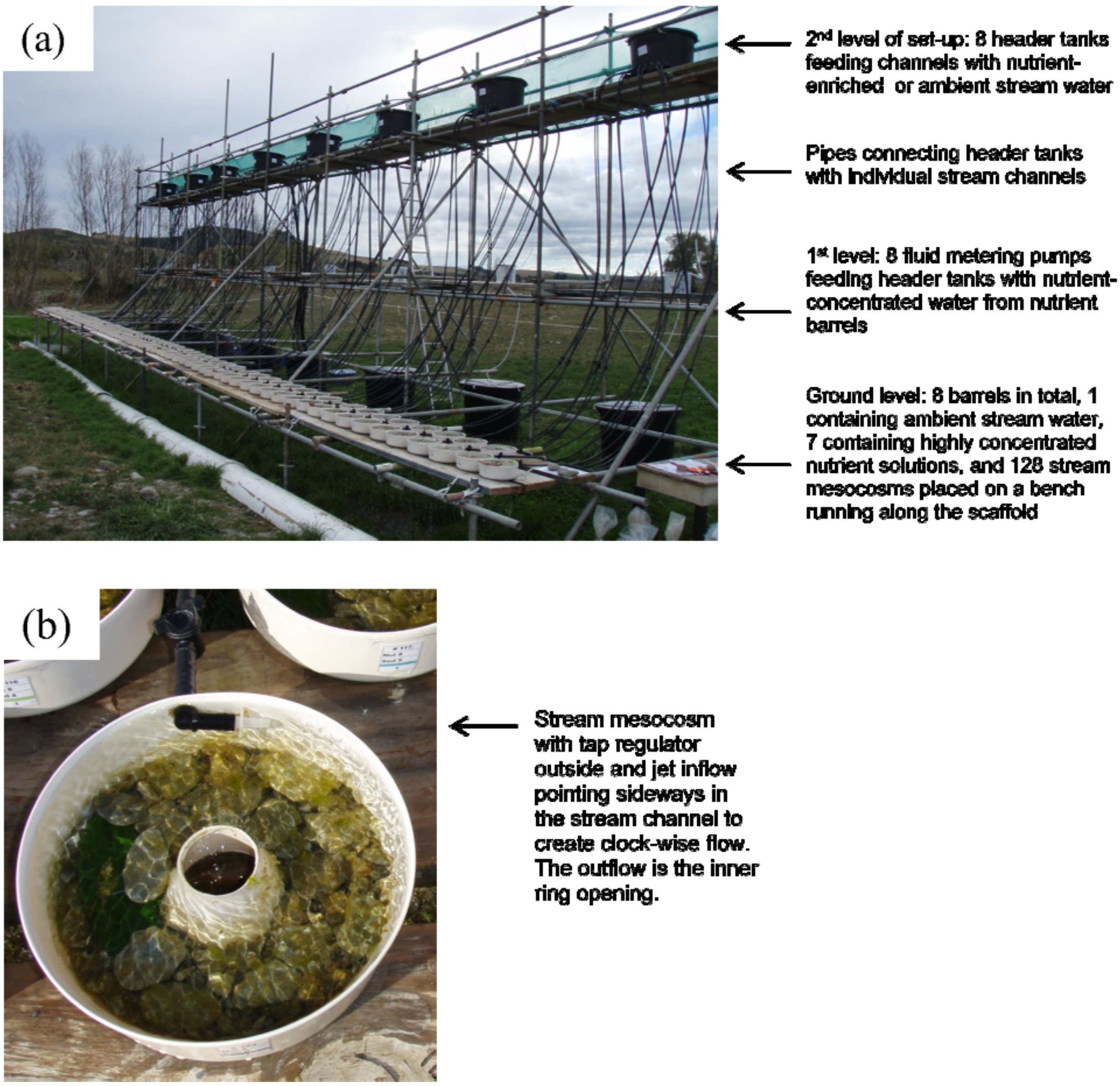


Figure 3.1 (a) Photo of the experimental set-up consisting of a three-level scaffold and 128 stream mesocosms. The eight nutrient treatment levels were randomly assigned to the eight header tanks and the eight sediment levels and replicates were randomly assigned within each nutrient block. (b) Photo of a circular stream mesocosm taken at the end of the two-week precolonisation period.

Nutrient enrichment was achieved by the combined addition of nitrogen (N) and phosphorus (P) because agricultural land use generally causes an increase in both elements and primary production in running waters is typically co-limited by these two nutrients (Francoeur, 2001; Elser *et al.*, 2007). The N:P ratio was kept constant across target nutrient concentrations because I could find no evidence of a systematic variation in ratio with land-use intensity. The lowest level of nutrients was determined by the ambient stream water conditions and the highest level was chosen to be above those generally recorded in highly enriched streams in intensive agricultural catchments in New Zealand (Hamill & McBride, 2003; Buck *et al.*, 2004; Larned *et al.*, 2004; Wilcock *et al.*, 2006). Enrichment levels covering a similar range have also been recorded in agricultural streams in Europe (Jarvie *et al.*, 2008; Friberg *et al.*, 2010) and North America (Smith *et al.*, 2007; Zheng *et al.*, 2008; Miltner, 2010; Yuan, 2010). The target nutrient concentrations from level 2 were set along an evenly spaced logarithmic scale to maximize statistical power and, given previous knowledge of response shapes to increasing nutrient concentrations (Smith *et al.*, 2007; Friberg *et al.*, 2010; Yuan, 2010), to provide a more informative regression line (Gotelli & Ellison, 2004). Hence, more levels fall in the lower concentration range where the change in an ecological response variable may be expected to be steepest or to follow a unimodal shape. Conversely, fewer levels fall in the range of extremely high concentrations where the change in an ecological response variable may be expected to be less steep or even zero. Accordingly, nutrient levels were set to be 1.9 times higher than those of the previous level except for level 2, for which I expected a similar concentration ratio but this was dependent on the actual ambient concentrations of the Kauru River. For dissolved inorganic nitrogen (DIN, see below), the target concentrations of the eight nutrient treatments were ambient, 113, 214, 406, 772, 1466, 2786, and 5293 µg/L, respectively. For dissolved reactive phosphorus (DRP), the corresponding targets were ambient, 9, 17, 32, 62, 117, 223 and 423 µg/L. These nutrient concentrations were achieved in each header tank by continuously dripping in a highly concentrated solution of nitrate ($NaNO_3$) and phosphate (KH_2PO_4), which had been made up in large barrels from which water was pumped by battery-driven fluid metering pumps (FMI CERAMPUMP® Lab Pump Model QBG). The nutrient barrels had to be refilled once during the experiment. Dissolved nutrient concentrations in each mesocosm were monitored weekly by taking a filtered water sample (ADVANTEC GC-50 glass fiber filters) to determine NO_2^--N, NO_3^--N and NH_4^+-N (combined and reported as DIN) and DRP using standard methods (APHA, 1999). Average nutrient values ± 1 SE across the 16 stream mesocosms of each treatment level are presented in Figure 3.2a. The N:P ratio was calculated from DIN and DRP and given as a mass ratio (Figure 3.2a), which ranged between 9.5 and 15.5 for nutrient levels 2 to 8. This ratio was higher, at 30.6, for level 1 (the ambient value for the Kauru River). The N:P ratio of nutrient levels 2 to 8 are likely to indicate co-limitation between N and P whereas level 1 is more likely to be P-limited according to a New Zealand study which classified a ratio between 7:1 and 15:1 by mass as co-limited and those above 15:1 as P-limited (McDowell *et al.*, 2009).

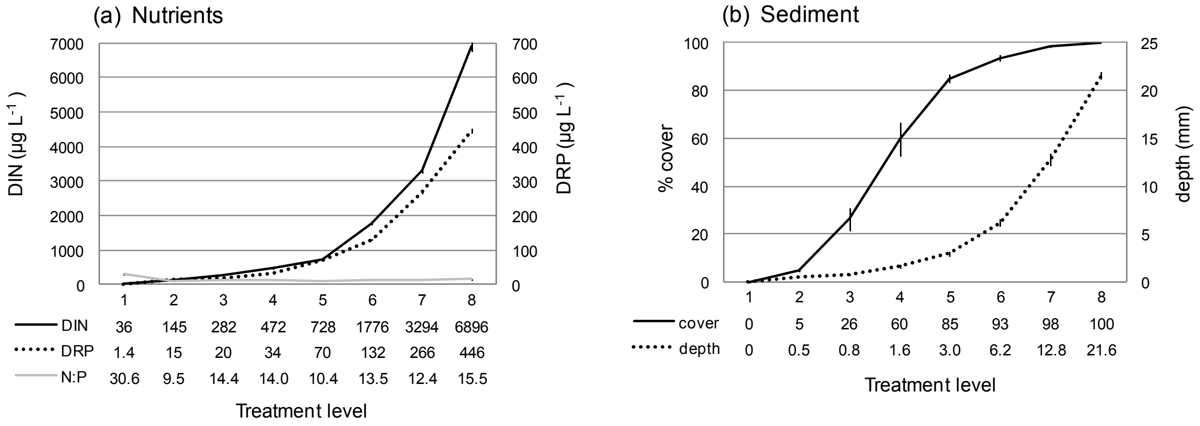


Figure 3.2 Actual stressor conditions achieved in the eight treatment levels (mean ± 1 SE for 16 stream mesocosms exposed to each level) of (a) nutrients and (b) deposited fine sediment. Note that where error bars are not visible they are very small. Deposited fine sediment was assessed once during the experiment (on day 8) as % sediment cover and sediment depth, and dissolved N and P concentrations were analyzed in water samples taken on three occasions (days 6, 12 and 16) and averaged for each mesocosm. N:P is the ratio of DIN and DRP by mass. For a comparison with the target levels of both stressors please see corresponding text.

Fine sediment levels were targeted to range between 0 and 100 % cover of the streambed and between 0 and 20 mm in depth. These sediment values were based on two surveys of agricultural streams in the Otago (Matthaei *et al.*, 2006) and Southland provinces (Chapter 2) of New Zealand's South Island. Trials were performed to determine the weight of sediment to be added to achieve these sediment cover and depth targets. No fine sediment was added to the lowest treatment level but from level 2 sediment levels were set along an evenly spaced logarithmic scale. The amount of sediment added to each treatment level was 0, 30, 54, 97, 175, 315, 567 and 1020 g, respectively. Natural fine sediment with an average grain size of 0.2 mm was sourced from the floodplain of the Taieri River, another Otago river, and amounts for each treatment level were weighed out in advance. Fine sediment was distributed equally within each stream mesocosm and the water flow was stopped briefly (ca. 2 min) during this process for sediment to settle on the bed. Almost all sediment had been deposited on the surface by the time flow was restarted and hardly any sediment was lost. The water flow initially redistributed the fine sediment, which then stayed in place for the remaining three weeks of the experiment with minimal further loss. The % cover of fine sediment on the bed surface and sediment depth in each mesocosm was recorded once (Figure 3.2b). The former was visually estimated and depth was given as an average value calculated from four measurements with a ruler at one random spot (where sediment was present) within each quarter section of the mesocosm.

Each circular stream mesocosm (25-cm external and 5-cm internal rim diameter, 9 cm high, nylon Microwave Ring Moulds; Interworld, Auckland, New Zealand) contained 500 ml of small to medium-sized gravel from the river floodplain, washed and sieved to remove particles smaller than 2 mm in diameter, and 15 randomly selected flat stones (b-diameter 3-4 cm) embedded in the surface of the gravel substratum. The gravel substratum plus surface stones was about 20 mm deep, and grain size of the benthic habitat (surface area 0.04 m^2) before addition of fine sediment ranged between 3 and 40 mm (average 23 mm) to simulate the natural substratum of small sheep/beef farmland streams in the Otago region (Matthaei *et al.*, 2006; Townsend *et al.*, 2008). To each mesocosm were added one mahoe leaf pack and cotton strips as part of the study of multiple-stressor effects on organic matter breakdown described in Chapter 5.

During the five-week experiment, the mesocosms were supplied with natural stream water pumped at a constant rate from the Kauru River. Two centrifugal pumps (Onga 415, capacity 300 L/min each) delivered water through 80 m of 50-mm polythene piping to a manifold, which split up flow equally through a further 10 m of 25-mm piping leading into eight header tanks sitting on the second level of a scaffold. The water level of the header tanks was regulated by ball-cocks and each tank fed 16 individual mesocosms with water by gravity through another 4 m of 13-mm polythene piping (Figure 3.1a). The average residence time of water in the header tanks was four minutes. The water entered each mesocosm through an adjustable inflow jet pointing sideways to create a circular flow (flow rates of 1.9 ± 0.1 L/min (mean ± 1 SD), measured once in a subset of five randomly selected mesocosms per header tank, and adjusted if necessary at least every second day) and flowed out over the inner circular opening of the mesocosm (Figure 3.1b). Current velocity was fastest towards the outer edge of the mesocosms (mean 0.13 ± 0.012 m/s (1 SD)) and slower near the inner edge. Keeping flow constant while adding different amounts of fine sediment necessarily influenced water depths in the mesocosms, which ranged from 31 to 59 mm with an average of 48 mm.

During the first two weeks all mesocosms were colonized by benthic algae and microorganisms arriving in inflowing water and a periphyton mat, showing rapid growth and with sloughing apparent, developed on the substratum surface (Figure 3.1b). Algae that grew on the sides of the mesocosms were removed every second day to avoid overgrowth of the mesocosm substrata. The mesocosms were also colonized by macroinvertebrates that could pass through a cylindrical 4.5-mm metal mesh cover that prevented the pump inflows in the river from clogging. Nylon nets (mesh size 500 µm) fitted at the inflow jet of one mesocosm per header tank for 49 hours starting from mid-day on day 16 caught an average of 56 macroinvertebrates (range 22-96). The average density of drifting invertebrates immigrating into the stream mesocosms from the Kauru River (about 1000 individuals per 100 m^3 averaged across each 49-h sampling period) was higher than those observed in studies in agricultural streams where drift sampling spanned 24 hours, both in New Zealand (James *et al.*, 2009; about 10 individuals per 100 m^3) and in Wales (Larsen & Ormerod, 2010; up to about 50 individuals per 100 m^3). This comparison indicates that natural colonisation of my experimental mesocosms was highly effective. Amongst those drifting individuals caught there were an average of 10 taxa (range 5-17), including caddisfly larvae longer than 7 mm, but with more than 60 % of individuals 2 mm or smaller. A total of 22 taxa arrived via the mesocosm inflows including the mayfly *Deleatidium* spp., the megalopteran *Archichauliodes diversus* Walker, eight caddisfly taxa (cased and free-living), three dipterans, two beetles, two gastropods, three microcrustaceans, Oligochaeta and two other taxa. To further enhance the richness and abundance of the macroinvertebrate community I also seeded macroinvertebrates from the Kauru River on the day before the stressors were introduced (day -1). These were collected by kick-net sampling (frame 60 cm wide, 40 cm tall; mesh size 200 µm) from 16 random bed patches in riffle habitats in an 80-m reach of the river. Each kick sample comprised roughly an area of 0.36 m^2, equivalent to the combined substratum surface area of eight mesocosms. These kick samples were added to the header tanks (two per tank) from where macroinvertebrates could be expected to distribute randomly to the mesocosms.

Water temperature in eight mesocosms and in the Kauru River at the pump inflows was measured by data loggers (Hobo Water Temp Pro; Onset Computer Corporation, Bourne, MA, USA) every 5 min and temperature in the mesocosms ranged from 6.9 to 22.2°C (mean 15.3°C) during the experiment. On average, the mesocosm temperatures were 0.78°C warmer than the water in the river.

3.3.3 Invertebrate sampling and response variables

On day 21, the water flow was stopped and the macroinvertebrate community in each mesocosm was sampled, by elutriating all substratum and retrieving macroinvertebrates from a 250-µm mesh-sized sieve. Samples were preserved in 90 % ethanol in the field, stained with Rose Bengal in the laboratory and randomly subsampled (25 %) for counting and identification under a stereomicroscope (Olympus SZ51, magnification 8–40x, Olympus

Corp., Tokyo, Japan) using standard keys. Subsamples from 47 mesocosms were counted in their entirety but for the other mesocosms half the subsample was counted in the case of small and abundant taxa (Oligochaeta, Cladocera, Copepoda, Nematoda). Six community composition variables were calculated: total invertebrate density, EPT (Ephemeroptera, Plecoptera and Trichoptera) density, % EPT, invertebrate taxon richness, EPT taxon richness and Simpson's evenness index (E). In addition, densities were calculated for each of the 13 most abundant taxa (out of a total of 42), which comprised 98 % of all individuals found (Oligochaeta, Chironomidae [excluding Tanypodinae], Nematoda, Cladocera, *Deleatidium* spp., Copepoda, *Potamopyrgus antipodarum*, *Hydora* spp., *Temnocephalus* spp., Tanypodinae, Ostracoda, *Psilochorema* spp. and *Oxyethira* spp. in order of abundance). Each of these taxa was found in at least 112 of the 128 mesocosms. I also examined biological traits related to life history, reproduction, attachment to substratum, body shape and flexibility, feeding habits and respiration, all of which have been shown to discriminate among land uses (Dolédec *et al.*, 2006). Each trait has two or more trait categories and scores were assigned for each taxon according to its affinity with that category (see more details in Dolédec *et al.*, 2006). I expressed these trait categories, from now on called traits for simplicity, in terms of the relative abundance of the categories by weighting the standardized trait scores with the raw abundances of the taxa, as is typically done to investigate stressor effects (Statzner & Bêche, 2010). For feeding habits and attachment to substratum, I included more than one category of the biological trait. Because I expressed these traits as relative abundances, they are technically not independent of each other. Furthermore, invertebrate traits are prone to be correlated across taxa because of phylogenetic constraints, discussed in the literature as the so-called trait syndrome (Poff *et al.*, 2006; Menezes *et al.*, 2010). However, Statzner & Bêche (2010) concluded that issues related to the trait syndrome were less of a concern in reality. Because of the potential non-independence of traits and phylogenetic constraints, I calculated Pearson's correlation coefficients between all invertebrate trait responses to inform the reader of potential inherent relationships between traits. Finally, I measured the maximum length of invertebrates (excluding cerci) to the nearest 1 mm to determine average body size per sample after multiplying the mid-point of the size class by the number of individuals in the class. Nematode and oligochaete worms were excluded from these measurements because individuals of these groups were often fragmented.

3.3.4 Data analysis

To determine the effects of the two manipulated stressors (fine sediment and nutrients) on macroinvertebrate community composition, I first performed exploratory non-metric multidimensional scaling (NMDS) with R package vegan in two-dimensional ordination space using the Bray-Curtis dissimilarity matrix, which was calculated from the relative abundances of all taxa identified (including rare taxa).

I then used experimental regression analysis (Gotelli & Ellison, 2004; Cottingham *et al.*, 2005) to further explore the relationships between macroinvertebrate variables and the two stressors. To test the competing hypotheses about different shapes described in the Introduction, I used a set of linear regression models for each response variable and adopted an information-theoretic model selection approach that allows inferences to be drawn based on the relative support for the models provided by the data (Johnson & Omland, 2004). I followed the protocol of Johnson & Omland (2004) to perform the model selection procedure in five steps, as described in detail in Chapter 2.

Step 1 involved the generation of biological hypotheses. My candidate set of competing models included the null model (intercept only), the global model (intercept plus five predictor terms: fine sediment S, nutrients N, interaction SxN, and the 2nd-order polynomial terms SxS and NxN) and simplified 'nested' versions of the global model (with one or more terms removed). Simplified models were obtained by removing terms in a hierarchical fashion but if an interaction or polynomial term was retained then the lower-order terms were retained as well (Quinn & Keough, 2002).

Step 2 fitted the models to the data. I used the eight levels of each stressor as numeric values (1-8) for each of the predictor variables. Transformations of the response variables were performed where needed to meet assumptions of multiple linear regression analysis. These included square-root transformation of the densities of Oligochaeta, Nematoda, *Hydora* spp., *Temnocephalus*, Ostracoda, *Psilochorema* and *Oxyethira* and natural log-transformation of the traits burrowers, scrapers and gill respiring as well as average body size. I only calculated the set of models for response variables where the global model provided a reasonable fit to the data (i.e. when a likelihood ratio test [F-test] comparing the fit of the global model with that of the null model was significant at $\alpha = 0.05$). In cases without a reasonable fit, I concluded that the response variable was not affected by either sediment or nutrients.

Step 3 selected the top models from among the candidate set by ranking all models using the small sample unbiased AIC_c (Akaike information criterion) and then selecting those models that were supported best by the data using the cut-off rule Δ $AIC_c \leq 6$ and also omitting those models that were merely a more complex version of a model ranked more highly (Richards *et al.*, 2011).

Step 4 involved the estimation of regression coefficients of the top model set and model averaging if there was more than one top model. Model averaging produced one final model for each biological response variable with regression coefficients (plus 95% CI) that were weighted averages derived from the top models using the ‘zero method’ of Burnham & Anderson (2002); see Nakagawa & Freckleton (2010) for justification). Levels 1 to 8 of both predictor variables were centred (by subtracting their mean value from each level value) and scaled (divided by their standard deviation). Centring improves the interpretability of regression coefficients when interaction and polynomial terms are present and deals with collinearity problems that arise when 1st-order terms are highly correlated with their interactions (Quinn & Keough, 2002; Schielzeth, 2010). Scaling permits the use of regression coefficients of predictor variables as measures of effect size (Schielzeth, 2010). I also scaled all invertebrate response variables, allowing the calculation of scale-independent standardized partial regression coefficients that can be used as measures of effect size when comparing models for different biological response variables (Schielzeth, 2010). I used the R extension package MuMIn (Bartoń, 2009) for calculation of AIC_c values, Akaike weights and model averaging.

Finally, Step 5 comprised the drawing of inferences from the averaged models for each biological response variable to test my stated hypotheses.

3.4 Results

NMDS plots (Figure 3.3) revealed a gradient pattern of increasing dissimilarities of macroinvertebrate communities with increasing levels of deposited fine sediment (Figure 3.3a), but no perceptible pattern with increasing nutrient levels (Figure 3.3b).

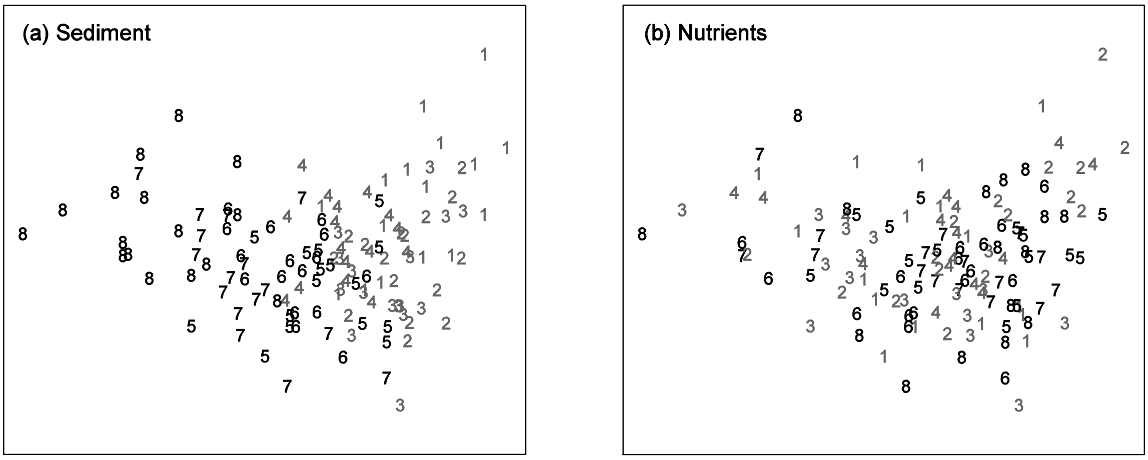


Figure 3.3 NMDS (non-metric multidimensional scaling) plots (2D stress = 0.14) of the 128 mesocosms based on dissimilarities in macroinvertebrate community composition between the mesocosms grouped by treatment levels (1-8) of (a) deposited fine sediment and (b) dissolved nutrients, and colour-coded for the four lower (1-4, grey) and higher levels (5-8, black).

At least one of the two stressors was an important predictor for 31 of the 32 macroinvertebrate response variables. The exception was *Potamopyrgus antipodarum*, as shown by the global model (1) having no better fit to the data than the null model (13, Table 3.1). For the remaining response variables, Table 3.1 presents the results of steps 2 and 3 of the model fitting procedure described in the Methods. Only a single top model was selected for 15 response variables and two or three top models for the others. Akaike weights of the 13 models add up to 1 and the weight of a model can be interpreted as the probability of this model, given the data, being the best model in the candidate set of models (Johnson & Omland, 2004). The Akaike weights of the selected models in Table 3.1 ranged from 0.08 to 0.64, the highest value being ascribed to the top model for *Deleatidium*. R^2 values were not used in model selection but are shown for each regression model in Table 3.1 to provide a general measure of model fit. R^2 values of the top models ranged from 0.04 (*Oxyethira*) to 0.71 (spherical body shape). For each response variable, Table 3.2 shows the final model (determined by model averaging, see Step 4 of the model fitting procedure) which can be used to determine the average value of the response for any sediment and nutrient level of interest. Figures 3.4, 3.5 and 3.6 present the standardized partial regression coefficients (plus 95% CIs) as standardized effect sizes (left hand panels) and generated three-dimensional response surfaces (right hand panels). For ease of comprehension, these figures show the surfaces of the non-scaled response variables along the non-centred and non-scaled levels of nutrients and sediment. Several of the final models included a term whose effect size was very small. If the partial regression coefficient of a predictor was less than 0.1, I considered it to be of no ecological significance. Note that the effect size of the main-effect term nutrients (N) or sediment (S) for several responses was less than 0.1, but this effect could not be neglected in the corresponding model because a higher-order term was present and larger than 0.1. Furthermore, given my hypotheses (see Introduction) I was interested only in positive, negative or subsidy-stress (hump-shaped) responses. In three cases (for burrower, respires using gills and average body size), there was a curvilinear relationship to sediment where the response variable remained more or less constant from zero to intermediate sediment levels but increased from intermediate to high levels so I grouped these curvilinear responses with those of a positive response. Moreover, in eight cases polynomial terms were present but the minimum or maximum was outside the range of my predictor variables or extremely close to level 1 and a subsidy effect barely perceptible; I grouped these curvilinear responses with those of a either positive (single individual reproduction) or negative response shape (Cladocera, *Deleatidium*, *Temnocephalus*, low body flexibility, spherical body shape, filter-feeder, EPT density).

Table 3.1 AIC$_c$ values, Akaike weights and R^2 values of models in the candidate set of 13 linear regression models calculated for each of 31 biological response variables where the global model (model 1) provided a statistically significantly better fit to the data than the null model (model 13). The predictor variables in the models are: S = level of deposited fine sediment (1-8, centred and scaled), N = nutrient level (1-8, centred and scaled), SxN = interaction term, SxS and NxN = 2nd-order polynomial terms. The response variables (also scaled) included (a) 12 invertebrate taxon densities (some of which were square-root transformed), (b) 12 relative trait representations (some of them log-transformed) and (c) seven community-level responses (average body size log-transformed, others untransformed). AIC$_c$ values and Akaike weights are results of Step 2 of the model selection procedure. Bold values indicate all models that were in the top model set identified in Step 3 of the model selection procedure. The underlined model is the best top model. For more details see Methods. Sample size is n = 128.

	(a)	Biological response variables (invertebrate densities)											
		Oligochaeta	Chironomidae	Nematoda	Cladocera	*Deleatidium*	Copepoda	*Hydora*	*Temnocephalus*	Tanypodinae	Ostracoda	*Psilochorema*	*Oxyethira*
Model	Predictor terms	AIC_c of models in candidate model set											
1	S+ N+ SxN+ SxS+ NxN	339.7	347.9	338.1	240.7	242.0	322.1	357.9	271.9	325.3	351.6	331.4	363.8
2	S+ N+ SxN+ SxS	340.6	354.5	338.1	238.5	248.4	320.0	358.4	269.8	330.4	349.4	341.7	363.9
3	S+ N+ SxN+ NxN	347.1	**345.7**	365.0	262.9	246.7	347.2	356.0	288.6	323.4	357.1	329.3	363.1
4	S+ N+ SxS+ NxN	**338.4**	349.6	338.4	238.7	**239.9**	320.4	356.7	271.3	323.1	350.2	330.6	364.9
5	S+ N+ SxN	347.8	352.3	364.5	260.7	252.7	345.2	356.5	286.5	328.5	355.0	339.7	363.2
6	S+ N+ SxS	**339.3**	356.0	338.4	**236.6**	246.3	318.4	357.2	269.3	328.2	**348.1**	340.8	365.0
7	S+ N+ NxN	345.8	**347.4**	364.8	260.9	**244.6**	345.5	354.8	287.9	**321.2**	355.8	**328.5**	364.2
8	S+ N	346.5	353.8	364.4	258.8	250.6	343.4	355.4	285.8	**326.3**	**353.7**	338.8	364.3
9	S+ SxS	**344.3**	354.9	**336.2**	261.4	248.7	**316.9**	356.0	**267.7**	329.5	358.3	343.9	363.2
10	N+ NxN	362.3	362.1	368.9	359.8	365.6	370.0	367.3	370.2	362.8	359.3	356.4	368.0
11	S	351.0	352.8	362.3	279.3	252.8	341.9	**354.2**	284.2	327.7	363.2	341.9	**362.5**
12	N	362.6	367.5	368.4	357.7	366.7	368.0	367.6	368.2	365.9	357.3	364.2	368.1
13	Null model	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3
Model	Predictor terms	Akaike weights of models in candidate model set											
1	S+ N+ SxN+ SxS+ NxN	0.20	0.16	0.16	0.07	0.23	0.04	0.04	0.06	0.06	0.08	0.10	0.09
2	S+ N+ SxN+ SxS	0.13	0.01	0.16	0.21	0.01	0.11	0.03	0.17	0.00	0.24	0.00	0.08
3	S+ N+ SxN+ NxN	0.00	**0.50**	0.00	0.00	0.02	0.00	0.10	0.00	0.16	0.00	0.30	0.13
4	S+ N+ SxS+ NxN	**0.38**	0.07	0.14	0.19	**0.64**	0.09	0.07	0.08	0.19	0.16	0.16	0.05
5	S+ N+ SxN	0.00	0.02	0.00	0.00	0.00	0.00	0.07	0.00	0.01	0.01	0.00	0.12
6	S+ N+ SxS	**0.24**	0.00	0.14	**0.54**	0.03	0.25	0.05	0.22	0.01	**0.46**	0.00	0.05
7	S+ N+ NxN	0.01	**0.21**	0.00	0.00	**0.06**	0.00	0.18	0.00	**0.49**	0.01	**0.44**	0.07
8	S+ N	0.01	0.01	0.00	0.00	0.00	0.00	0.13	0.00	**0.04**	**0.03**	0.00	0.07
9	S+ SxS	**0.02**	0.00	**0.40**	0.00	0.01	**0.51**	0.10	**0.48**	0.01	0.00	0.00	0.12
10	N+ NxN	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
11	S	0.00	0.01	0.00	0.00	0.00	0.00	**0.24**	0.00	0.02	0.00	0.00	**0.17**
12	N	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
13	Null model	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
Model	Predictor terms	R^2 of models in candidate model set											
1	S+ N+ SxN+ SxS+ NxN	0.25	0.20	0.26	0.66	0.65	0.35	0.14	0.56	0.33	0.18	0.30	0.10
2	S+ N+ SxN+ SxS	0.24	0.15	0.25	0.66	0.63	0.35	0.12	0.56	0.29	0.18	0.23	0.08
3	S+ N+ SxN+ NxN	0.20	**0.20**	0.08	0.58	0.63	0.19	0.14	0.49	0.33	0.13	0.30	0.09
4	S+ N+ SxS+ NxN	**0.25**	0.18	0.25	0.65	**0.65**	0.35	0.13	0.55	0.33	0.18	0.29	0.08
5	S+ N+ SxN	0.18	0.15	0.06	0.58	0.61	0.19	0.12	0.49	0.29	0.13	0.23	0.07
6	S+ N+ SxS	**0.23**	0.12	0.24	**0.65**	0.63	0.35	0.11	0.55	0.29	**0.18**	0.22	0.06
7	S+ N+ NxN	0.19	**0.18**	0.06	0.58	**0.63**	0.19	0.13	0.48	**0.33**	0.12	**0.29**	0.06
8	S+ N	0.17	0.12	0.05	0.58	0.61	0.19	0.11	0.48	**0.29**	**0.12**	0.22	0.05
9	S+ SxS	**0.19**	0.12	**0.24**	0.57	0.61	**0.34**	0.11	**0.55**	0.27	0.09	0.19	0.06
10	N+ NxN	0.06	0.06	0.01	0.08	0.04	0.00	0.03	0.00	0.06	0.08	0.10	0.02
11	S	0.13	0.12	0.05	0.50	0.59	0.19	**0.11**	0.48	0.27	0.04	0.19	**0.04**
12	N	0.04	0.01	0.00	0.08	0.01	0.00	0.01	0.00	0.02	0.08	0.03	0.00
13	Null model	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

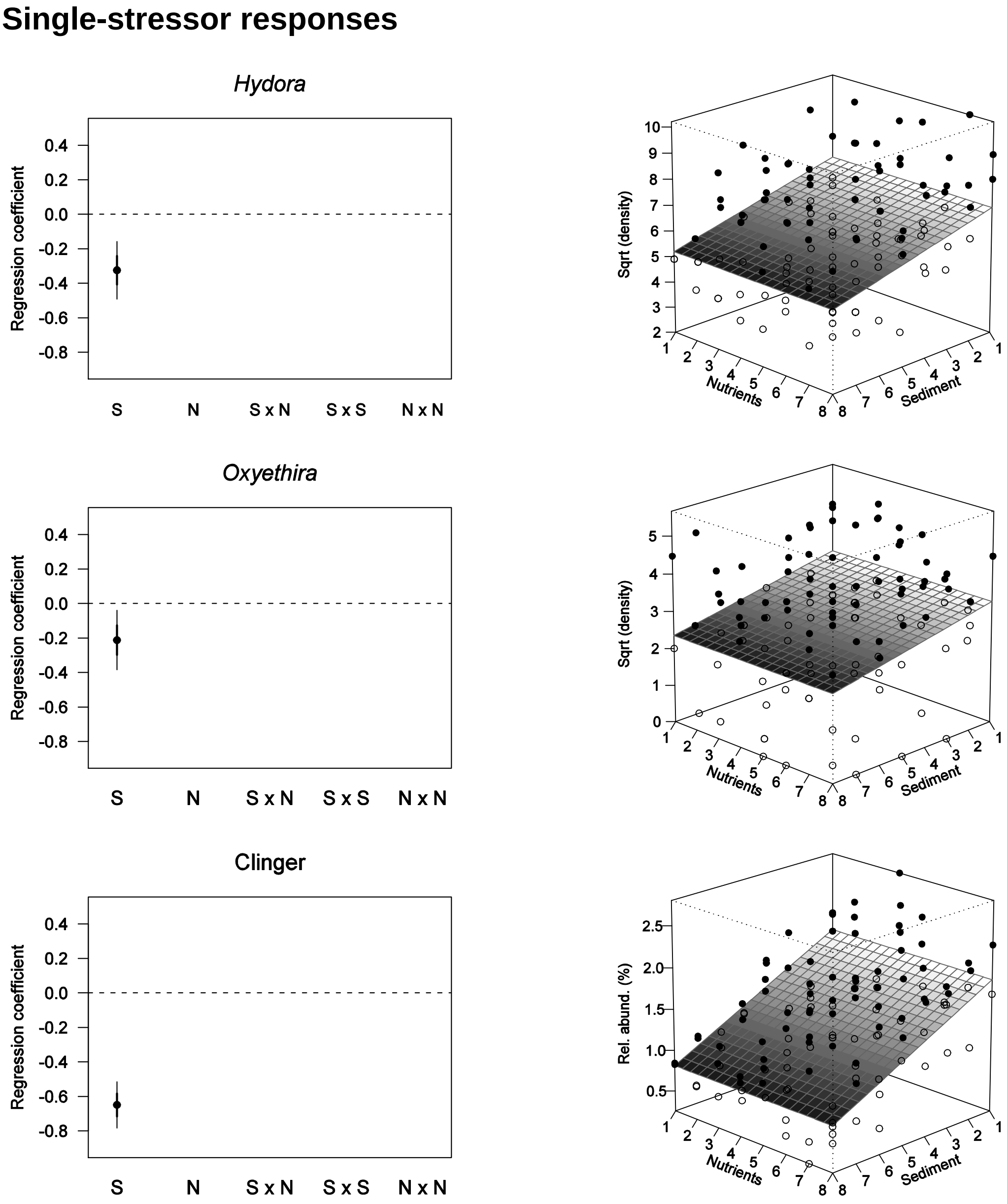
	(b)	Biological response variables (relative trait representations)											
		>2 reprod.cycles/ individual	Single ind. reprod.	Surface eggs	Burrower	Clinger	Low body flexibility	Spherical body shape	Grazer	Deposit-feeder	Filter-feeder	Predator	Respires using gills
Model	Predictor terms	AIC$_c$ of models in candidate model set											
1	S+ N+ SxN+ SxS+ NxN	329.1	271.9	300.3	293.4	303.8	319.7	218.9	312.4	265.3	244.7	273.9	361.1
2	S+ N+ SxN+ SxS	327.6	269.7	298.3	291.9	302.6	318.4	216.9	310.1	263.2	243.8	271.6	358.9
3	S+ N+ SxN+ NxN	341.9	278.0	298.1	307.7	302.1	321.3	235.8	312.4	263.1	264.8	273.0	370.4
4	S+ N+ SxS+ NxN	327.2	269.9	298.4	291.2	302.5	317.5	216.8	310.2	263.1	242.6	271.6	359.1
5	S+ N+ SxN	340.4	275.8	296.1	306.2	300.9	320.0	233.8	310.2	261.0	263.7	270.8	368.2
6	S+ N+ SxS	**325.8**	**267.7**	296.4	**289.8**	301.3	**316.2**	**214.8**	308.0	261.1	**241.8**	269.4	**357.0**
7	S+ N+ NxN	340.1	275.9	296.2	305.5	300.8	319.2	233.8	310.2	260.9	262.8	270.8	368.5
8	S+ N	338.6	273.8	**294.2**	304.1	299.6	317.8	231.8	308.0	258.9	261.7	268.7	366.3
9	S+ SxS	**330.5**	278.7	302.3	**291.8**	299.9	324.3	248.7	**307.2**	258.9	253.7	268.5	**358.8**
10	N+ NxN	365.0	364.3	365.8	367.9	369.5	362.9	357.7	369.7	370.5	364.6	370.0	366.9
11	S	342.6	284.1	**300.2**	305.7	**298.3**	325.7	261.3	**307.3**	**256.8**	271.6	**267.8**	367.9
12	N	363.5	362.2	363.8	366.2	368.0	361.3	355.7	367.6	368.4	363.0	367.9	364.8
13	Null model	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3
Model	Predictor terms	Akaike weights of models in candidate model set											
1	S+ N+ SxN+ SxS+ NxN	0.09	0.06	0.02	0.07	0.02	0.06	0.07	0.02	0.01	0.10	0.01	0.06
2	S+ N+ SxN+ SxS	0.19	0.19	0.05	0.14	0.04	0.11	0.19	0.05	0.02	0.16	0.04	0.17
3	S+ N+ SxN+ NxN	0.00	0.00	0.06	0.00	0.05	0.03	0.00	0.02	0.02	0.00	0.02	0.00
4	S+ N+ SxS+ NxN	0.22	0.17	0.05	0.21	0.04	0.17	0.20	0.05	0.02	0.29	0.04	0.15
5	S+ N+ SxN	0.00	0.01	0.15	0.00	0.08	0.05	0.00	0.05	0.05	0.00	0.06	0.00
6	S+ N+ SxS	**0.45**	**0.52**	0.13	**0.43**	0.07	**0.34**	**0.53**	0.16	0.05	**0.44**	0.12	**0.43**
7	S+ N+ NxN	0.00	0.01	0.14	0.00	0.09	0.08	0.00	0.05	0.06	0.00	0.06	0.00
8	S+ N	0.00	0.02	**0.38**	0.00	0.16	0.15	0.00	0.15	0.16	0.00	0.18	0.00
9	S+ SxS	**0.04**	0.00	0.01	**0.15**	0.14	0.01	0.00	**0.23**	0.16	0.00	0.19	**0.17**
10	N+ NxN	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	S	0.00	0.00	**0.02**	0.00	**0.31**	0.00	0.00	**0.22**	**0.46**	0.00	**0.28**	0.00
12	N	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
13	Null model	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Model	Predictor terms	R^2 of models in candidate model set											
1	S+ N+ SxN+ SxS+ NxN	0.31	0.56	0.45	0.48	0.44	0.36	0.71	0.40	0.58	0.64	0.55	0.12
2	S+ N+ SxN+ SxS	0.31	0.56	0.45	0.48	0.43	0.36	0.71	0.40	0.58	0.64	0.55	0.12
3	S+ N+ SxN+ NxN	0.23	0.53	0.45	0.41	0.43	0.34	0.66	0.39	0.58	0.58	0.55	0.03
4	S+ N+ SxS+ NxN	0.31	0.56	0.45	0.48	0.43	0.36	0.71	0.40	0.58	0.64	0.55	0.12
5	S+ N+ SxN	0.22	0.53	0.45	0.41	0.43	0.34	0.66	0.39	0.58	0.57	0.55	0.03
6	S+ N+ SxS	**0.31**	**0.56**	0.45	**0.48**	0.43	**0.36**	**0.71**	0.40	0.58	**0.64**	0.55	**0.12**
7	S+ N+ NxN	0.23	0.53	0.45	0.41	0.43	0.34	0.66	0.39	0.58	0.58	0.55	0.03
8	S+ N	0.22	0.53	**0.45**	0.41	0.43	0.34	0.66	0.39	0.58	0.57	0.55	0.03
9	S+ SxS	**0.27**	0.51	0.41	**0.46**	0.42	0.30	0.61	**0.39**	0.58	0.60	0.55	**0.09**
10	N+ NxN	0.04	0.05	0.04	0.02	0.01	0.06	0.10	0.01	0.00	0.05	0.00	0.03
11	S	0.18	0.48	**0.41**	0.39	**0.42**	0.28	0.57	**0.38**	**0.58**	0.53	**0.54**	0.00
12	N	0.04	0.05	0.04	0.02	0.00	0.05	0.09	0.01	0.00	0.04	0.00	0.03
13	Null model	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

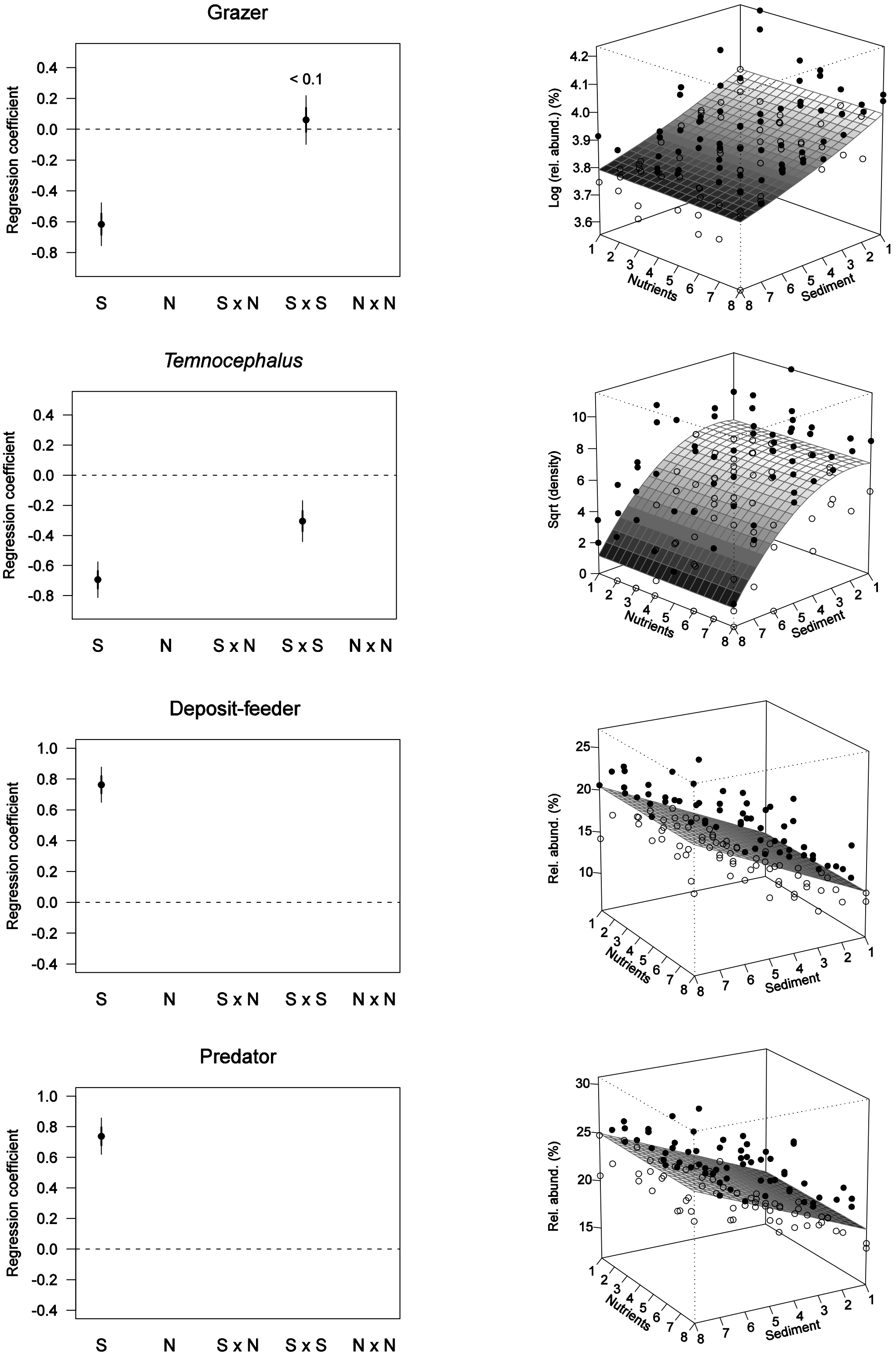
	(c)	Biological response variables (community level)						
		Invertebrate density	EPT density	% EPT	Invert. richness	EPT richness	Evenness	Average body size
Model	Predictor terms	AIC_c of models in candidate model set						
1	S+ N+ SxN+ SxS+ NxN	315.2	240.3	307.5	314.7	322.0	305.5	326.9
2	S+ N+ SxN+ SxS	317.4	250.0	305.5	321.3	333.7	306.3	324.7
3	S+ N+ SxN+ NxN	337.2	244.4	305.3	**313.1**	**320.3**	320.3	349.3
4	S+ N+ SxS+ NxN	**314.5**	**239.0**	305.3	316.5	323.6	**304.3**	325.1
5	S+ N+ SxN	338.7	253.5	303.3	319.7	332.0	320.8	347.1
6	S+ N+ SxS	**316.6**	248.6	303.3	322.8	335.0	**305.1**	**322.9**
7	S+ N+ NxN	336.2	**243.1**	303.1	**314.8**	**322.0**	319.1	347.5
8	S+ N	337.7	252.2	**301.2**	321.2	333.3	319.6	345.3
9	S+ SxS	320.8	250.7	310.2	320.7	333.9	**307.4**	**326.9**
10	N+ NxN	363.4	364.5	364.9	364.8	359.7	366.1	366.2
11	S	340.8	254.1	308.1	**319.1**	332.3	321.3	348.3
12	N	364.2	366.8	362.9	368.4	367.6	365.8	364.1
13	Null model	366.3	366.3	366.3	366.3	366.3	366.3	366.3
Model	Predictor terms	Akaike weights of models in candidate model set						
1	S+ N+ SxN+ SxS+ NxN	0.30	0.30	0.02	0.20	0.21	0.20	0.07
2	S+ N+ SxN+ SxS	0.10	0.00	0.05	0.01	0.00	0.13	0.20
3	S+ N+ SxN+ NxN	0.00	0.04	0.05	**0.46**	**0.48**	0.00	0.00
4	S+ N+ SxS+ NxN	**0.44**	**0.57**	0.05	0.08	0.09	**0.35**	0.17
5	S+ N+ SxN	0.00	0.00	0.13	0.02	0.00	0.00	0.00
6	S+ N+ SxS	**0.15**	0.00	0.14	0.00	0.00	**0.23**	**0.49**
7	S+ N+ NxN	0.00	**0.08**	0.15	**0.19**	**0.21**	0.00	0.00
8	S+ N	0.00	0.00	**0.40**	0.01	0.00	0.00	0.00
9	S+ SxS	0.02	0.00	0.00	0.01	0.00	**0.08**	**0.07**
10	N+ NxN	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	S	0.00	0.00	0.01	**0.02**	0.00	0.00	0.00
12	N	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	Null model	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Model	Predictor terms	R^2 of models in candidate model set						
1	S+ N+ SxN+ SxS+ NxN	0.38	0.66	0.42	0.39	0.35	0.43	0.32
2	S+ N+ SxN+ SxS	0.36	0.62	0.42	0.34	0.28	0.42	0.32
3	S+ N+ SxN+ NxN	0.26	0.64	0.42	**0.38**	**0.35**	0.35	0.18
4	S+ N+ SxS+ NxN	**0.38**	**0.65**	0.42	0.37	0.33	**0.42**	0.32
5	S+ N+ SxN	0.23	0.61	0.42	0.34	0.27	0.33	0.18
6	S+ N+ SxS	**0.35**	0.62	0.42	0.32	0.26	**0.41**	**0.32**
7	S+ N+ NxN	0.25	**0.64**	0.42	**0.36**	**0.33**	0.34	0.18
8	S+ N	0.23	0.60	**0.42**	0.32	0.25	0.33	0.18
9	S+ SxS	0.32	0.61	0.38	0.32	0.25	**0.39**	**0.29**
10	N+ NxN	0.05	0.05	0.04	0.04	0.08	0.03	0.03
11	S	0.19	0.59	0.38	**0.32**	0.25	0.31	0.15
12	N	0.03	0.01	0.04	0.00	0.01	0.02	0.03
13	Null model	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3.2 Intercept and regression coefficients for the final model (the top model, or averaged for the top model set) for each of the 31 biological response variables. Transformations of response variables are shown in parentheses and all of them are scaled. The predictor variables in the models are S = level of deposited fine sediment (1-8, centred and scaled), N = nutrient level (1-8, centred and scaled), SxN = interaction term, SxS and NxN = 2nd-order polynomial terms. The average of levels 1+2+3 etc. up to level 8 plus the corresponding standard deviation of both predictor variables are as follows: mean = 4.50, SD = 2.30. Sample size is n = 128.

		Standardized partial regression coefficients					
Biological response variable	SD	Intercept	S	N	SxN	SxS	NxN
Invertebrate densities:							
Oligochaeta (sqrt)	5.32	5.39	0.357	-0.204		-0.278	-0.092
Chironomidae	115.6	3.46	-0.339	-0.086	-0.111		-0.274
Nematoda (sqrt)	5.94	3.56	-0.217			-0.499	
Cladocera	192.3	2.17	-0.708	-0.284		-0.309	
Deleatidium	124.5	2.58	-0.771	0.116		-0.146	-0.179
Copepoda	88.7	2.87	-0.433			-0.453	
Hydora (sqrt)	1.74	3.49	-0.325				
Temnocephalus (sqrt)	2.80	2.18	-0.694			-0.305	
Tanypodinae	16.7	1.86	-0.522	-0.139			-0.212
Ostracoda (sqrt)	1.71	3.13	-0.200	-0.289		-0.246	
Psilochorema (sqrt)	1.28	3.67	-0.433	0.181			-0.309
Oxyethira (sqrt)	1.42	1.98	-0.212				
Relative trait representations (%):							
> 2 reproductive cycles/individual	6.33	11.4	0.428	-0.178		-0.337	
Single individual reproduction	4.21	9.86	0.695	-0.219		0.197	
Lays eggs at water surface	4.28	2.61	-0.643	0.180			
Burrower (log)	0.142	23.9	0.623	-0.098		0.308	
Clinger	0.53	2.53	-0.650				
Low body flexibility	3.91	4.03	-0.533	0.232		-0.161	
Spherical body shape	2.70	2.78	-0.753	-0.308		-0.249	
Grazer (log)	0.108	36.0	-0.616			0.061	
Deposit-feeder	4.10	3.81	0.763				
Filter-feeder	3.21	3.40	-0.729	-0.204		-0.300	
Predator	3.30	6.44	0.738				
Respires using gills (log)	0.167	18.9	0.066	0.120		0.332	
Community-level responses:							
Invertebrate density	743	4.15	-0.441	-0.180		-0.412	-0.127
EPT density	148	2.74	-0.769	0.087		-0.152	-0.210
% EPT	4.82	2.73	-0.613	0.206			
Invertebrate taxon richness	1.96	9.35	-0.566	0.003	-0.095		-0.233
EPT taxon richness	1.35	4.83	-0.496	0.081	-0.100		-0.314
Evenness	0.063	5.64	-0.555	0.127		-0.329	0.072
Average body size (log)	0.146	2.63	0.382	0.161		0.435	

Ten of the 31 final models were single-stressor models (Figure 3.4), in every case showing a relationship with fine sediment only (no term with nutrients included or effect size for nutrients less than 0.1). Five invertebrate responses showed a linear or curvilinear decline with rising levels of fine sediment (*Hydora*, *Oxyethira*, clinger, grazer, *Temnocephalus*), three showed a linear or curvilinear increase (deposit-feeders, predators, burrowers) and two showed subsidy-stress responses to sediment (Nematoda, Copepoda).




Grazer
Regression coefficient
< 0.1
S
N
S x N
S x S
N x N
Log (rel. abund.) (%)
Nutrients
Sediment
Temnocephalus
Regression coefficient
Sqrt (density)
Deposit-feeder
Regression coefficient
Rel. abund. (%)
Predator
Regression coefficient
Rel. abund. (%)

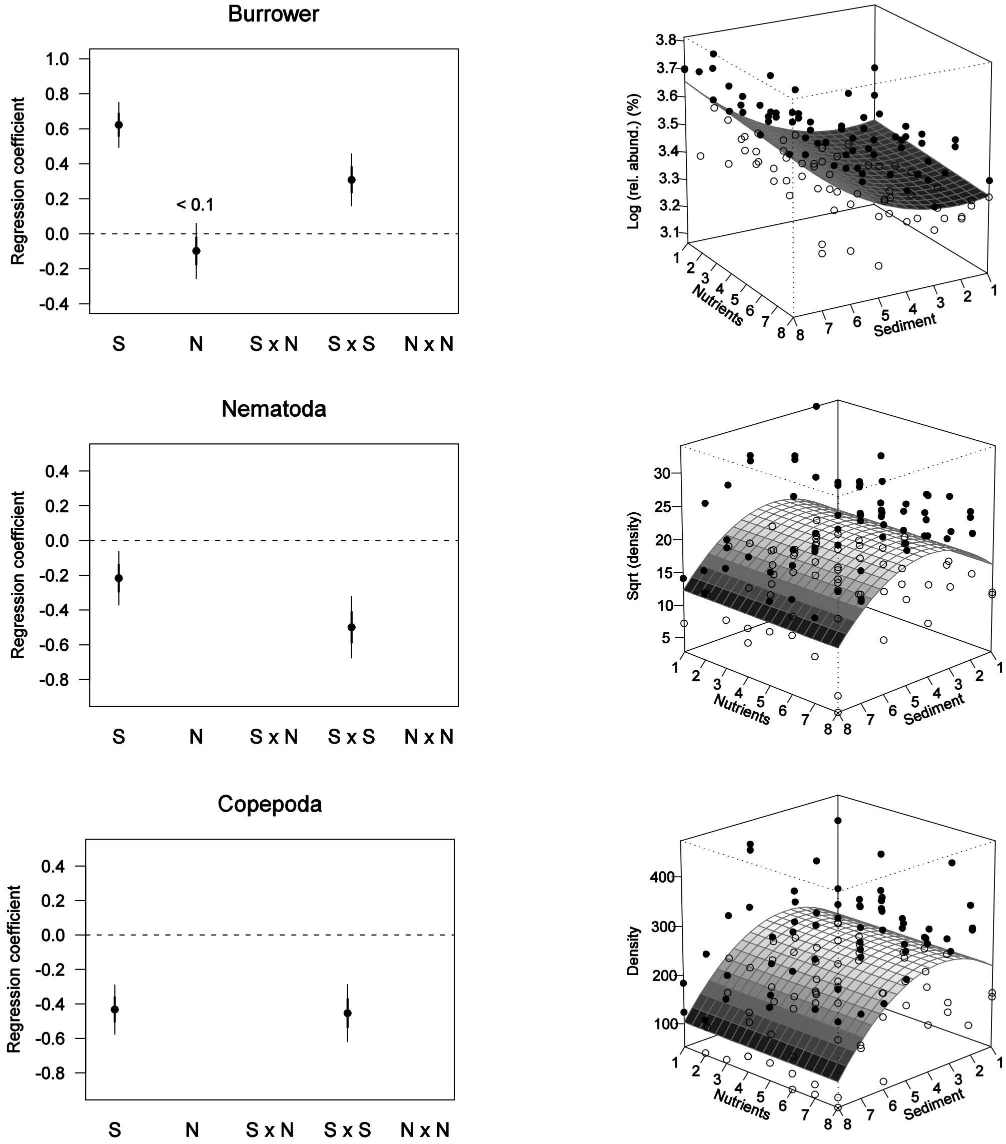
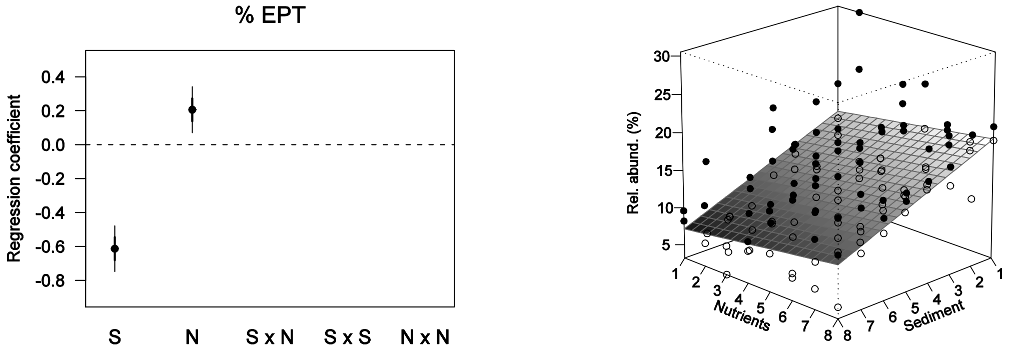
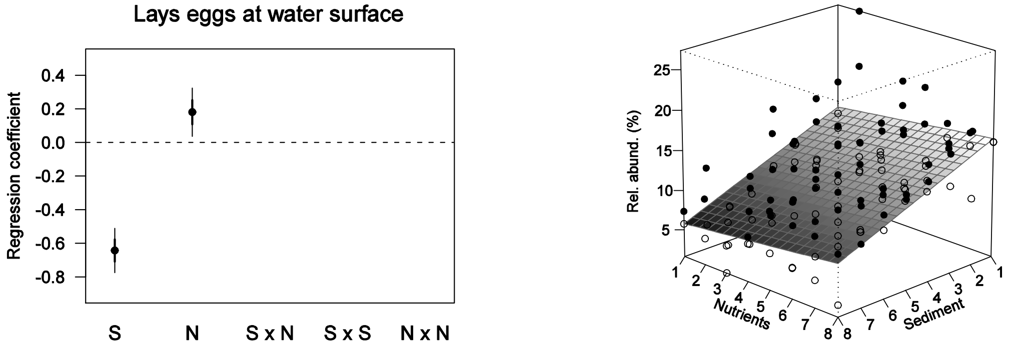


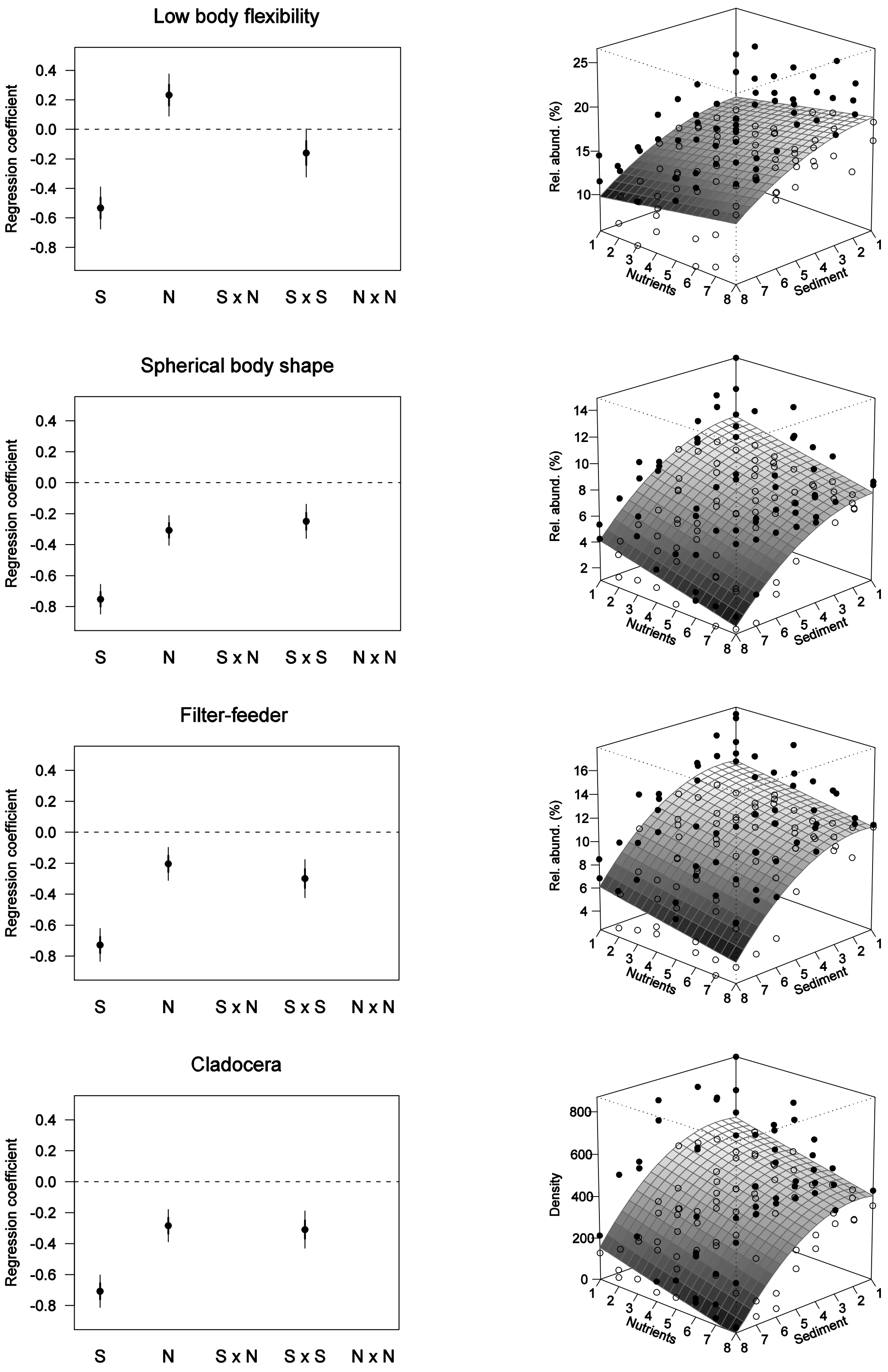
Figure 3.4 Relationships between stressor levels and invertebrate response variables for which the model selection procedure revealed a single-stressor response (no nutrient term in the final model or effect size of nutrient term less than 0.1). Standardized partial regression coefficients (plus 95% CIs) of the terms in the final model are shown on the left and three-dimensional response surfaces on the right. Note that these coefficients are also standardized effect sizes of the predictors (for example, a negative or positive effect size of sediment (S) is the reduction or increase in the response in unit standard deviations due to the main effect of sediment at the mean level of nutrients when sediment increases from the mean by one standard deviation). See Table 3.2 for details of transformations used for response variables, all of which were scaled. The predictor variables in the models are S = level of deposited fine sediment (levels 1-8, centred and scaled), N = nutrient level (levels 1-8, centred and scaled), SxN = interaction term, SxS and NxN = 2nd-order polynomial terms. The response surfaces are plotted for the entire range of stressor levels. For ease of comprehension, all three axes of the response surfaces have been plotted with non-centred and non-scaled values. Densities are given as numbers of individuals per mesocosm (surface area 0.04 m^2). Please note that the highest nutrient and sediment levels are in the front corner of each 3D plot. The data points are the observed values in the 128 mesocosms with data points that lie above the surface shown as solid circles and those below it as open circles.

Nineteen final models were simple multiple-stressor models where both stressors affected the response variable but without interactive effects between them (no interaction term in the final model or one with an effect size of less than 0.1). Combinations of different shapes to fine sediment and nutrients were manifold (Figure 3.5). Overall, to fine sediment there were 11 linear or curvilinear negative responses (% EPT, lays eggs at water surface, low body flexibility, spherical body shape, filter-feeder, Cladocera, Tanypodinae, *Psilochorema*, *Deleatidium*, total EPT, invertebrate richness), three curvilinear positive (single individual reproduction, average body size, respires using gills) and five subsidy-stress responses (Ostracoda, >2 reproductive cycles/individual, Oligochaeta, evenness, total invertebrates). To nutrients there were seven negative linear (single individual reproduction, spherical body shape, filter-feeder, Cladocera, Ostracoda, >2 reproductive cycles/individual, Oligochaeta), six positive linear (% EPT, lays eggs at water surface, low body flexibility, average body size, respires using gills, evenness) and six subsidy-stress responses (Tanypodinae, *Psilochorema*, *Deleatidium*, total EPT, invertebrate richness, total invertebrates).

Simple multiple-stressor responses


% EPT


Lays eggs at water surface

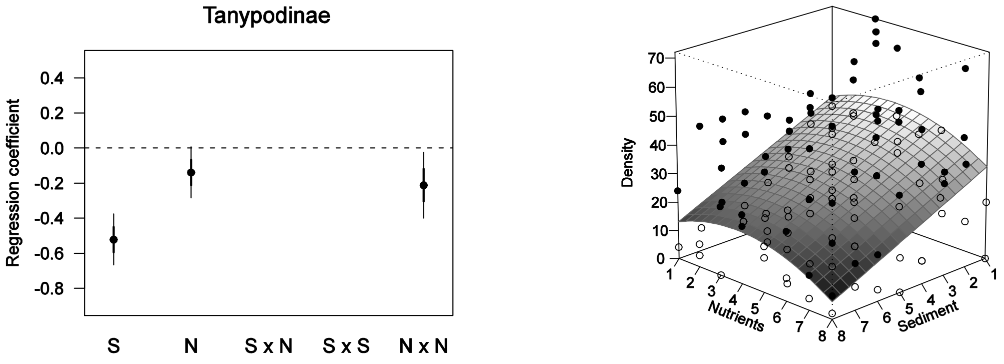


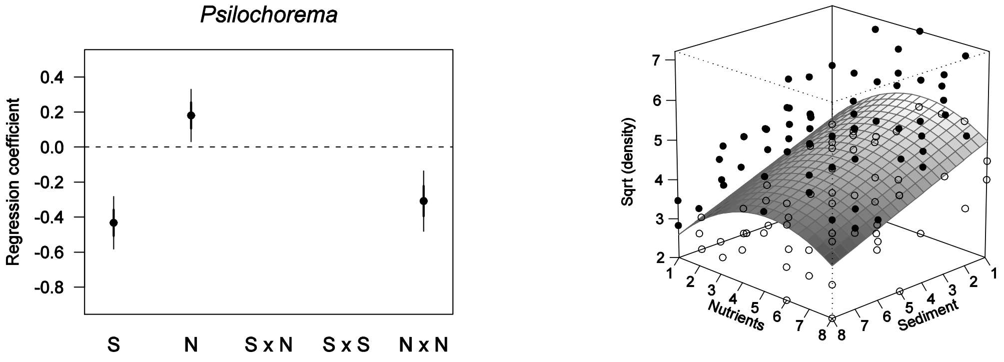
Low body flexibility

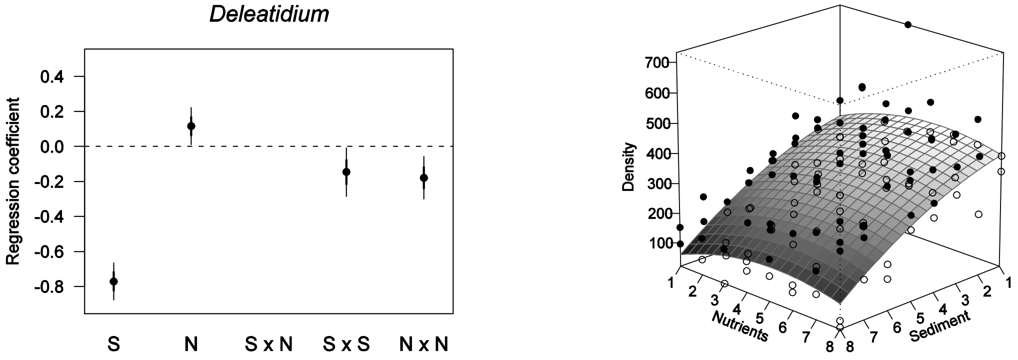
Spherical body shape

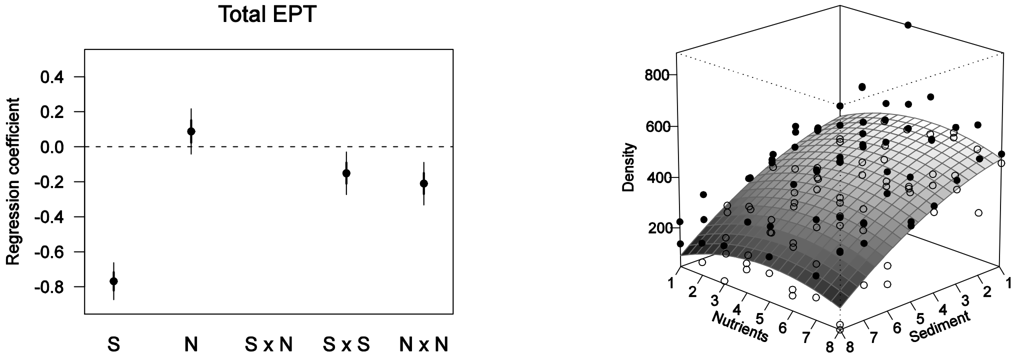
Filter-feeder

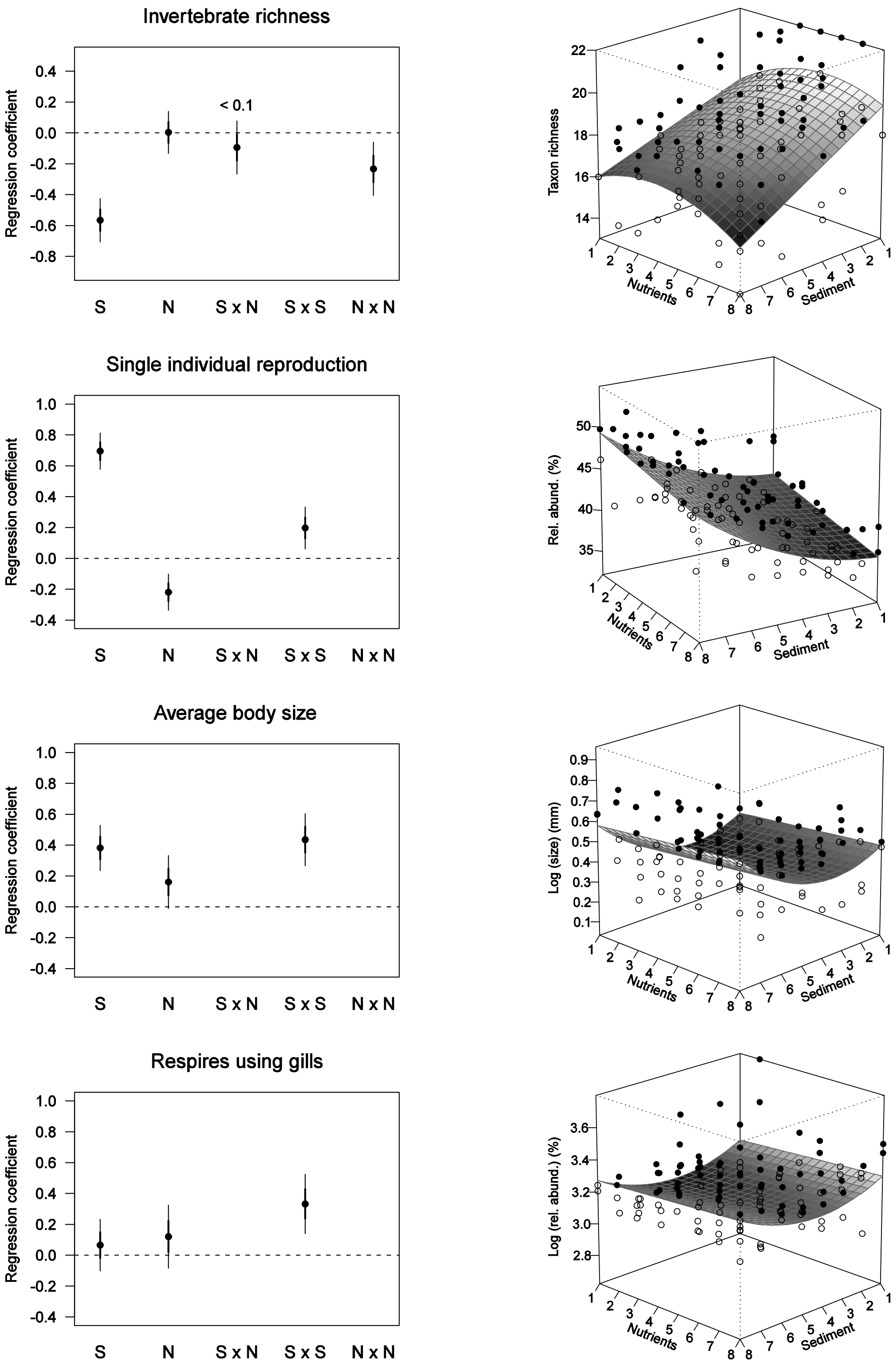
Cladocera

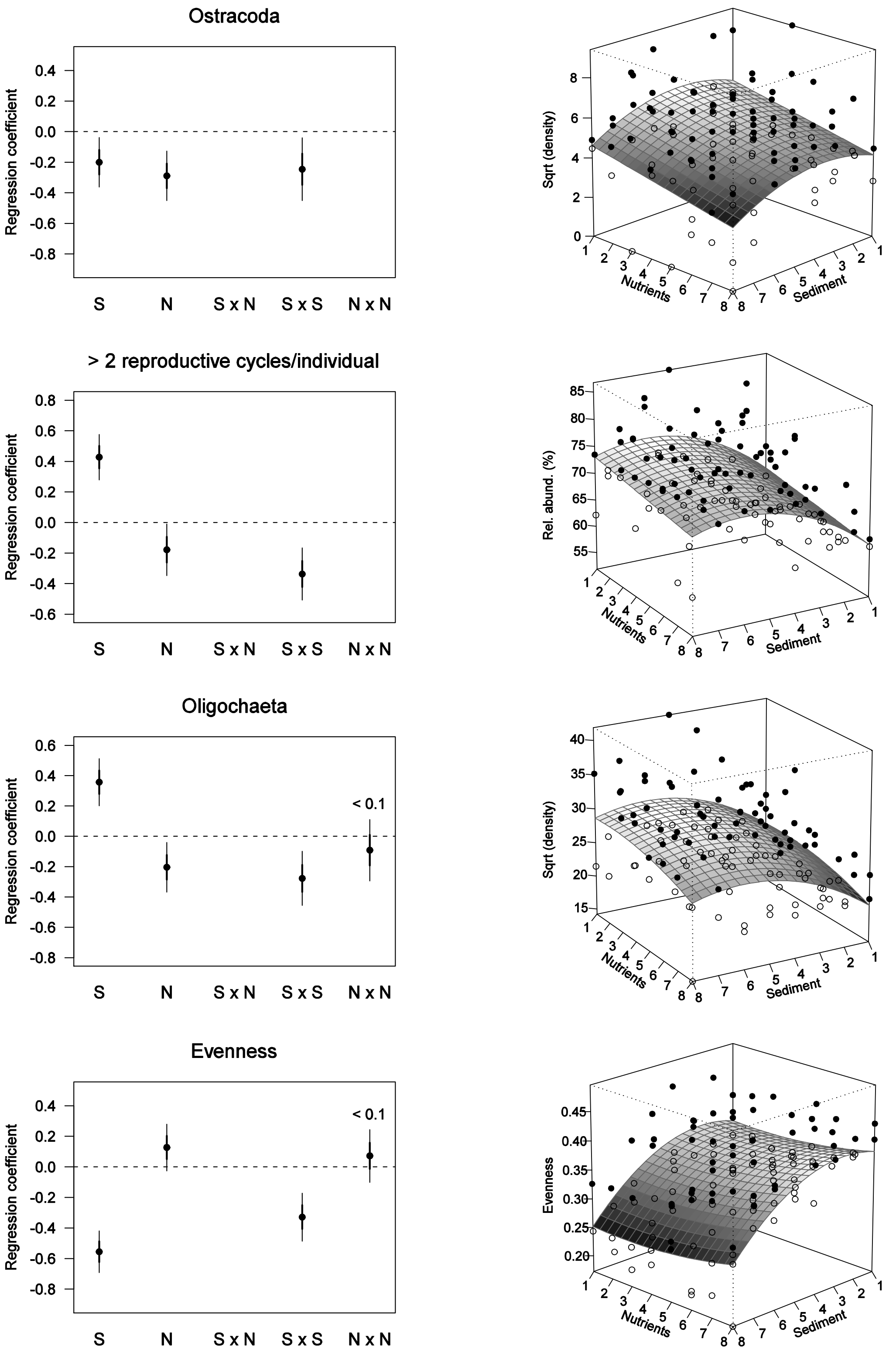

Tanypodinae
Regression coefficient
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
S
N
S x N
S x S
N x N
Density
70
60
50
40
30
20
10
0
Nutrients
Sediment
1 2 3 4 5 6 7 8


Psilochorema
Regression coefficient
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
S
N
S x N
S x S
N x N
Sqrt (density)
7
6
5
4
3
2
Nutrients
Sediment
1 2 3 4 5 6 7 8


Deleatidium
Regression coefficient
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
S
N
S x N
S x S
N x N
Density
700
600
500
400
300
200
100
Nutrients
Sediment
1 2 3 4 5 6 7 8


Total EPT
Regression coefficient
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
S
N
S x N
S x S
N x N
Density
800
600
400
200
Nutrients
Sediment
1 2 3 4 5 6 7 8


Invertebrate richness
Regression coefficient
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
< 0.1
S
N
S x N
S x S
N x N
Taxon richness
14
16
18
20
22
Nutrients
Sediment
1
2
3
4
5
6
7
8
Single individual reproduction
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
Rel. abund. (%)
35
40
45
50
Average body size
Log (size) (mm)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Respires using gills
Log (rel. abund.) (%)
2.8
3.0
3.2
3.4
3.6


Ostracoda
Regression coefficient
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
S
N
S x N
S x S
N x N
Sqrt (density)
8
6
4
2
0
Nutrients
Sediment
1 2 3 4 5 6 7 8
> 2 reproductive cycles/individual
Regression coefficient
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
S
N
S x N
S x S
N x N
Rel. abund. (%)
85
80
75
70
65
60
55
Nutrients
Sediment
Oligochaeta
Regression coefficient
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
< 0.1
S
N
S x N
S x S
N x N
Sqrt (density)
40
35
30
25
20
15
Nutrients
Sediment
Evenness
Regression coefficient
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
< 0.1
S
N
S x N
S x S
N x N
Evenness
0.45
0.40
0.35
0.30
0.25
0.20
Nutrients
Sediment

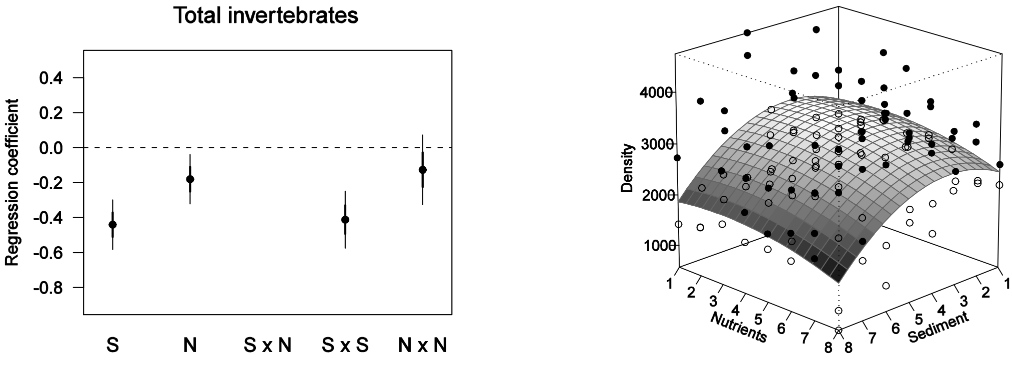


Figure 3.5 Relationships between stressor levels and invertebrate response variables for which the model selection procedure revealed a simple multiple-stressor response (no interaction term in the final model or effect size of interaction term less than 0.1). Standardized effect size estimates (standardized partial regression coefficients) plus 95% CIs of the terms in the final model are shown on the left and three-dimensional response surfaces on the right. See Table 3.2 and Figure 3.4 for more details.

Only two invertebrate variables showed complex multiple-stressor responses where the relationship along the sediment gradient depended on nutrient level, and vice versa. Both EPT richness and Chironomidae declined linearly with sediment but followed a subsidy-stress pattern in relation to nutrients (Figure 3.6). However, the decline with sediment steepened with increasing levels of nutrients and the maximum response to nutrients (i.e. the inflection point at which the subsidy turned into a stress effect) was pushed toward lower values of nutrients with increasing levels of sediment. Because the effects of each individual stressor became stronger at increasing levels of a second stressor, this interaction can be called synergistic.

Complex multiple-stressor responses

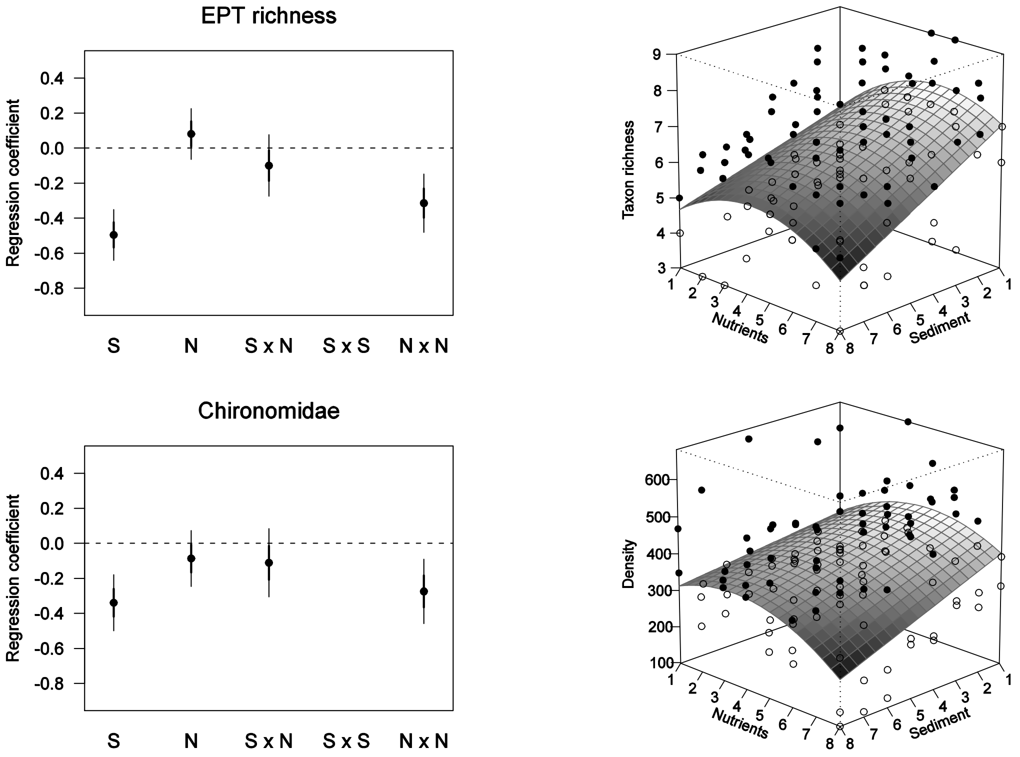


Figure 3.6 Relationships between stressor levels and invertebrate response variables for which the model selection procedure revealed a complex multiple-stressor response (with interaction term and its effect size 0.1 or greater in the final model). Standardized effect size estimates (standardized partial regression coefficients) plus 95% CIs of the terms in the final model are shown on the left and three-dimensional response surfaces on the right. See Table 3.2 and Figure 3.4 for more details.

Table 3.3 summarises all cases with single-stressor, simple multiple-stressor and complex multiple-stressor models by assigning them to three shape categories (negative, positive, subsidy-stress) for each stressor.

Table 3.3 Invertebrate response variables categorized by response shape to each individual stressor and ordered by level of biological organization. All cases for each shape category are included, whether from best models that included a single stressor or best models that showed simple or complex multiple-stressor outcomes. See Table 3.2 for details of transformations used for response variables.

			Biological response variables		
Stressor	Shape category	# of responses/category	Invertebrate density	Relative trait representation	Community level
Sediment	negative (linear or curvilinear) ⇩	18	Chironomidae Cladocera *Deleatidium* *Hydora* *Temnocephalus* Tanypodinae *Psilochorema* *Oxyethira*	Lays eggs at water surface Clinger Low body flexibility Spherical body shape Grazer Filter-feeder	EPT density % EPT Invertebrate richness EPT richness
	positive (linear or curvilinear) ⇧	6		Single ind. reproduction Burrower Deposit-feeder Predator Respires using gills	Average body size
	subsidy-stress ↷	7	Oligochaeta Nematoda Copepoda Ostracoda	> 2 reprod. cycles/ind.	Invertebrate density Evenness
Nutrients	negative (linear) ⇩	7	Oligochaeta Cladocera Ostracoda	> 2 reprod. cycles/ind. Single ind. reproduction Spherical body shape Filter-feeder	
	positive (linear) ⇧	6		Lays eggs at water surface Low body flexibility Respires using gills	% EPT Evenness Average body size
	subsidy-stress ↷	8	Chironomidae *Deleatidium* Tanypodinae *Psilochorema*		Invertebrate density EPT density Invertebrate richness EPT richness

Pearson’s correlation coefficients among the 12 invertebrate trait response variables showed in several instances that traits were highly correlated (r ≥ 0.80) (Table 3.4).

Table 3.4 Pearson's correlation coefficient matrix of invertebrate trait response variables (data transformation as for analysis and given in parenthesis), values ≥ 0.80 in bold.

	Reproduction			Habit		Body form		Functional feeding group				Respiration
	> 2 reprod. cycles/ ind.	Single ind. reprod.	Lays eggs at water surface	Burrower (log)	Clinger	Low body flexibility	Spherical body shape	Grazer (log)	Deposit-feeder	Filter-feeder	Predator	Respires using gills (log)
> 2 reprod. cycles/ind.	1	0.55	-0.79	0.24	-0.45	-0.48	-0.15	**-0.84**	0.71	-0.11	0.76	-0.59
Single ind. Reprod.		1	**-0.80**	**0.89**	-0.57	**-0.82**	-0.48	-0.77	**0.82**	-0.57	0.73	-0.16
Lays eggs at water surface			1	-0.65	0.61	0.73	0.40	**0.89**	**-0.83**	0.41	**-0.82**	0.55
Burrower (log)				1	-0.56	-0.64	-0.46	-0.62	0.65	-0.52	0.57	-0.11
Clinger					1	0.49	0.50	0.56	-0.65	0.49	-0.59	0.09
Low body flexibility						1	0.42	0.62	-0.78	0.63	-0.60	0.20
Spherical body shape							1	0.43	-0.70	**0.94**	-0.68	-0.30
Grazer (log)								1	**-0.90**	0.40	**-0.94**	0.58
Deposit-feeder									1	-0.73	**0.96**	-0.27
Filter-feeder										1	-0.63	-0.33
Predator											1	-0.36
Respires using gills (log)												1

3.5 Discussion

3.5.1 Response shapes and subsidy-stress hypothesis

My first hypothesis had predicted that each stressor might produce negative or positive effects or a subsidy to invertebrate response variables at low levels but a stressor effect at high levels. This hypothesis was supported by the range of stressor response shapes I found along each of the two tested gradients. At the lowest level of biological organization, the taxon level, the densities of eight common invertebrate taxa declined in a linear or curvilinear fashion with increasing fine sediment, and three with increasing nutrients. None of the taxon densities showed a strictly positive response to either sediment or nutrients, indicating that none of the abundant taxa in the mesocosm community preferred conditions under highly augmented levels of fine sediment or nutrients. However, subsidy-stress patterns were common along both gradients.

My subsidy-stress hypothesis in relation to nutrients is based on the assumption that increasing nutrients stimulate primary production, increasing resource availability to invertebrates initially but causing harm when algae proliferate (Quinn, 2000; Hillebrand, 2002). An increase in algal food supply should most directly affect the density of grazing invertebrates, as reported in previous studies (Riley *et al.*, 2003; Niyogi *et al.*, 2007). In my experiment New Zealand's most widespread invertebrate grazer, the mayfly *Deleatidium*, followed a subsidy-stress pattern although the subsidy effect was barely perceptible, providing only weak evidence for the proposed underlying mechanism. The relative representation of the grazing trait was also unaffected by nutrient concentrations in this experiment as well as in my field survey (Chapter 2). Perhaps increasing nutrient concentrations did not produce an increase in food supply, which is in line with standing stocks of algal biomass (chlorophyll a) being unaffected by increasing nutrients in both my field survey and experiment (Chapters 2 & 4). A more pronounced subsidy-stress pattern was found for the predatory trichopteran *Psilochorema*, with densities peaking at the same nutrient level (5.2) as for *Deleatidium*. Chironomidae and its grazing/predatory subfamily Tanypodinae also became somewhat more abundant as nutrient concentrations increased to intermediate levels, confirming previous observations by Biggs *et al.* (2000) who suggested that chironomids benefit from elevated algal biomass for its contribution as habitat rather than as an energy source.

At the community level, the subsidy-stress hypothesis was supported with hump-shaped responses of total invertebrate and EPT densities. The maximum invertebrate density along the nutrient gradient was reached at level 2.9 but density was only 2.2 % higher than at the non-augmented level 1 (when sediment was held constant at level 1). A larger subsidy effect with an increase of 21 % compared to that at level 1 was found for EPT density which peaked at nutrient level 5.0, suggesting that some of the EPT taxa were successful in utilizing additional algal resources directly or indirectly. Finally, a subsidy-stress response was also found for both total invertebrate and EPT richness with an average gain of 1.5 taxa in both instances at levels 5.2 and 5.4, respectively, compared to the numbers of taxa predicted at nutrient level 1 (and sediment held constant at level 1). Although my experiment revealed subsidy effects of augmented nutrient concentrations on richness, in accord with some previous survey-based research (Riley *et al.*, 2003; Yuan & Norton, 2003), my own field survey (Chapter 2) and two other observational studies (Heino *et al.*, 2007; Niyogi *et al.*, 2007) failed to reveal subsidies on richness measures, suggesting that such effects are variable or difficult to detect in non-manipulative studies. Subsidy effects, on the other hand, have been more consistently reported for densities or biomasses of individual taxa and the entire invertebrate community and for the relative abundance of individuals in the EPT orders (Quinn & Hickey, 1990b; Riley *et al.*, 2003; Niyogi *et al.*, 2007; Chapter 2).

My subsidy-stress hypothesis in relation to sediment is based on the assumption that fine sediment augmentation can initially increase habitat heterogeneity and provide additional habitat for taxa that can burrow and live in fine sediment, while further augmentation decreases habitat heterogeneity by filling up interstitial spaces and smothering substratum, reducing or eliminating habitat for most other taxa. I found an initial increase in densities of the burrowing taxa Oligochaeta and Copepoda and of further taxa with other modes of attachment to substratum, namely Nematoda and Ostracoda, all of which also responded positively to fine sediment addition during a larger-scale (50-m reaches) field experiment (Matthaei *et al.*, 2006). Burrowers compared to other groups seemed to be enhanced as fine sediment increased but only noticeably from levels 2 or 3 (equivalent to 5-26 % sediment cover and 0.5-0.8 mm depth) onwards, whereas clingers to the substratum gradually decreased with increasing sediment. Interestingly, while initially fine sediment provided additional habitat for burrowing taxa, a further increase beyond sediment level 3 eventually seemed to represent less suitable habitat indicated by decreasing densities. This was the case even for oligochaete worms, which benefitted most from the added sediment with a maximum density increase of 70 % compared to sediment level 1. The point of change where a subsidy turned into a stress effect for this most sediment-tolerant taxon in my experiment was at sediment level 6.0 where fine sediment covered 93 % of the bed surface and was 6.2 mm deep. Invertebrates with certain morphologies, notably low body flexibility and spherical shape, were also negatively influenced by sediment addition probably because of an inability to penetrate into fine sediment. Finally, fine sediment not only affects habitat but also influences food availability with consequences for representation of functional feeding groups. Grazers and filter-feeders declined proportionately along almost the entire sediment addition gradient (by 10.0 % and 7.1 %, respectively), in contrast to deposit-feeders and predators which increased by 9.5 % and 7.4 %, respectively. Overall grazers were the dominant feeding group with 54 % representation at low sediment levels, but they were increasingly less well represented at higher sediment levels, probably because fine sediment is unstable and sustains less algal biomass or algae of lower food quality (Burkholder, 1996) than coarser substrata or because fine sediment contaminates the food resources (Broekhuizen *et al.*, 2001). Filter-feeders, on the other hand, might have declined because fine sediment clogs their filtering apparatus (Wood & Armitage, 1997). Both grazers and filter-feeders also showed strong negative relationships to deposited fine sediment in an American study (Rabeni *et al.*, 2005). At the community level, total invertebrate density showed a subsidy-stress response to increasing sediment, as previously reported (Matthaei *et al.*, 2006; Larsen *et al.*, 2009). At sediment level 3.3, total density peaked and was 10.4 % higher than at level 1 (nutrients held constant at level 1), followed by a decline with further sediment increase so that density at the highest sediment level was 65.2 % of that at level 1. Community evenness also increased initially, but only slightly, and declined from sediment level 2.6 onwards, probably both because sensitive taxa were lost (as indicated by the negative response of EPT richness) and sediment-tolerant taxa became more dominant in the community.

The three invertebrate traits related to reproduction ('> 2 reproductive cycles per individual', 'single individual reproduction' and 'lays eggs at water surface') responded to both stressors. However, I consider the experimental period of 21 days to be too short for completion of the cycle from oviposition to recruitment, although asexual reproduction might occur. Consequently, the significant patterns for reproduction traits are likely to have resulted from phylogenetic constraints (Poff *et al.*, 2006) where the reproductive traits co-varied with other traits that were direct determinants of the occurrence of certain taxa. Each of the three trait responses relating to reproduction was correlated strongly ($r \geq 0.80$) with up to three functional feeding traits, and the variation in the feeding traits was generally better accounted for by the two stressor gradients than variation in the reproductive traits. Nevertheless, the responses of the reproductive traits to the sediment gradient agree with the results of a previous study (Dolédec *et al.*, 2006) and my own field survey (Chapter 2). In all three studies, the relative proportion of 'lays eggs at the water surface' decreased with sediment while 'more than two reproductive cycles per individual' as well as 'single individual reproduction' increased, the latter two probably reflecting the advantage of species that can reproduce quickly and in isolation and, hence, are resilient to stressor perturbations (Townsend & Hildrew, 1994; Dolédec *et al.*, 2006). In contrast, the responses of the reproductive traits to the nutrient gradient were opposite to those found for sediment and also not in agreement with my field survey results (Chapter 2) and Dolédec *et al.*'s study (2006). Therefore, these responses are more likely to be a spurious consequence of the trait syndrome problem (Poff *et al.*, 2006). Finally, the trait 'respires using gills' was the least correlated with any of the other traits investigated and can be expected to be directly or indirectly affected by the focal stressors within the time span of my experiment. However, its variation was poorly explained by the stressors, consistent with the results of my field survey (Chapter 2).

3.5.2 Single-stressor and multiple-stressor responses

My second hypothesis, which predicted that invertebrate variables would show a range of responses to sediment and nutrients including no effect, single-stressor effect, simple multiple-stressor effect (no interaction) or complex multiple-stressor effect (interaction), was also supported. The majority of response variables followed a single or simple multiple-stressor effect, with only two variables showing a complex multiple-stressor pattern and a single variable unresponsive to either stressor.

Single-stressor effects were found for ten invertebrate variables, all in the taxon-level or trait category, and the operational stressor was always deposited fine sediment. It is notable that, in contrast to taxon and trait responses, all six of our taxonomic community-level response variables were affected by nutrients. Perhaps nutrients affected rare taxa more strongly than common and widespread taxa so that nutrient effects emerged only for aggregate metrics. King & Baker (2010) acknowledged that sometimes the response signal to stressor gradients can be enhanced by aggregating taxa but, in contrast to my results, they more frequently found that the signal was diluted by incorporation of abundant and tolerant taxa.

Nineteen invertebrate variables showed simple multiple-stressor responses (without interaction), and their common feature is that the response shape along each individual stressor gradient stayed the same across all levels of the other stressor. In other words, the combined effect of both stressors is simply additive. All combinations of possible response shapes along the two stressor gradients were realised (negative, positive and hump-shaped). In a few cases, similar three-dimensional response shapes of two (deposit-feeder and predator, ‘lays eggs at the water surface’ and % EPT, EPT and *Deleatidium* densities) or three invertebrate variables (Cladocera, filter-feeder and spherical body shape) possibly reflected a strong correlation between these response variables. In two extreme cases, I found a correlation coefficient of 0.99 between paired response variables. This very strong correlation occurred for the relative representation of the trait ‘lays eggs at the water surface’ and % EPT (because nine of eleven taxa with this trait were in the EPT orders) and for EPT and *Deleatidium* densities (because 80 % of all individuals in the EPT orders belonged to the genus *Deleatidium*).

A complex multiple-stressor effect occurs when the two stressors interact and the combined effect cannot be predicted based on knowledge of single-stressor effects. In my experiment, I found only weak interactive effects (predicted with low certainty because the 95

% CI of the partial regression coefficient included zero) for two invertebrate variables, EPT richness and Chironomidae. Both responded in a similar fashion. Neither overall response shape to the two stressors changed, but the strength of the negative effect became stronger at higher levels of the other stressor, signifying synergistic interactions. Along the sediment gradient the fitted model showed an average loss of 1.4 taxa from zero to maximum sediment at the lowest nutrient concentrations, compared to a loss of 2.7 taxa when nutrient concentrations were at their highest. At the same time, nutrient enrichment produced a subsidy-stress response with a maximum gain of 1.5 taxa at nutrient level 5.4 compared to level 1 and a decline thereafter, although richness remained higher than for the lowest nutrient level when sediment was absent. Due to interactive effects, however, the maximum gain of taxa was only 0.8 taxa (peaking at the lower nutrient level of 4.2 and declining thereafter), and the lowest number of 4.4 EPT taxa occurred when both nutrients and fine sediment were at their highest.

Strong complex multiple-stressor responses were rarer in this experiment than in my field survey (Chapter 2). For example, in my survey I found strong interactive effects for *Deleatidium* with fine sediment having no effect on relative abundance at low nutrient concentrations but a strong negative effect at high nutrient concentrations. I also found a subsidy-stress response of % EPT to increasing nutrients at low sediment levels but a strictly negative response at high sediment levels. This discrepancy may be partly due to the different temporal scales of the experimental and field survey approaches. In the field, stressors may have been operating for months or longer, but in the experiment they only had 21 days to exert an effect, a period much shorter than the life cycle of most macroinvertebrates. Twenty-one days was a timeframe recommended for investigating multiple-stressor effects on stream communities (Culp & Baird, 2006), therefore my manipulative period was probably long enough to simulate and test the effect of a sediment pulse and an episode of augmented nutrients on the presence and colonization of macroinvertebrates. However, this period was most likely too short to reveal sublethal stressor effects on certain parts of the invertebrate life cycles that did not take place during my experiment, such as those related to reproduction. The different spatial scales might also be partly responsible for discrepancies between mesocosm and field survey results. Thus, complex multiple-stressor patterns may have been more difficult to detect in mesocosms subjected throughout the experimental period to quite high colonization rates including the continuous supply of sensitive taxa from the relatively unpolluted Kauru River.

Overall, augmented fine sediment and nutrients mainly acted in concert as multiple stressors in my experiment, and there was little evidence for strong interactive effects between the two stressors. Increasing levels of both stressors altered the densities of certain invertebrate taxa, leading to changes in community structure and in the relative representation of functional traits which, in turn, may influence ecosystem functioning in agricultural streams. These results imply that in a multiple-stressor situation management could be improved if stressor-specific indicators (ecological responses that react to only one stressor) were available. In my experiment, sediment was the only relevant stressor in a few cases that all involved the densities of common invertebrate taxa or the representation of invertebrate traits. Hence, stressor-specific invertebrate indicator variables may be more likely to be found for fine sediment than for nutrients and at the taxon or trait level, which might render these invertebrate metrics more useful tools for bio-diagnostic monitoring than the traditional community-level metrics.

3.5.3 Stressors compared: deposited fine sediment vs. nutrients

My final hypothesis had predicted that fine sediment augmentation would produce stronger stressor effects than nutrient augmentation, and this was strongly supported at all levels of biological organization. Fine sediment had strictly negative effects on the densities of eight common invertebrate taxa and subsidy-stress effects on the remaining four taxa, most of them known to be pollution-tolerant. At the community-level too I observed a subsidy-stress response by total invertebrate density and strictly negative effects of increasing fine sediment on EPT density, % EPT, total invertebrate taxon richness and EPT richness. A sediment-induced shift in community structure, with pollution-tolerant taxa becoming more dominant at the expense of sensitive taxa, was manifested by a decline in community evenness from sediment level 2.6 onwards. This pattern can also be seen clearly in the corresponding NMDS plot, with community structure becoming increasingly dissimilar from that at the lowest sediment level as the amount of deposited fine sediment increased.

By contrast, nutrient augmentation had strictly negative effects on only three common taxa and subsidy-stress responses were found for four taxa, including two known to be pollution-tolerant (Chironomidae and its subfamily Tanypodinae) but also caddisfly *Psilochorema* and the mayfly *Deleatidium*, both pollution-sensitive. In fact, despite a reduction after an initial subsidy effect, the densities of the latter two taxa at the highest nutrient level were still higher than those at the lowest level and therefore showed overall a positive response to increased nutrient concentrations. At the community-level, EPT density showed the same subsidy-stress pattern while % EPT actually responded positively along the entire nutrient gradient. Only total invertebrate density followed a subsidy-stress pattern along the nutrient gradient similar to that for sediment, with lowest densities at the highest nutrient treatment and therefore overall negative effects. While richness measures were strictly negatively influenced by fine sediment, both total invertebrate and EPT taxon richness followed subsidy-stress responses to nutrients. Furthermore, richness at the highest nutrient concentrations was similar to that at the lowest level with an estimated difference of no more than one taxon. A clear shift in community structure along the entire nutrient gradient could not be detected in the corresponding NMDS plot, probably because of pronounced subsidy-stress responses of the majority of common taxon densities and richness variables.

Overall, the effect size of sediment was considerably larger than that of nutrients at the centre of each gradient for almost all invertebrate response variables, and sediment effects were predicted with greater certainty (as demonstrated by their 95 % CIs not including zero), supporting the findings of my field survey (Chapter 2). Thus, fine sediment can be considered a more pervasive stressor than dissolved nutrients. Fine sediment had immediate detrimental impacts on individual invertebrate taxa and community structure, including taxon richness and the partitioning of functional feeding groups, which in turn may affect ecosystem functioning. Invertebrates have been shown to respond to experimental fine sediment addition in less than a day through drift to escape unsuitable habitat and feeding conditions (Suren & Jowett, 2001; Larsen & Ormerod, 2010). Invertebrates have also been shown to be sensitive to relatively small amounts of deposited fine sediment with a suggested upper limit of 10 % cover (Bryce *et al.*, 2010) for protection of sensitive species, which is in agreement with this experiment showing a noticeable change in EPT density even between sediment level 1 and 2.

Even though most invertebrate variables responded to increasing nutrient concentrations, the effects on most taxa and community composition were only weakly negative or even positive, in contrast to the predominantly negative sediment effects. These findings suggest that a short-term 21-day exposure to augmented nutrients was not detrimental to invertebrate community structure. Augmented nutrient concentrations are known to have deleterious effects on the biotic integrity of streams (Camargo & Alonso, 2006) and nutrient criterion values of 440 µg DIN /L and 40 µg TP /L (approximately as high as treatment level 4) have been suggested to protect stream condition (Miltner, 2010). Therefore, I conclude that for negative effects to become detectable, invertebrate communities need to be exposed for a longer period of time than in my experiment. Exposure for 21 days to the highest nutrient levels might have caused higher drift losses because of direct toxicity, given that levels 7 and

8 both exceeded the maximum level of 2000 µg NO_3-N /L that is assumed to protect most sensitive invertebrate taxa from toxic effects (Camargo *et al.*, 2005). However, 21 days were probably too short for augmented nutrient concentrations to cause negative effects via indirect mechanistic pathways, involving the negative consequences of eutrophication (Camargo & Alonso, 2006) and changes in competitive interactions among species due to alterations in food quality (Evans-White *et al.*, 2009). For the same reasons, my experiment may not have revealed strong interactions between fine sediment and nutrients, which have been found in observational studies (Townsend *et al.*, 2008; Chapter 2). Furthermore, interactions between nutrient concentrations and other environmental variables explained more than half of the variation in fish and macroinvertebrate metrics in a study by Wang *et al.* (2007) while augmented nutrient concentration by itself had only a small effect on these metrics. Wang *et al.* (2007) concluded that augmented nutrient concentrations can be considered to play a key role in the decline of stream condition in interaction with other stressors, a conclusion at least partly supported by the results of my mesocosm experiment.

3.5.4 Potential stressor thresholds

Knowledge about thresholds of harm for environmental stressors is crucial for successful freshwater management and often stressor-response relationships have been quantified with a view to defining such thresholds and develop appropriate criteria to guide freshwater management (Wang *et al.*, 2007; Evans-White *et al.*, 2009; Smith & Tran, 2010). An ecological threshold, defined as a point where a small change in an environmental variable produces a large ecological response (Groffman *et al.*, 2006; Dodds *et al.*, 2010), is an obvious threshold beyond which a system should not move to avoid dramatic changes, even potentially to an alternate state (Scheffer *et al.*, 2001). However, thresholds of harm are not necessarily bound to such drastic nonlinear responses and can also be defined where there is a gradual change in an ecological variable across a stressor gradient based on detection of a negative impact on one or more key species or an important ecosystem function (Hilderbrand *et al.*, 2010). Finally, in instances where an ecological variable follows a subsidy-stress response a potential threshold of harm can be defined as the inflection point where the effect of an environmental driver turns from a subsidy to stress (Niyogi *et al.*, 2007; Withers & Jarvie, 2008).

Although augmented nutrient concentrations in my experiment did not reveal many strong detrimental effects on invertebrate response variables, the transition from a subsidy to a stress response may be indicative of ecosystem change (Odum *et al.*, 1979) and thus define a stressor threshold. Amongst four community-level variables widely used as indicators of ecological stream condition (EPT density, % EPT, total invertebrate and EPT taxon richness), the lowest inflection point was found for EPT density at a nutrient level of 5.0. This potential stressor threshold equates to nutrient concentrations of 728 µg DIN /L and 70 µg DRP /L. The inflection points for total invertebrate and EPT taxon richness, at least at low sediment levels, were at nutrient levels of 5.2 and 5.4 and those of the common mayfly *Deleatidium* and the caddisfly *Psilochorema* behaved similarly (both 5.2). These findings suggest that managers should try to keep below such nutrient concentrations to avoid negative impacts on sensitive invertebrate taxa and overall stream condition. The nutrient threshold values derived from my experiment are of similar order to those based on macroinvertebrate responses determined using field surveys. The latter range from 300, 440 and 500 µg/L for NO_3-N, DIN and TN, respectively, up to 1,680 µg TN /L, and 50 to 80 µg dissolved P /L or 30 to 40 µg TP /L (Wang *et al.*, 2007; Evans-White *et al.*, 2009; Miltner, 2010; Smith & Tran, 2010). This similarity is encouraging considering the temporal and spatial limitations of the experimental approach and given that threshold estimates are to a large extent dependent on the statistical method applied (Wang *et al.*, 2007; Dodds *et al.*, 2010). In my field survey, inflection points occurred at 144 and 107 µg DIN /L for % EPT and relative abundance of the grazing caddisfly *Pycnocentrodes*, respectively, and therefore at lower concentrations than those of the other surveys and in my experiment. Higher thresholds in experiments could be due to detrimental effects on invertebrates of augmented nutrient concentrations, and potential interactions with sediment, taking longer than the experiment's three weeks to take effect. Spatial-scale effects contributing to a discrepancy between survey and experimental results include the continuous supply of sensitive taxa from the unimpacted source stream and the small scale of the mesocosms, reducing the potential for top-down influences on invertebrates and periphyton to interact with the stressors (Biggs *et al.*, 2000). Furthermore, the rapid turnover of water within the small mesocosms may have prevented secondary negative effects of increased algal productivity and/or algal community changes on invertebrates via decreases in water quality. Increased algal productivity can decrease water quality due to high rates of photosynthesis and respiration that can cause stressful diel pH and dissolved oxygen concentrations in streams (dependent on pH buffering capacity and re-aeration of the stream); however, there was no support for increased algal production because standing stocks of algal biomass (chlorophyll a) seemed unaffected by nutrients in both my field survey and experiment (Chapters 2 & 4). On the other hand, changes in algal community structure with increasing nutrients were apparent in the mesocosms (Chapter 4) with potentially more toxin-producing algae in the community when nutrients were high; nevertheless, rapid turnover of water may have diluted any negative effects on invertebrates.

In agreement with results from my field survey, none of the four commonly used invertebrate indicators of ecological stream condition showed subsidy-stress patterns across the fine sediment gradient so the subsidy-stress threshold concept cannot be applied here. However, the marked and consistent decline of common pollution-sensitive taxa with increasing sediment suggests that inputs should be controlled to avoid fine sediment deposition on the streambed. To provide potential sediment thresholds, I have adopted a benchmark approach, similar to Cormier *et al.* (2008), and determined thresholds as the sediment levels where the proportional reduction of expected EPT density was between 5 and 10 %. I chose EPT density because this was the stream condition variable most strongly affected by sediment, with density being 80 % lower at the highest than the lowest sediment level. Accordingly, potential stressor thresholds were defined at levels 1.9 and 2.6 for a proportional reduction of the mean expected EPT density of 5 and 10 %, respectively. Thus, deposited fine sediment should be kept well below level 3 and ideally not exceed level 2, the latter amounting to 5 % sediment cover and 0.5 mm sediment depth on average. It is again encouraging to note that these values agree very well with empirical sediment thresholds developed by other researchers using different approaches. For example, Zweig & Rabeni (2001) and Larsen *et al.* (2009) conducted field surveys and derived sediment tolerance values for individual common invertebrate taxa from 50 % cumulative abundance data, and in both studies the most sensitive taxa were assigned tolerance values of 5 % sediment cover or less. In another survey combined with a literature review, Bryce *et al.* (2010) defined a threshold of 10 % fine sediment cover from optimum sediment tolerance values for the most sediment-sensitive EPT taxa.

CHAPTER 4 Multiple-stressor effects on periphyton communities: A mesocosm experiment

4.1 Summary

Augmented deposited fine sediment and inorganic nutrient concentrations have been shown to act in concert and sometimes produce complex multiple-stressor responses of stream macroinvertebrate communities in both field surveys and experiments. By contrast multiple-stressor effects on the periphyton community are relatively underexplored, even though periphyton organisms can be expected to be more directly affected by nutrients than macroinvertebrates. Moreover, periphyton likely plays a major role in the mechanistic pathway for nutrient effects on macroinvertebrates. Using the same replicated regression design as in the previous chapter, I determined the shapes of periphyton responses across both stressor gradients after 20 days of exposure. I tested (1) the subsidy-stress hypothesis for each stressor and (2) whether sediment and nutrients operated as single or multiple stressors and whether they interacted. Further, I focused on ecological guilds of three distinct algal growth forms (low profile, high profile, motile), testing the hypotheses that (3) sediment augmentation produces a proportional increase in motile algae and a decrease in high-profile algae, and (4) nutrient augmentation produces proportional increases in both motile and high-profile algae. Along the fine sediment gradient subsidy-stress patterns were found only for densities of two common *Nitzschia* species, whereas the remaining algal variables responded either negatively (six common taxa, seven community-level variables) or positively (four community variables). By contrast, subsidy-stress patterns along the nutrient gradient occurred frequently both at the algal taxon and community levels, strongly supporting the subsidy-stress hypothesis for nutrient enrichment. Overall, more periphyton variables responded to nutrients than to sediment. Single-stressor responses were common but sediment and nutrients mainly acted as multiple stressors and typically in a simple additive way, possibly because their underlying mechanisms are very different from one another. However, complex multiple-stressor interactions were also found. Thus, the proportion of cyanobacteria was unaffected by increasing sediment or nutrients across their wide stressor gradients when operating in isolation but increased markedly when both stressors acted in concert. Knowledge of such complex multiple-stressor responses of potentially harmful algal species should prove useful to resource managers in avoiding unexpected negative outcomes. My hypothesis about the representation of algal growth forms across the sediment gradient was fully supported, whereas the related hypothesis regarding the nutrient gradient received only partial support. The motile guild, as predicted, increased in prevalence with rising nutrients at the expense of the high profile guild but only up to an asymptote at intermediate nutrient levels. At intermediate nutrient levels the patterns reversed, with representation of the high

profile guild increasing, also as predicted, and the motile guild decreasing with a further rise in nutrients. Due to this subsidy-stress response pattern, the representation of the motile growth form could be a useful ecological indicator for detecting early signs of nutrient enrichment while being less suitable for detecting more severe cases of enrichment.

4.2 Introduction

Many streams and rivers in agricultural landscapes are in poor ecological condition and degradation is, in most cases, a result of the operation of multiple stressors. Two major stressors are augmented inorganic nutrient concentrations (nitrogen and phosphorus) and deposited fine sediment on the streambed, which have been shown sometimes to interact in complex ways to affect macroinvertebrate communities (Townsend *et al.*, 2008; Matthaei *et al.*, 2010; Chapters 2 & 3). Less attention has been paid to the individual and combined effects of augmented nutrients and fine sediment on the periphyton compartment of the ecosystem (which comprises microscopic benthic algae, bacteria and fungi), although periphyton communities can be expected to be more directly affected by nutrients than macroinvertebrates (Kelly & Whitton, 1998; Dodds, 2007). Furthermore, changes in periphyton structure and function due to augmented nutrient concentrations, which are themselves relatively underexplored (Larned, 2010), are the main proposed mechanistic pathway for nutrient effects on macroinvertebrates. These effects include alteration of the food base and, if periphyton growth is excessive, negative consequences associated with eutrophication, including habitat degradation, hypoxia and sometimes the occurrence of toxin-producing cyanobacteria (Miltner & Rankin, 1998; Camargo & Alonso, 2006). Changes to the periphyton due to increased fine sediment deposition, potentially interacting with augmented nutrients, may have further bottom-up effects on macroinvertebrates. Hence, unravelling the effects of augmented nutrients and fine sediment on periphyton attributes might also shed light on the mechanisms underlying those observed at higher trophic levels such as macroinvertebrate communities.

Knowledge of potential multiple-stressor interactions is also important when periphyton attributes serve as indicators of ecosystem stress due to anthropogenic pollution. Algal community attributes are commonly used as indicators for inorganic nutrient pollution and eutrophication (Kelly & Whitton, 1995; Whitton, 1999; Biggs, 2000; Dodds, 2007) but not much is known about the extent to which increased deposition of fine sediment can interact and confound these responses. The frequency of physical disturbance, which is typically high in substrata with unstable and moving fine particles, is, together with resource supply, one of the two major abiotic determinants of algal community structure and functioning (Biggs *et al.*, 1998). Here I focus on augmented levels of deposited fine sediment as an anthropogenically-derived stressor. However, the periodic deposition and movement of fine sediment has similar consequences for algal communities as natural physical disturbance in setting communities back to early-successional stages and hence preventing a thick algal mat from developing (Peterson, 1996).

Algal taxa have acquired different traits reflecting the trade-offs between constraints of disturbance and resource supply and various morphological adaptations are thought to confer advantages in relation to one or the other (McCormick, 1996; Biggs *et al.*, 1998). For this reason Passy (2007) classified diatom taxa into three distinct growth morphologies (low profile, high profile, motile), chosen to reflect their differential potentials to tolerate nutrient limitation and/or physical disturbance, and tested responses across gradients of nutrients and current velocity (the latter as a proxy for disturbance). Her study supported the hypothesised behaviour of the three guilds across the nutrient gradient. The low profile guild dominated the community under low nutrient supply, where the development of a thick algal mat was prevented, but declined as nutrient supply increased, most likely because low profile taxa faced nutrient depletion and shading within a multi-layered algal mat. Here the high profile guild gained dominance. Motile cells, in contrast, can physically escape resource-depleted microhabitats and this guild increased in abundance with nutrient augmentation because most motile taxa are competitive under nutrient-enriched conditions (Pringle, 1990; McCormick, 1996). The predictable behaviour of the guilds led Passy (2007) to conclude that they may be good indicators of anthropogenic pollution. To build further on her work, it is important to consider the responses of the low profile, high profile and motile guilds along multiple-stressor gradients.

Both inorganic nutrients and fine sediment can be considered ‘usable inputs’ to stream ecosystems, as defined by Odum *et al.* (1979), with the potential to produce subsidy-stress responses. An increase in inorganic nutrient availability from low to intermediate levels may produce subsidy effects for certain taxa (Biggs *et al.*, 1998; Chetelat *et al.*, 1999) and the community as a whole, manifested in increased primary production (Biggs, 2000; Dodds *et al.*, 2002) and changes to algal stoichiometry (Liess & Hillebrand, 2006; Liess *et al.*, 2009). Experiments and field surveys have also revealed increases in diatom species richness and evenness with increasing nutrient availability (Pringle, 1990; Stevenson *et al.*, 2008; Liess *et al.*, 2009) and theory suggests that release from nutrient limitation and the presence of an extensive vertical resource gradient within a thick multi-story mat may account for such responses (Biggs & Smith, 2002; Passy, 2008). At higher nutrient concentrations, however, algal growth may become saturated and the community dominated by eutrophic species (often called nutrient-tolerant); now a further increase in nutrient supply will produce no further subsidy but can potentially have negative effects on algal response variables. Across the whole nutrient gradient, therefore, a subsidy-stressor response may result. Responses to augmented nutrients, however, can be modified by the presence of deposited fine sediment. For example, Pringle (1990) found that the response of sessile taxa to experimentally added nutrients depended on substratum type. Sessile taxa responded positively to nutrients on glass slides but not when growing on sand-agar slides and the author noted that this was because the fine substratum sustained a community with a dense layer of motile cells in its upperstory, preventing proliferation of cells in the understory. Hillebrand & Kahlert (2002) also reported that benthic algae growing on fine sediment were less affected by nutrient addition than those growing on hard substrata, possibly due to less favourable physical conditions or reduced access to water-column nutrients in fine sediment habitat. Fine sediment has also been reported to sustain less algal biomass because the movement of fine particles crushes or buries algal cells (Burkholder, 1996). On the other hand, deposition of small amounts of fine sediment on coarse substrata could increase habitat heterogeneity and thus algal species richness or might provide a relative subsidy to species that can tolerate fine sediment and benefit from reduced competition from taxa that are strong competitors when fine sediment is absent. Hence, stressor-response relationships between fine sediment and periphyton community attributes may also take positive, negative or subsidy-stress shapes.

The aim of this study was to investigate the individual and combined effects of augmented nutrient and surface sediment on periphyton response variables in a mesocosm experiment. In addition to algal responses to these two potential stressors, the bacterial community of the periphyton mat was investigated using a community DNA-fingerprinting technique. The combined effects of agricultural stressors on these bacterial communities are largely unknown (Lear & Lewis, 2009). I used a broad range of stressor levels along both gradients to permit precise definitions of stressor response patterns and tested the following hypotheses that are based on the findings of previous multiple-stressor research in New Zealand streams (Townsend *et al.*, 2008; Matthaei *et al.*, 2010; Chapters 2 & 3): (1) augmented sediment and nutrients can act as a subsidy to periphyton response variables at low levels but as a stressor at high levels, and (2) periphyton variables show a range of responses to sediment and nutrients including no effect, single-stressor effect, simple multiple-stressor effect (no interaction) or complex multiple-stressor effect (interaction). In addition, based on findings of predictable changes in algal growth forms along environmental gradients (Biggs *et al.*, 1998; Passy, 2007) I tested the specific hypotheses that (3) fine sediment augmentation produces a proportional increase in motile algae and a decrease in high-profile algae, and (4) that nutrient augmentation produces proportional increases in both motile and high-profile algae.

4.3 Methods

4.3.1 Study site and experimental design

The study site and experimental design of the mesocosm experiment to test algal response variables across gradients of deposited fine sediment and nutrient concentrations are described in detail in Chapter 3 and illustrated in Figure 3.1. During the five-week experiment the mesocosms were supplied with natural stream water pumped at a constant rate from the Kauru River, a 3rd-order stream of nutrient-poor status and with diverse macroinvertebrate and algal communities (Herrmann, 2009; Liess *et al.*, 2009). During the two-week pretreatment period all mesocosms were colonized without augmentation by benthic algae and microorganisms arriving in inflowing water and a periphyton mat, showing rapid growth and with sloughing apparent, developed on the substratum surface (Figure 3.1b). Macroinvertebrates entered the mesocosms in the stream water but were also actively introduced one day before imposition of the stressor treatments. Fine sediment with an average grain size of 0.2 mm was added (on day 0) and stayed in the mesocosms for the three-week manipulative period. Augmented nutrient concentrations were achieved in each header tank by continuously dripping in a highly concentrated solution of nitrate ($NaNO_3$) and phosphate (KH_2PO_4) for the experimental period. The actual stressor conditions achieved in the mesocosms can be found in Figure 3.2 along with the N:P ratio calculated from DIN and DRP and given as a mass ratio, which ranged between 9.5 and 15.5 for nutrient levels 2 to 8. This ratio was higher, at 30.6, for level 1 (the ambient value for the Kauru River). The N:P ratios of nutrient levels 2 to 8 are likely to indicate potential co-limitation between N and P whereas level 1 is more likely to be P-limited, according to a study of New Zealand streams that classified ratios between 7:1 and 15:1 by mass as co-limited and those above 15:1 as P-limited (McDowell *et al.*, 2009).

4.3.2 Periphyton sampling and response variables

On day 20 of the manipulative period, the percent cover of macroscopically obvious algal growth in each mesocosm was visually estimated by one person. This was done by estimating the surface area of the substratum covered by algae in 5 % increments in each quarter of the mesocosm and averaging for the four quarters. I also sampled periphyton from a standardised circular surface area within a core (plastic tubing, 25 mm high) with an inner diameter of 27 mm using a 3-ml cut-off plastic pipette to transfer all organic material on the surface into a 50-ml Falcon tube. For mesocosms containing substantial amounts of fine sediment (levels 5-8), at least 2 mm of the top fine sediment cover was included in the sample. The flow in the mesocosm was stopped briefly during this procedure by closing the tap regulator at the inflow. From each mesocosm, I took two samples from random locations within the same quarter of each mesocosm to optimise consistency in flow conditions across all mesocosms. Both samples were put on ice in the field and stored in the freezer at -20°C until further analysis. One sample was used for algal taxonomic analysis and the other for analysis of photosynthetic pigments as well as for bacterial community analysis (see below). Sample preparation for all analyses involved homogenisation of the cells within the algal slurry by blending (Omni Mixer, Ivan Sorval Inc., Newton, CT, U.S.A.) for a minimum of 20 seconds and until obvious clumps of algae were broken up; this was done to minimise subsampling error.

For algal taxonomic analysis, a second preparation step involved ultrasonication treatment in a bath filled with ice for three minutes at 40 kHz (Transsonic T 1040/H, Elma GmbH & Co KG, Singen, Germany) to detach algal cells from fine sediment (Voltolina, 1991; Wulff *et al.*, 1997). After sonication, algal cells were preserved with formalin (final concentration 2 %) and stored in the dark until taxonomic identification. For most taxa, the natural counting units were cells. For filamentous cyanobacteria with very small cells, 10 μm long units were counted (but referred to as cells for simplicity from now on). Cell counts were performed on a 125 to 500 μl (depending on cell density) subsample, withdrawn from the centre of the homogenised sample after fine sediment had been allowed to settle for 15 seconds, and filled up with deionised water to 2 ml in a 2ml-settling chamber. Care was taken that cells settled evenly across the surface of the chamber, which was checked by scanning the surface at low magnification. Following methods described in Biggs & Kilroy (2000), cell counts of a minimum of 300 live cells were performed within a known number of randomly located grid fields at 400x magnification under an inverted light microscope (Axiovert 25 CFL, ZEISS, Jena, Germany) using standard keys to identify to the lowest taxonomic level possible. Most diatoms were identified to species level whereas green algae and cyanobacteria were identified to genus level. I calculated total algal density and the densities of individual taxa in cells per mm^2 of the sampled mesocosm surface.

Twenty taxa (out of a total of 62) comprised 95 % of all cells in the mesocosms and 16 of them occurred in more than 100 mesocosms and hence were considered common enough for analysis using their densities as response variables. These taxa were, in order of relative abundance, *Synedra ulna* var. *biceps* (12.5 %), *Scenedesmus* spp. (12.4 %), *Melosira varians* (11.8 %), *Synedra* spp. (9.2 %), *Nitzschia* spp. (7.4 %), *Nitzschia amphibia* (6.7 %), *Nitzschia dissipata* (5.4 %), *Encyonema minutum* (5.0 %), *Fragilaria vaucheriae* (3.7 %), *Nitzschia sp.1* (3.3 %), *Navicula capitoradiata* (2.9 %), *Ankistrodesmus* spp. (1.6 %), *Cymbella tumida* (1.3 %), *Navicula cryptotenella* (1.1 %), *Navicula cryptocephala* (0.9 %), *Gomphonema minutum* (0.8 %). The taxa *Synedra acus/ulna* (comprising *S. acus* and *S. ulna*) and *Nitzschia gracilis/palea* (comprising *N. gracilis* and *N. palea*) were created because the conspecifics were difficult to distinguish. I also determined the community variables algal taxon richness (rarefied using R package vegan), Simpson's evenness and the relative abundances (given in percent) of broad taxonomic groups: diatoms, green algae divided into filamentous and non-filamentous green algae, and cyanobacteria. Overall, diatoms were dominant and comprised 75.3 % of all cells counted (made up of 47 taxa), non-filamentous green algae were the second-most abundant group with 14.7 % (8 taxa), followed by filamentous green algae with 5.5 % (5 taxa) and cyanobacteria, which were the least abundant group with 4.5 % (2 taxa). All algal taxa found in the mesocosms and their naming authorities are listed in the Appendix.

In addition to algal taxonomic analysis, I determined the relative abundances (in percent) of ecological algal guilds possessing one of three growth forms (high profile, low profile or motile) which have been reported to respond to nutrient conditions and habitat in a previous study of diatom communities (Passy, 2007). All taxa were assigned to one of the three guilds (see Appendix) according to instructions given by Passy (2007), taking into account personal observations and information in the literature, particularly the work of Lange *et al.* (2011) that previously assigned taxa to guilds in the same Kauru River mesocosm system. Overall the high profile guild was dominant with a representation of 50.2 % (made up of 22 taxa), the motile guild followed with 28.1 % (21 taxa) and least abundant was the low profile guild with 21.7 % (19 taxa). See Table 4.1 for summary statistics of all periphyton response variables considered. Because I expressed the broad taxonomic groups and the ecological guilds as relative abundances, they are technically not independent of each other. Therefore, I calculated Pearson's correlation coefficients between response variables to indicate potential interrelationships.

Chlorophyll was extracted with ethanol and absorption at 665 nm and 750 nm wavelengths before and after acidification was measured spectrophotometrically with a microplate reader (FLUOStar Omega, BMG LABTECH GmbH, Offenburg, Germany) to estimate chlorophyll a (chl a) corrected for phaeophytin as a measure of live algal biomass (Biggs & Kilroy, 2000). The estimate of phaeophytin, a degradation product of chlorophyll a, was also used to calculate the chlorophyll a:phaeophytin ratio as a measure of the vitality of the algal mat (Peterson, 1996). Furthermore, absorption was measured at 480 nm for calculation of the 480/665 absorption ratio in each sample because this index of the pigment ratio carotenoid:chlorophyll has been suggested to be a measure of the nutrient status of algae (Heath *et al.*, 1990).

A subsample of 1.2 ml was taken from the periphyton sample after the homogenisation step, transferred into an Eppendorf cup using a sterile pipette tip and frozen for later microbiological analysis. The bacterial community of the periphyton mat was characterised using Automated Ribosomal Intergenic Spacer Analysis (ARISA), a community DNA-fingerprinting technique (Lear *et al.*, 2008). This method measures the proportion of different intergenic spacer fragment lengths in the 16S–23S region of the bacterial rRNA gene. Fragments in this particular intergenic spacer region are highly variable in length between bacterial species and hence each fragment length can be tentatively assumed to be associated with one species. The species were not identified but the total number of different fragment lengths detected per sample was used as an index of bacterial species richness. From the ARISA data set I also calculated Simpson's evenness for the bacterial community (Table 4.1). Detailed descriptions of the DNA extraction method, ARISA and quantitative methods used to determine bacterial community composition can be found in Lear *et al.* (2008).

Table 4.1 Summary statistics of the periphyton response variables.

Periphyton variables	Mean	Min	Max
Algal densities (cells/mm^2):			
Total algal cell density	9560	1293	46054
Synedra ulna var.*biceps*	1081	0	6843
Scenedesmus spp.	1077	0	4641
Melosira varians	1022	0	4935
Synedra acus/ulna	802	159	3829
Nitzschia gracilis/palea	642	25	3451
Nitzschia amphibia	584	4	3570
Nitzschia dissipata	473	0	3171
Encyonema minutum	433	0	2231
Fragilaria vaucheriae	322	0	1571
Nitzschia sp. 1	286	0	1751
Navicula capitoradiata	249	0	2483
Ankistrodesmus spp.	135	0	666
Cymbella tumida	112	0	660
Navicula cryptotenella	91	0	363
Navicula cryptocephala	80	0	595
Gomphonema minutum	72	0	481
Taxonomic groups:			
% Diatoms	76	17	97
% Filamentous greens	5	0	50
% Non-filamentous greens	16	1	46
% Cyanobacteria	3	0	73
Ecological guilds:			
% High profile guild	48	20	90
% Low profile guild	23	4	50
% Motile guild	29	4	69
Community-level variables:			
Algal cover	70	23	98
Chlorophyll a (mg/m^2)	89	9	312
Chl a:Phaeophytin	2	0.42	23
Carotenoid:Chl a	1	0.61	1.19
Algal taxon richness (rarefied)	22.6	13.8	29.6
Algal evenness	0.38	0.12	0.58
Bacterial species richness	38	2	139
Bacterial evenness	0.39	0.10	0.99

4.3.3 Data analysis

To determine the effects of the two manipulated stressors (deposited fine sediment and nutrients) on algal community composition, I first performed exploratory non-metric multidimensional scaling (NMDS) with R package vegan using the Bray-Curtis dissimilarity matrix, calculated from the relative abundances of all algal taxa identified (including rare taxa). The same procedure was performed for the bacterial community using the ARISA data set. Data were not transformed for this type of analysis.

I then used experimental regression analysis (Gotelli & Ellison, 2004; Cottingham *et al.*, 2005) to explore the relationships between individual periphyton response variables (Table 4.1) and the two stressors. To test the competing hypotheses about different response shapes (described in the Introduction), I used a set of linear regression models for each response variable and adopted an information-theoretic model selection approach that allows inferences to be drawn based on the relative support for the models provided by the data (Johnson & Omland, 2004). I followed the protocol of Johnson and Omland (2004) to perform the model selection procedure in five steps in the same fashion as described in detail in Chapter 3. Transformations of the response variables were performed where necessary to meet the assumptions of linear regression analysis as follows: percent algal cover and percent diatoms were arcsin-transformed ($\sin^{-1}$(sqrt(response/100))); chlorophyll:phaeophytin, *Nitzschia gracilis/palea* and *N. amphibia*, bacterial species richness were log-transformed; percent cyanobacteria and *Synedra ulna* var. *biceps* were log(x+1)-transformed; all other variables were square-root transformed except for algal taxon richness and algal evenness, which did not need transformation. All response variables were also scaled by dividing each transformed value by one standard deviation of the sample population. Predictor variables were both scaled and centred, to allow calculation of standardized partial regression coefficients as described in more detail in Chapter 3.

4.4 Results

NMDS plots of the algal community (Figure 4.1) indicate that the algal communities associated with the four higher sediment and nutrient levels (levels 5-8, black) differed from those in the four lower sediment and nutrient levels (levels 1-4, grey), respectively, by the positioning of these groups on opposite sides of the plots. However, the gradient pattern of increasing dissimilarity with increasing levels of deposited fine sediment (Figure 4.1a) was less distinct than that with increasing levels of nutrients (Figure 4.1b). NMDS plots of the bacterial community are not shown because the 3D stress value of 0.28 was too large for useful interpretation.

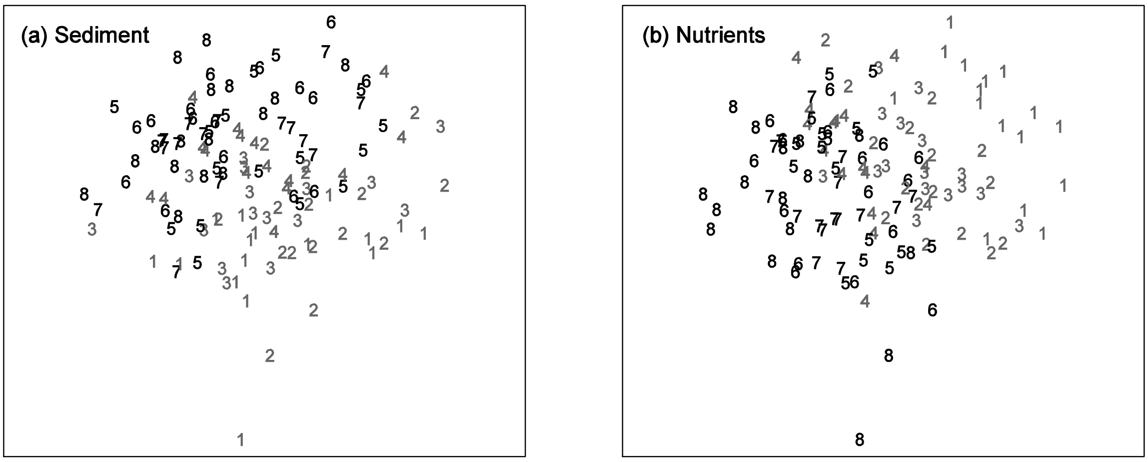


Figure 4.1 NMDS (non-metric multidimensional scaling) plots (3D stress = 0.14) of the 128 mesocosms based on dissimilarities in algal community composition between the mesocosms grouped by treatment levels (1-8) of (a) deposited fine sediment and (b) dissolved nutrients, and colour-coded for the four lower (1-4, grey) and higher levels (5-8, black).

At least one of the two stressors was an important predictor for 28 of the 32 periphyton response variables. The exceptions were *Navicula cryptocephala* and *Gomphonema minutum*, algal taxon richness and bacterial evenness. In these cases, the global model (1) did not statistically significantly fit the data better than the null model (13, Table 4.2) and they were excluded from further analysis. For the remaining response variables, Table 4.2 presents the results of steps 2 and 3 of the model fitting procedure described in the Methods. Only a single top model was selected for nine response variables and two, three or four top models for the others. Akaike weights of the 13 models sum to 1 and the weight of a model can be interpreted as the probability of this model, given the data, being the best model in the candidate set of models (Johnson & Omland, 2004). The Akaike weights of the selected models in Table 4.2 ranged from 0.02 to 0.72, the highest value being ascribed to the top model for percent filamentous green algae. R^2 values were not used in model selection but are shown for each regression model in Table 4.2 to provide a general measure of model fit. R^2 values of the top models ranged from 0.10 (*Cymbella tumida*, bacterial species richness) to 0.65 (algal cover). For each response variable, Table 4.3 shows the final model (determined by model averaging, see Step 4 of the model fitting procedure in Chapter 3, section 3.3.4) which can be used to determine the average value of the response for any sediment and nutrient level of interest. Figures 4.2, 4.3 and 4.4 present the standardized partial regression coefficients (plus 95% CIs) as standardized effect sizes (left hand panels) and three-dimensional response surfaces (right hand panels). For ease of comprehension, these figures show the surfaces of the non-scaled response variables along the non-centred and non-scaled levels of nutrients and sediment. Several of the final models included a term whose effect size was very small. If the partial regression coefficient of a predictor was less than 0.1, I considered it to be of no ecological significance. Note that the effect size of the main-effect term nutrients (N) for response percent motile guild was less than 0.1, but this effect could not be neglected in the corresponding model because a higher-order term was present and larger than 0.1. Furthermore, given my hypotheses (see Introduction) I was interested only in positive, negative or subsidy-stress (hump-shaped) responses. In one case (for chlorophyll:phaeophytin), there was a curvilinear relationship to nutrients where the response variable remained more or less constant from zero to intermediate nutrient levels but increased from intermediate to high levels. Consequently, I grouped this curvilinear response with those of a positive response. Moreover, in five cases polynomial terms were present but the minimum or maximum was outside the range of my predictor variables or extremely close to level 1 and a subsidy effect was barely perceptible; I grouped these curvilinear responses with those of either negative (*Synedra acus/ulna*, percent diatoms, carotenoid:chl a) or positive response shapes (*Scenedesmus*, percent cyanobacteria).

Table 4.2 AIC_c values, Akaike weights and R^2 values of models in the candidate set of 13 linear regression models calculated for each of 28 biological response variables where the global model (model 1) provided a statistically significantly better fit to the data than the null model (model 13). The predictor variables in the models are: S = level of deposited fine sediment (1-8, centred and scaled), N = nutrient level (1-8, centred and scaled), SxN = interaction term, SxS and NxN = 2nd-order polynomial terms. The response variables (also scaled) comprise a visual estimate of algal cover, three variables from photosynthetic pigment analysis, total algal cell density and the cell densities of 14 algal taxa, the percentages of diatoms, filamentous green algae, non-filamentous green algae and cyanobacteria in the community, the percentages of high profile, low profile and motile guilds in the community, algal evenness and bacterial species richness. For data transformations see Methods or Table 4.3. AIC_c values and Akaike weights are results of Step 2 of the model selection procedure. Bold values indicate all models that were in the top model set identified in Step 3 of the model selection procedure. The underlined model is the best top model. For more details see Methods.

		Periphyton response variables											
		Algal cover	Chlorophyll a	Chl a:Phaeophytin	Carotenoid:Chl a	Algal cell density	*Synedra ulna* var.*biceps*	*Scenedesmus* spp.	*Melosira varians*	*Synedra acus/ulna*	*Nitzschia gracilis/palea*	*Nitzschia amphibia*	*Nitzschia dissipata*
Model	Predictor terms	AIC$_c$ of models in candidate model set											
1	S + N + SxN + SxS + NxN	242.9	305.9	268.9	328.2	348.0	304.6	333.9	335.5	340.3	349.0	272.6	339.0
2	S + N + SxN + SxS	243.2	305.4	298.3	330.1	352.0	302.4	336.7	335.3	343.7	366.5	340.6	368.7
3	S + N + SxN + NxN	**240.7**	304.3	268.9	**327.9**	346.9	303.8	332.0	333.7	342.5	349.9	270.6	343.3
4	S + N + SxS + NxN	245.1	304.2	**266.7**	328.2	345.8	304.0	333.6	333.5	**339.0**	347.2	270.4	**337.4**
5	S + N + SxN	**241.0**	303.9	297.8	**329.8**	350.9	301.6	334.8	333.6	345.7	367.0	338.5	371.6
6	S + N + SxS	245.3	303.7	296.1	330.1	349.9	301.8	336.4	333.4	**342.4**	364.7	338.4	367.1
7	S + N + NxN	**242.9**	302.7	**266.8**	**327.9**	**344.8**	303.2	331.8	331.8	**341.2**	348.2	**268.4**	341.7
8	S + N	**243.2**	302.2	295.7	**329.8**	348.8	**301.0**	334.6	331.7	**344.4**	365.3	336.4	370.0
9	S + SxS	261.0	302.2	367.5	361.2	348.2	348.7	369.5	331.7	349.0	367.5	337.4	365.3
10	N + NxN	362.4	369.2	**269.3**	336.3	365.0	330.6	**330.8**	368.7	358.6	**346.6**	319.4	**339.8**
11	S	258.9	**300.8**	366.3	360.5	**347.1**	347.5	367.6	**330.0**	350.7	368.0	335.4	368.3
12	N	361.2	368.1	297.2	337.9	368.1	328.5	**333.5**	368.1	361.1	363.6	367.5	368.0
13	Null model	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3
Model	Predictor terms	Akaike weights of models in candidate model set											
1	S + N + SxN + SxS + NxN	0.10	0.02	0.11	0.17	0.07	0.04	0.07	0.02	0.21	0.11	0.07	0.24
2	S + N + SxN + SxS	0.09	0.03	0.00	0.06	0.01	0.13	0.02	0.02	0.04	0.00	0.00	0.00
3	S + N + SxN + NxN	**0.30**	0.05	0.11	**0.20**	0.12	0.06	0.17	0.05	0.07	0.07	0.18	0.03
4	S + N + SxS + NxN	0.03	0.06	**0.34**	0.16	0.20	0.06	0.07	0.06	**0.41**	0.28	0.21	**0.52**
5	S + N + SxN	**0.26**	0.06	0.00	**0.08**	0.02	0.19	0.04	0.05	0.01	0.00	0.00	0.00
6	S + N + SxS	0.03	0.07	0.00	0.06	0.03	0.17	0.02	0.06	**0.08**	0.00	0.00	0.00
7	S + N + NxN	**0.10**	0.12	**0.34**	**0.19**	**0.34**	0.09	0.19	0.13	**0.14**	0.17	**0.55**	0.06
8	S + N	**0.09**	0.14	0.00	**0.07**	0.05	**0.26**	0.05	0.14	**0.03**	0.00	0.00	0.00
9	S + SxS	0.00	0.15	0.00	0.00	0.06	0.00	0.00	0.14	0.00	0.00	0.00	0.00
10	N + NxN	0.00	0.00	**0.10**	0.00	0.00	0.00	**0.31**	0.00	0.00	**0.38**	0.00	**0.16**
11	S	0.00	**0.30**	0.00	0.00	**0.11**	0.00	0.00	**0.32**	0.00	0.00	0.00	0.00
12	N	0.00	0.00	0.00	0.00	0.00	0.00	**0.08**	0.00	0.00	0.00	0.00	0.00
13	Null model	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Model	Predictor terms	R^2 of models in candidate model set											
1	S + N + SxN + SxS + NxN	0.65	0.43	0.57	0.32	0.20	0.43	0.29	0.28	0.25	0.20	0.56	0.26
2	S + N + SxN + SxS	0.64	0.42	0.45	0.30	0.16	0.43	0.26	0.27	0.22	0.06	0.24	0.05
3	S + N + SxN + NxN	**0.65**	0.42	0.56	**0.31**	0.20	0.43	0.29	0.28	0.22	0.18	0.56	0.22
4	S + N + SxS + NxN	0.64	0.42	**0.57**	0.31	0.20	0.43	0.28	0.28	**0.24**	0.19	0.56	**0.25**
5	S + N + SxN	**0.64**	0.42	0.44	**0.29**	0.16	0.43	0.26	0.26	0.19	0.04	0.23	0.01
6	S + N + SxS	0.63	0.42	0.45	0.28	0.16	0.43	0.25	0.26	**0.21**	0.06	0.24	0.04
7	S + N + NxN	**0.64**	0.42	**0.56**	**0.30**	**0.20**	0.42	0.27	0.27	**0.22**	0.17	**0.56**	0.22
8	S + N	**0.63**	0.41	0.44	**0.27**	0.16	**0.42**	0.25	0.26	**0.19**	0.04	0.23	0.00
9	S + SxS	0.58	0.41	0.02	0.07	0.16	0.16	0.01	0.26	0.16	0.02	0.23	0.04
10	N + NxN	0.06	0.01	**0.55**	0.24	0.04	0.27	**0.27**	0.01	0.09	**0.17**	0.33	**0.21**
11	S	0.58	**0.41**	0.02	0.06	**0.15**	0.15	0.01	**0.26**	0.13	0.00	0.23	0.00
12	N	0.06	0.00	0.43	0.21	0.00	0.27	**0.24**	0.00	0.06	0.04	0.01	0.00
13	Null model	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4.2. continued

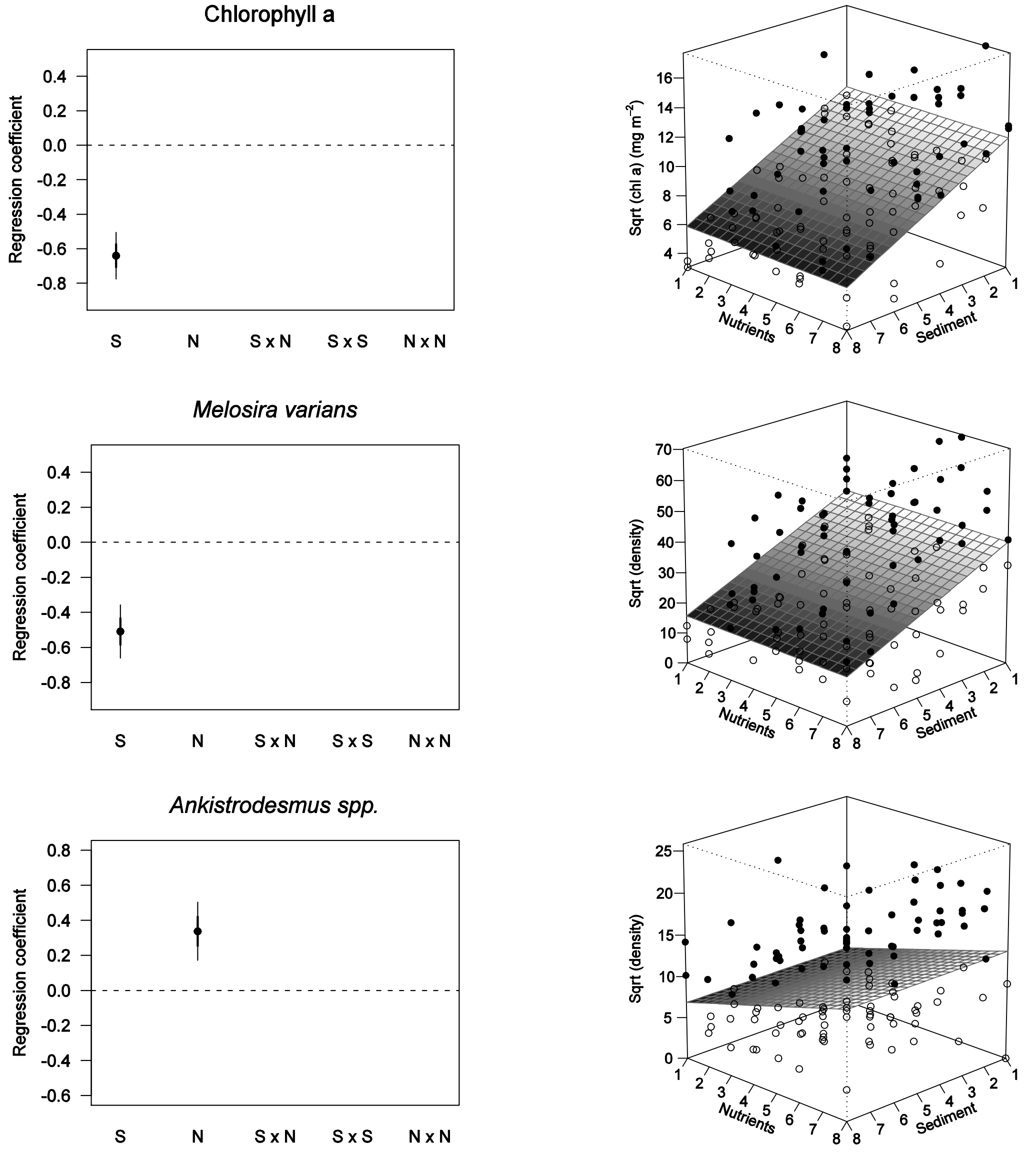
	Periphyton response variables															
	Encyonema minutum	*Fragilaria vaucheriae*	*Nitzschia* sp. 1	*Navicula capitoradiata*	*Ankistrodesmus* spp.	*Cymbella tumida*	*Navicula cryptotenella*	% Diatoms	% Filamentous greens	% Non-filamentous greens	% Cyanobacteria	% High profile guild	% Low profile guild	% Motile guild	Algal evenness	Bacterial species richness
Model	AIC$_c$ of models in candidate model set															
1	300.9	349.6	**292.9**	345.3	360.6	354.03	352.2	299.1	304.3	283.3	350.9	313.4	330.4	309.8	340.4	335.9
2	316.5	368.5	305.2	356.7	358.4	352.41	358.7	306.9	313.1	281.7	353.1	339.8	328.3	352.0	366.1	334.0
3	**298.7**	**348.8**	311.2	**343.3**	358.6	352.21	350.0	299.1	312.8	281.2	**348.7**	311.3	328.6	**307.6**	**338.3**	333.7
4	301.3	349.6	**292.9**	345.3	359.0	354.58	350.0	297.8	**302.2**	282.5	353.4	313.0	328.2	312.2	341.1	333.7
5	314.4	367.5	321.5	354.7	356.4	**350.63**	356.6	306.7	320.7	279.6	**351.0**	337.7	326.5	349.8	364.0	331.8
6	316.7	368.2	305.1	356.5	356.8	352.99	356.6	305.6	310.9	280.9	355.5	339.0	326.1	353.1	366.3	331.8
7	**299.2**	**348.8**	311.0	**343.3**	357.0	352.79	**347.9**	297.8	310.6	280.4	**351.3**	**310.9**	326.4	**310.0**	**339.0**	331.5
8	314.6	367.2	321.1	354.5	354.8	**351.23**	354.5	305.5	318.6	**278.9**	**353.4**	336.9	324.4	350.9	364.2	**329.6**
9	356.5	367.4	342.7	367.9	370.2	364.99	362.2	369.1	310.6	366.5	363.2	348.6	369.1	351.9	367.9	343.0
10	318.7	**349.0**	328.9	**344.2**	355.1	356.46	355.1	**296.2**	362.7	286.0	357.3	337.8	326.0	333.4	**340.2**	331.9
11	354.4	366.4	354.2	366.0	368.2	363.22	360.2	368.1	318.2	364.5	361.1	346.5	367.2	349.8	365.8	340.9
12	331.6	367.1	337.4	355.1	**353.0**	**354.91**	361.2	303.8	367.3	**284.5**	359.2	358.7	**323.9**	367.6	364.8	**330.1**
13	366.3	366.3	366.3	366.3	366.3	366.34	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	366.3	340.8
Model	Akaike weights of models in candidate model set															
1	0.14	0.16	**0.51**	0.11	0.01	0.05	0.06	0.10	0.24	0.03	0.14	0.11	0.01	0.19	0.13	0.01
2	0.00	0.00	0.00	0.00	0.03	0.11	0.00	0.00	0.00	0.07	0.05	0.00	0.03	0.00	0.00	0.03
3	**0.42**	**0.24**	0.00	**0.30**	0.03	0.12	0.18	0.10	0.00	0.09	**0.44**	0.33	0.03	**0.58**	**0.38**	0.04
4	0.11	0.16	**0.49**	0.11	0.02	0.04	0.18	0.18	**0.72**	0.05	0.04	0.14	0.03	0.06	0.09	0.04
5	0.00	0.00	0.00	0.00	0.08	**0.27**	0.01	0.00	0.00	0.20	**0.14**	0.00	0.08	0.00	0.00	0.09
6	0.00	0.00	0.00	0.00	0.06	0.08	0.01	0.00	0.01	0.11	0.01	0.00	0.10	0.00	0.00	0.09
7	**0.33**	**0.24**	0.00	**0.29**	0.06	0.09	**0.52**	0.18	0.01	0.13	**0.12**	**0.41**	0.08	**0.17**	**0.26**	0.11
8	0.00	0.00	0.00	0.00	0.16	**0.20**	0.02	0.00	0.00	**0.29**	**0.04**	0.00	0.23	0.00	0.00	**0.28**
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	**0.21**	0.00	**0.19**	0.15	0.01	0.01	**0.42**	0.00	0.01	0.01	0.00	0.11	0.00	**0.14**	0.09
11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	**0.41**	**0.03**	0.00	0.01	0.00	**0.02**	0.00	0.00	**0.29**	0.00	0.00	**0.22**
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Model	R^2 of models in candidate model set															
1	0.45	0.19	**0.48**	0.22	0.12	0.17	0.18	0.46	0.43	0.52	0.19	0.39	0.31	0.41	0.25	0.12
2	0.37	0.05	0.42	0.13	0.12	0.16	0.12	0.41	0.38	0.52	0.16	0.24	0.31	0.16	0.07	0.12
3	**0.45**	**0.18**	0.39	**0.22**	0.12	0.16	0.18	0.45	0.38	0.52	**0.19**	0.39	0.30	**0.41**	**0.25**	0.12
4	0.44	0.18	**0.47**	0.21	0.12	0.15	0.18	0.45	**0.43**	0.51	0.15	0.38	0.31	0.39	0.23	0.12
5	0.37	0.04	0.33	0.13	0.12	**0.16**	0.12	0.40	0.33	0.52	**0.16**	0.24	0.30	0.16	0.07	0.12
6	0.35	0.03	0.41	0.12	0.12	0.14	0.12	0.41	0.38	0.51	0.13	0.23	0.31	0.14	0.05	0.12
7	**0.44**	**0.17**	0.38	**0.21**	0.12	0.14	**0.18**	0.44	0.38	0.51	**0.15**	**0.38**	0.30	**0.39**	**0.23**	0.12
8	0.35	0.03	0.32	0.12	0.12	**0.14**	0.12	0.40	0.33	**0.51**	**0.13**	0.23	0.30	0.14	0.05	**0.12**
9	0.10	0.02	0.20	0.02	0.00	0.04	0.06	0.01	0.37	0.03	0.06	0.16	0.01	0.14	0.02	0.02
10	0.33	**0.15**	0.28	**0.19**	0.11	0.10	0.11	**0.44**	0.06	0.48	0.10	0.23	0.29	0.25	**0.21**	0.10
11	0.10	0.02	0.11	0.02	0.00	0.04	0.06	0.00	0.32	0.03	0.06	0.16	0.01	0.14	0.02	0.02
12	0.25	0.01	0.22	0.10	**0.11**	**0.10**	0.06	0.40	0.01	**0.48**	0.07	0.07	**0.29**	0.01	0.03	**0.10**
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

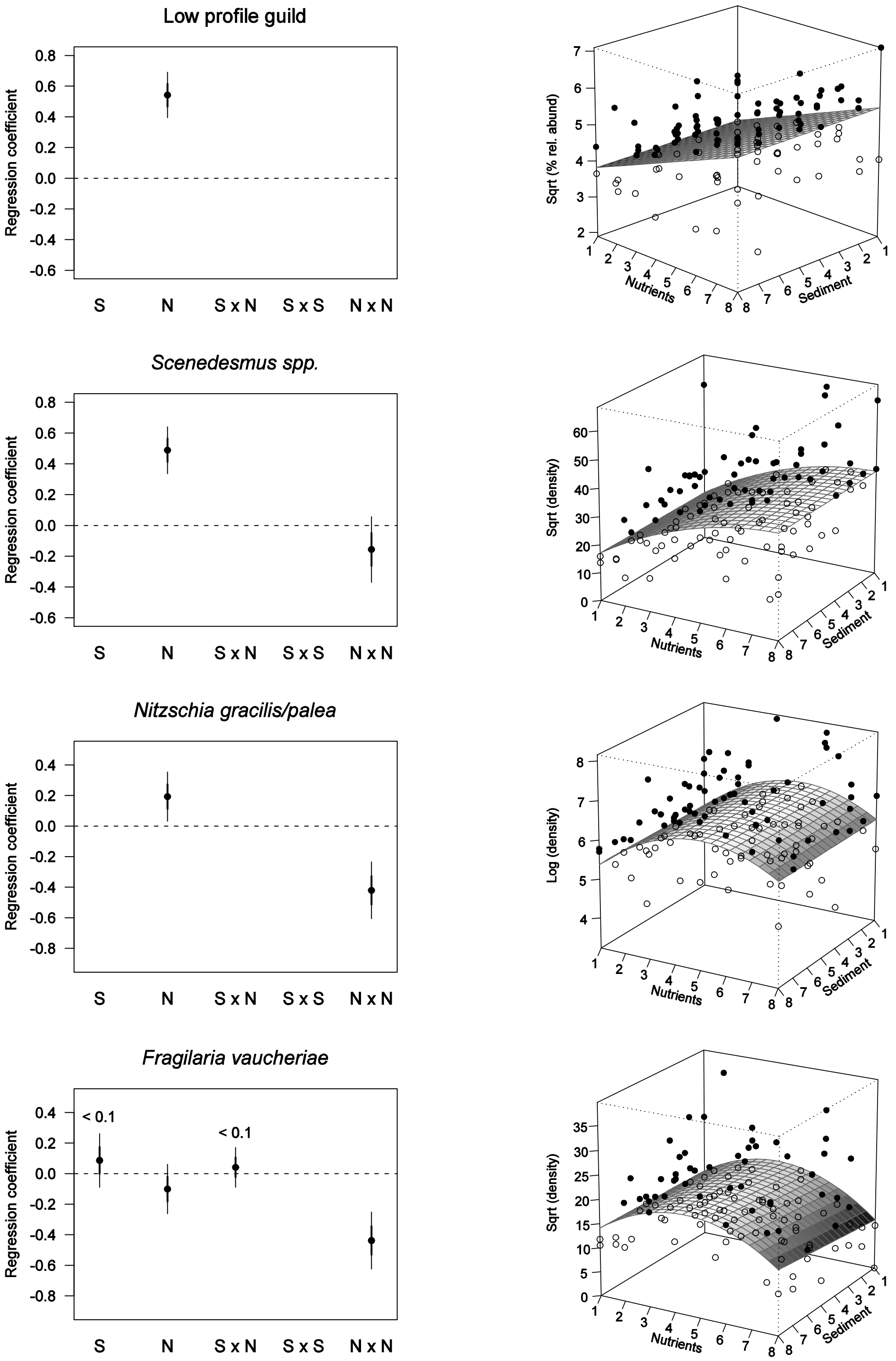
Table 4.3 Intercepts and regression coefficients for the final model (the top model, or averaged for the top model set) for each of the 28 biological response variables. Transformations of response variables are shown in parentheses and all of them are scaled. The predictor variables in the models are S = level of deposited fine sediment (1-8, centred and scaled), N = nutrient level (1-8, centred and scaled), SxN = interaction term, SxS and NxN = 2nd-order polynomial terms. The average of levels 1+2+3 etc. up to level 8 plus the corresponding standard deviation of both predictor variables are as follows: mean = 4.50, SD = 2.30. Sample size is n = 128.

		Standardized partial regression coefficients					
Periphyton response variables	SD	Intercept	S	N	SxN	SxS	NxN
Algal densities (cells/mm^2):							
Synedra ulna var.*biceps* (log(x+1))	0.99	6.70	-0.389	-0.518			
Scenedesmus spp. (sqrt)	13.53	2.37		0.488			-0.156
Melosira varians (sqrt)	15.69	1.78	-0.509				
Synedra acus/ulna (sqrt)	7.62	3.89	-0.360	-0.236		-0.138	-0.176
Nitzschia gracilis/palea (log)	0.87	7.44		0.193			-0.421
Nitzschia amphibia (log)	1.37	4.81	-0.477	-0.083			-0.653
Nitzschia dissipata (sqrt)	9.86	2.66	0.029	-0.056		-0.173	-0.528
Encyonema minutum (sqrt)	10.71	2.00	-0.323	-0.500	0.061		-0.331
Fragilaria vaucheriae (sqrt)	6.61	2.96	0.087	-0.101	0.041		-0.438
Nitzschia sp.1 (sqrt)	10.13	1.97	0.324	-0.464	-0.049	-0.345	-0.288
Navicula capitoradiata (sqrt)	8.53	1.90	0.105	-0.314	-0.045		-0.341
Ankistrodesmus spp. (sqrt)	6.03	1.65		0.337			
Cymbella tumida (sqrt)	5.91	1.49	-0.187	-0.317	0.073		
Navicula cryptotenella (sqrt)	4.98	1.92	0.250	-0.235			-0.278
Taxonomic groups:							
% Diatoms (arcsin)	0.17	6.62		-0.630			-0.242
% Filamentous greens (sqrt)	1.54	0.54	-0.570	-0.095		0.255	0.259
% Non-filamentous greens (sqrt)	1.35	2.81	0.164	0.694			
% Cyanobacteria (log(x+1))	0.93	0.51	0.236	0.264	0.137		0.147
Ecological guilds:							
% High profile guild (sqrt)	1.07	5.98	-0.397	-0.271			0.449
% Low profile guild (sqrt)	1.00	4.64		0.542			
% Motile guild (sqrt)	1.24	4.79	0.368	-0.083	-0.113		-0.569
Algal evenness	0.10	4.40	0.116	-0.168	-0.065		-0.492
Community-level variables:							
Bacterial species richness (log)	0.92	3.56	0.075	0.324			
Algal cover (arcsin)	0.21	4.85	-0.758	-0.235	-0.084		-0.051
Chlorophyll a (sqrt)	3.10	2.87	-0.641				
Chl a:Phaeophytin (log)	0.70	0.18	-0.112	0.653		-0.044	0.399
Carotenoid:Chl a (sqrt)	0.05	17.06	-0.246	-0.461	-0.057		0.124
Algal cell density (sqrt)	27.40	3.60	-0.392	-0.041			-0.175

Nine of the 28 final models were single-stressor models (Figure 4.2), where only terms with sediment or nutrients were included or the effect size for the second stressor was less than 0.1. Two algal responses (chlorophyll a, *Melosira varians*) showed a linear decline with increasing levels of fine sediment. Four responses showed an increase with increasing nutrient levels (*Ankistrodesmus*, percent low profile guild, *Scenedesmus*, bacterial species richness) and two showed subsidy-stress responses to nutrients (*Nitzschia gracilis/palea*, *Fragilaria vaucheriae*).

Single-stressor responses




Low profile guild
Regression coefficient
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
S
N
S x N
S x S
N x N
Sqrt (% rel. abund)
Nutrients
Sediment
Scenedesmus spp.
Sqrt (density)
Nitzschia gracilis/palea
Log (density)
Fragilaria vaucheriae
< 0.1
< 0.1
-0.8

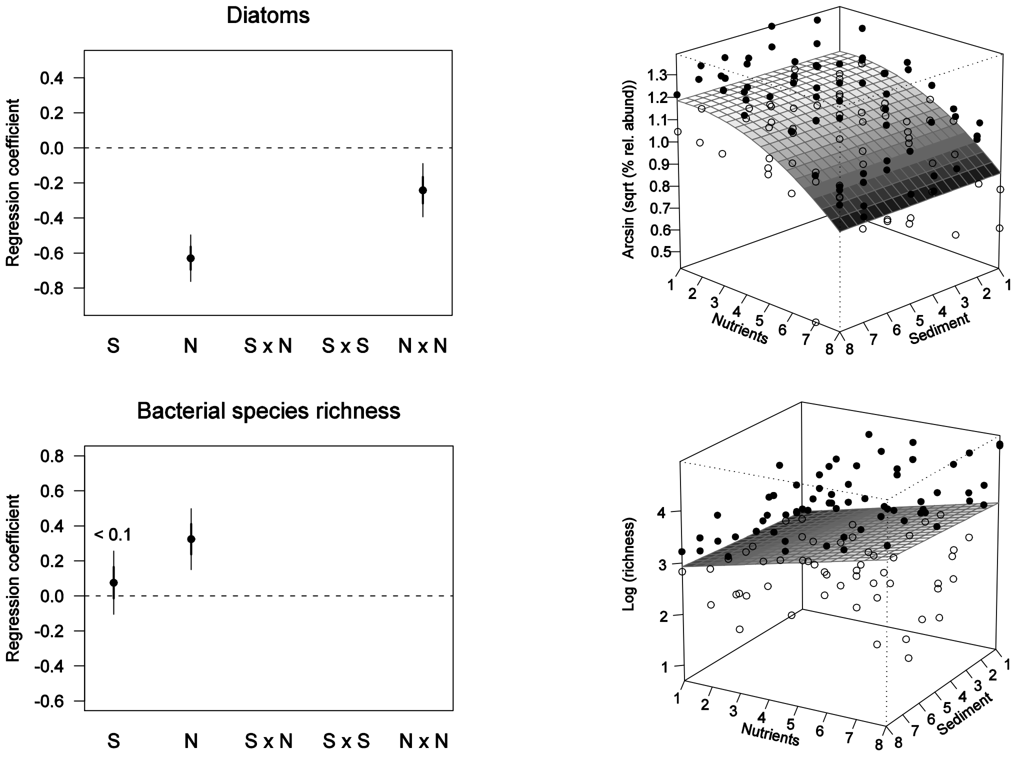
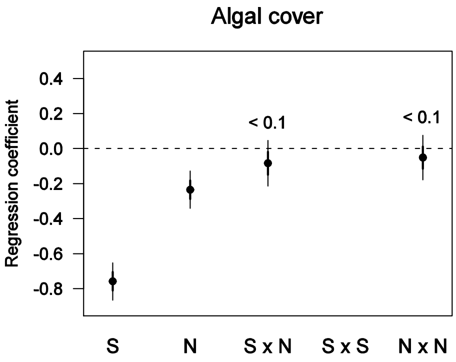
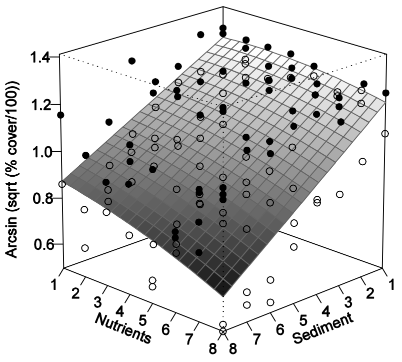


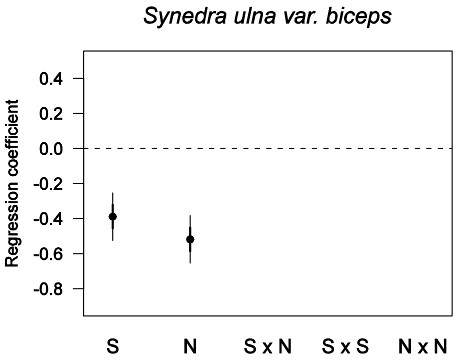
Figure 4.2 Relationships between stressor levels and periphyton response variables for which the model selection procedure revealed a single-stressor response (no nutrient term in the final model or effect size of nutrient term less than 0.1). Standardized partial regression coefficients (plus 95% CIs) of the terms in the final model are shown on the left and three-dimensional response surfaces on the right. Note that these coefficients are also standardized effect sizes of the predictors (for example, a negative or positive effect size of sediment (S) is the reduction or increase in the response in unit standard deviations due to the main effect of sediment at the mean level of nutrients when sediment increases from the mean by one standard deviation). See Table 4.3 for details of transformations used for response variables, all of which were scaled. The predictor variables in the models are S = level of deposited fine sediment (levels 1-8, centred and scaled), N = nutrient level (levels 1-8, centred and scaled), SxN = interaction term, SxS and NxN = 2nd-order polynomial terms. The response surfaces are plotted for the entire range of stressor levels. For ease of comprehension, all three axes of the response surfaces have been plotted with non-centred and non-scaled values. Densities are given as numbers of cells (or counting units) per mm^2; see Methods for details. Please note that the highest nutrient and sediment levels are in the front corner of each 3D plot. The data points are the observed values in the 128 mesocosms with data points that lie above the surface shown as solid circles and those below it as open circles.

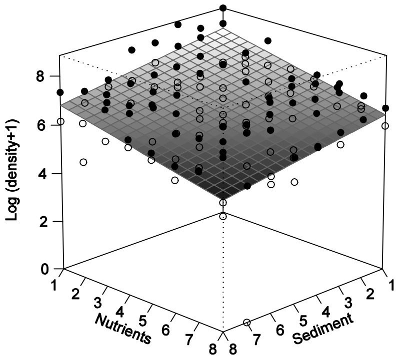
Seventeen final models were simple multiple-stressor models where both stressors affected the response variable but without interactive effects (no interaction term in the final model or interaction term with an effect size less than 0.1). Combinations of different shapes to fine sediment and nutrients were manifold (Figure 4.3). Overall, for fine sediment there were ten negative linear or curvilinear responses (algal cover, *Synedra ulna* var. *biceps*, *Cymbella tumida*, carotenoid:chlorophyll, algal cell density, *Nitzschia amphibia*, *Encyonema minutum*, chlorophyll:phaeophytin, percent filamentous green algae, percent high profile guild), four positive linear responses (percent non-filamentous green algae, *Navicula cryptotenella*, *Navicula capitoradiata*, algal evenness; the latter two responses being weak) and two subsidy-stress responses (*Nitzschia dissipata*, *Nitzschia* sp.1). For nutrients there were six negative linear or curvilinear responses (algal cover, *Synedra ulna* var. *biceps*, *Cymbella tumida*, carotenoid:chlorophyll, percent filamentous greens, percent high profile guild), two positive linear or curvilinear (chlorophyll:phaeophytin, percent non-filamentous green algae) and nine subsidy-stress responses (algal cell density, *Nitzschia amphibia*, *Encyonema minutum*, *Navicula capitoradiata*, *Navicula cryptotenella*, algal evenness, *Synedra acus/ulna*, *Nitzschia dissipata*, *Nitzschia* sp.1).

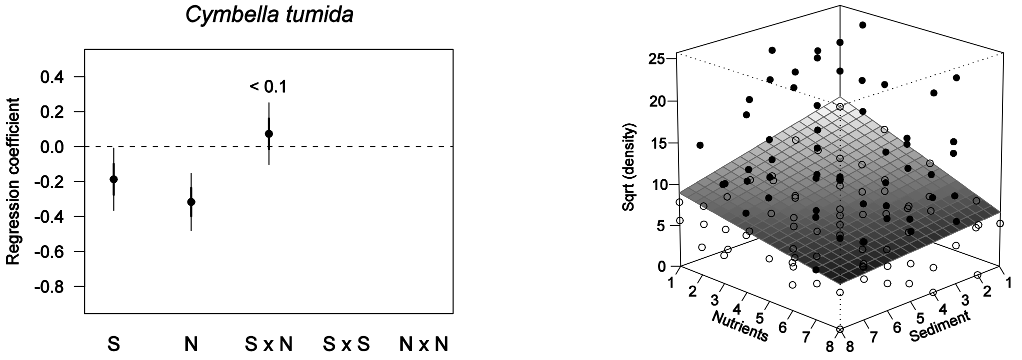
Simple multiple-stressor responses

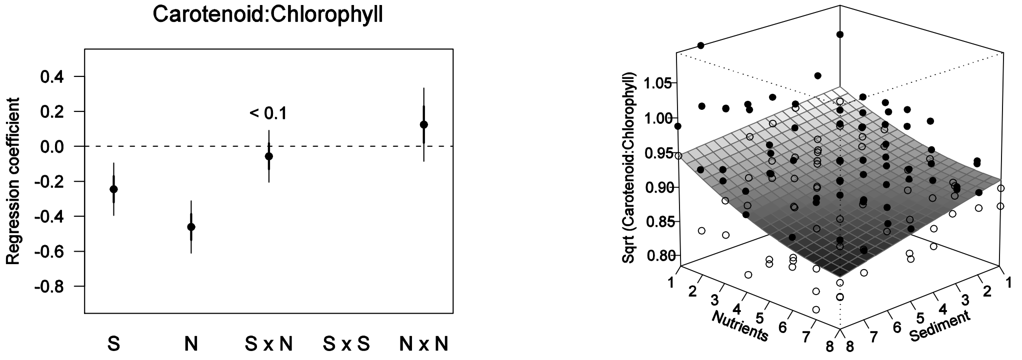


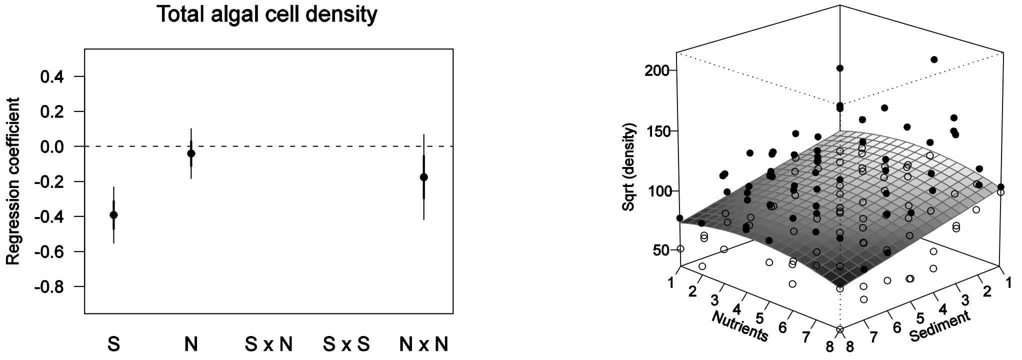


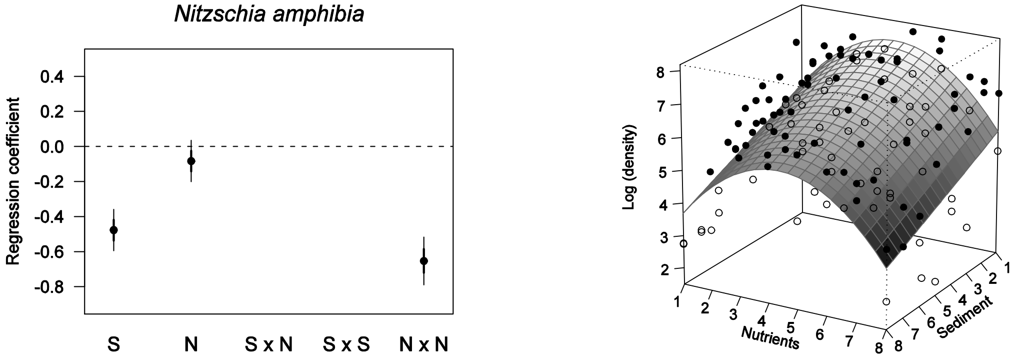


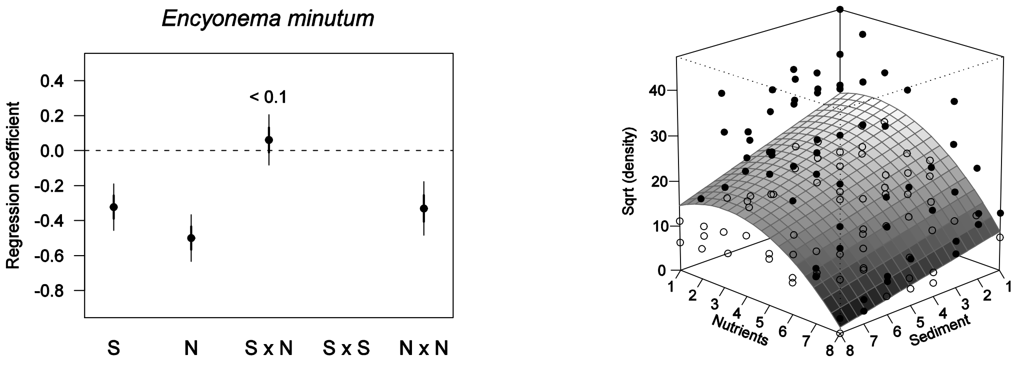



Cymbella tumida

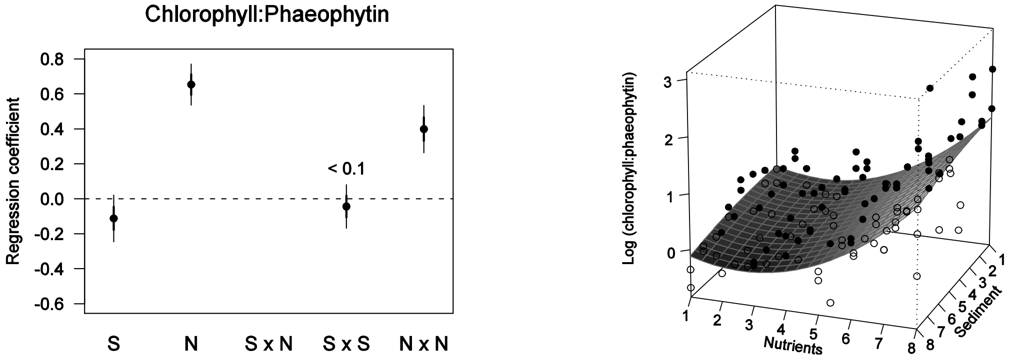

Carotenoid:Chlorophyll


Total algal cell density

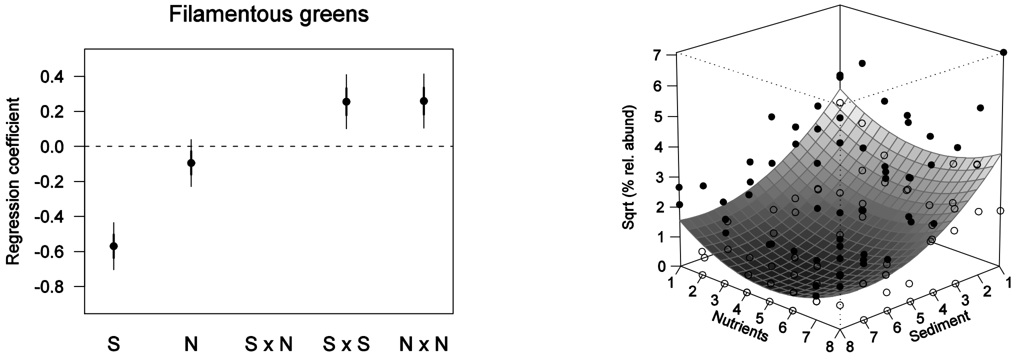

Nitzschia amphibia



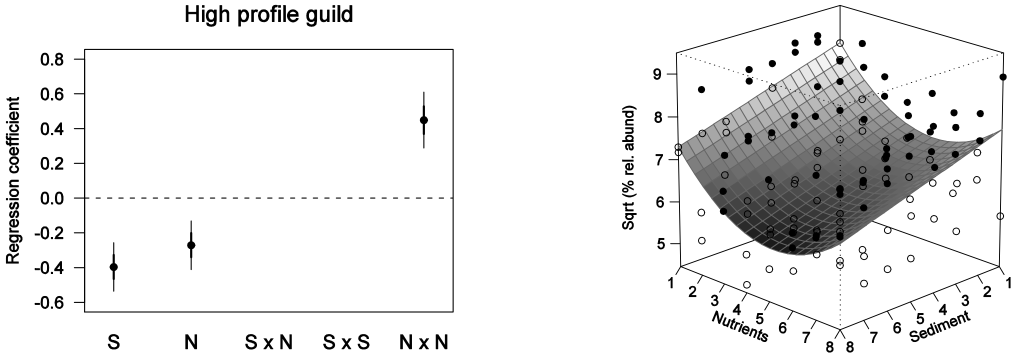
Encyonema minutum



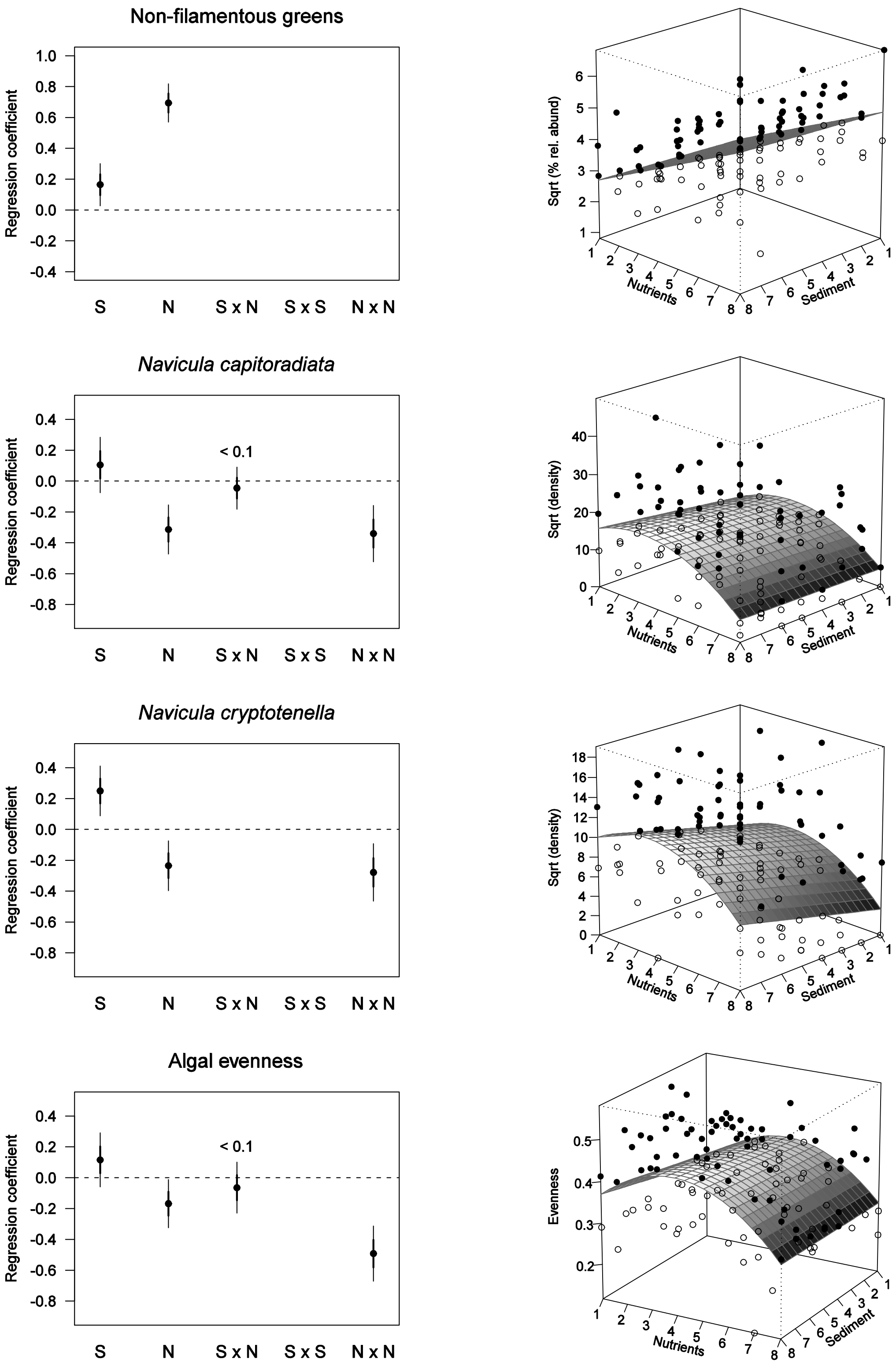
Chlorophyll:Phaeophytin



Filamentous greens



High profile guild


Non-filamentous greens
Regression coefficient
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
S
N
S x N
S x S
N x N
Sqrt (% rel. abund)
Nutrients
Sediment
Navicula capitoradiata
< 0.1
Sqrt (density)
Navicula cryptotenella
Algal evenness
Evenness

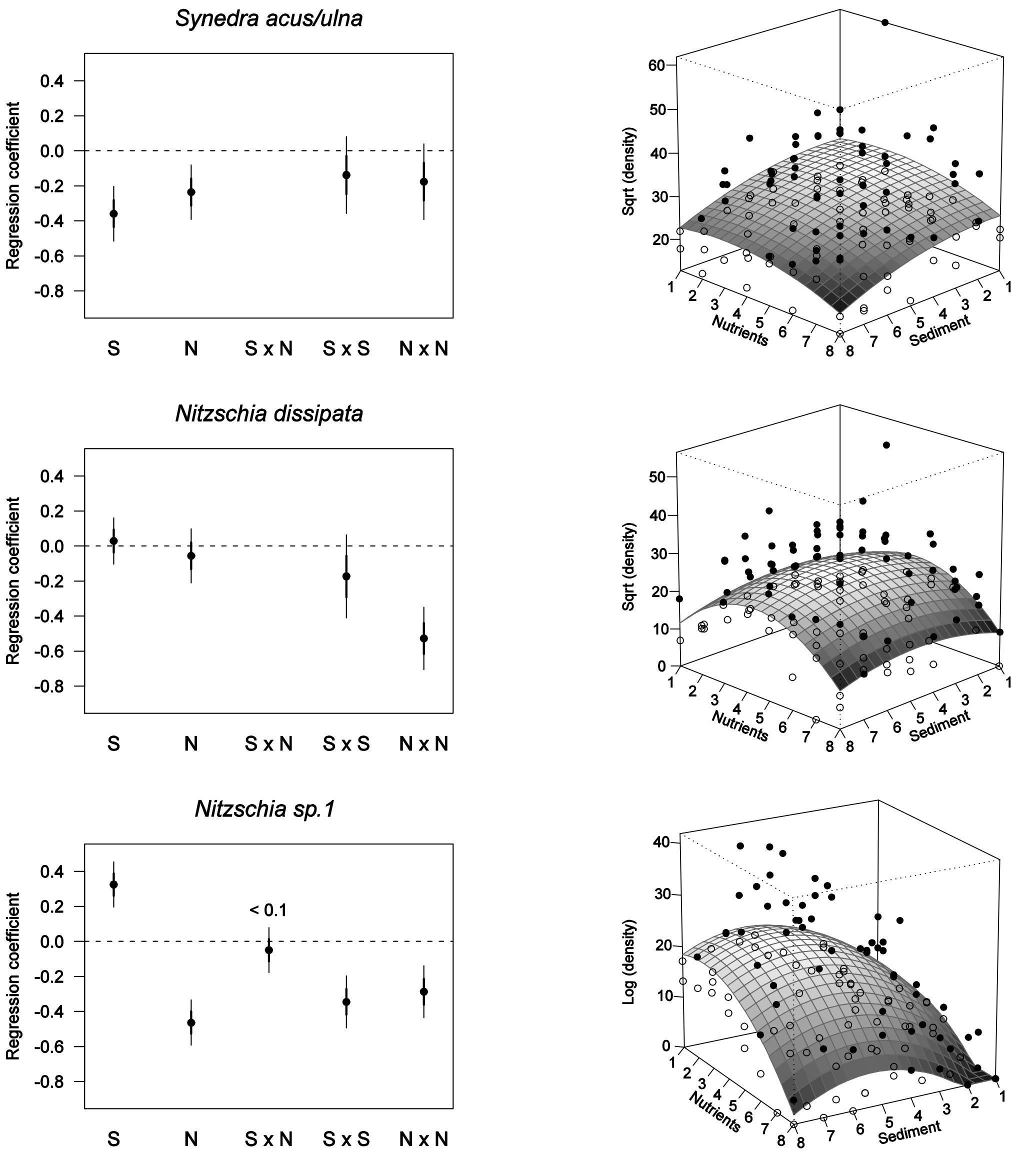
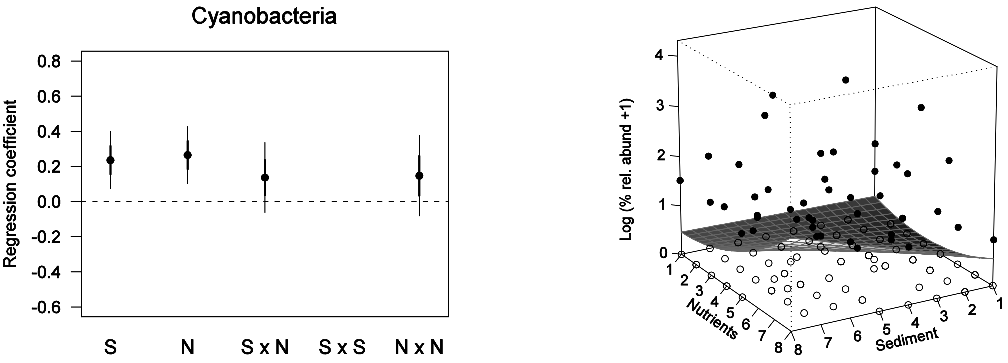


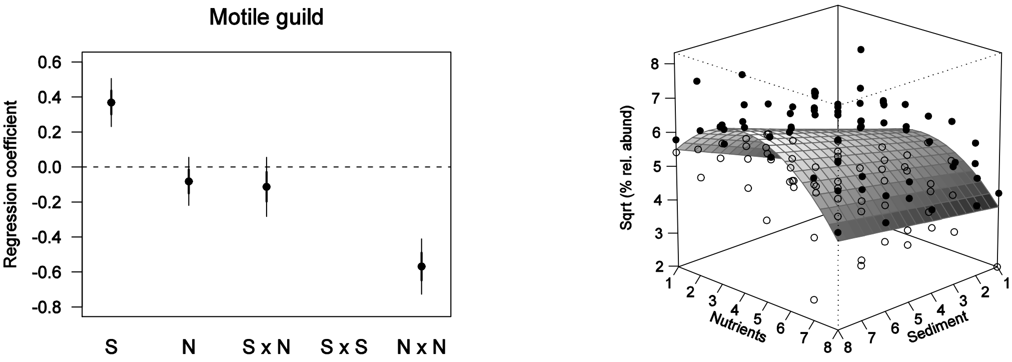
Figure 4.3 Relationships between stressor levels and periphyton response variables for which the model selection procedure revealed a simple multiple-stressor response (no interaction term in the final model or effect size of interaction term less than 0.1). Standardized effect size estimates (standardized partial regression coefficients) plus 95% CIs of the terms in the final model are shown on the left and three-dimensional response surfaces on the right. See Table 4.3 and Figure 4.2 for more details.

Only two periphyton variables (percent cyanobacteria, percent motile guild) showed complex multiple-stressor responses where the relationship along the sediment gradient depended on nutrient level, and vice versa (in both cases predicted with low certainty because the 95 % CI of the partial regression coefficient of the interaction term included zero) (Figure 4.4). The effect of sediment on percent cyanobacteria was barely perceptible at low levels of nutrients and the effect of nutrients was barely perceptible at low sediment. However, percent cyanobacteria increased with rising sediment levels at high nutrient levels and with rising nutrients at high sediment. This interaction can be classified as positive synergistic. The interactive effects of fine sediment and nutrients on percent motile guild were quite complex. Fine sediment generally had a positive effect on percent motile guild but at higher nutrient levels this effect became weaker. Nutrients, in contrast, produced a subsidy-stress response and the maximum value reached along the nutrient gradient was pushed toward lower values of nutrients with increasing levels of sediment. Because at the centre of the gradient fine sediment and nutrients have opposing effects on the motile guild but sediment exerts a sronger effect than nutrients, an interaction term that is opposing to the sediment effect signalises that this interaction can be categorised as antagonistic.

Complex multiple-stressor responses



Cyanobacteria



Motile guild

Figure 4.4 Relationships between stressor levels and periphyton response variables for which the model selection procedure revealed a complex multiple-stressor response (with interaction term and its effect size 0.1 or greater in the final model). Standardized effect size estimates (standardized partial regression coefficients) plus 95% CIs of the terms in the final model are shown on the left and three-dimensional response surfaces on the right. See Table 4.3 and Figure 4.2 for more details.

Table 4.4 summarises all cases with single-stressor, simple multiple-stressor and complex multiple-stressor models by assigning them to three shape categories (negative, positive, subsidy-stress) for each stressor.

Table 4.4 Periphyton response variables categorized by response shape to each individual stressor and ordered by level of biological organization. All cases for each shape category are included, whether from best models that included a single stressor or best models that showed simple or complex multiple-stressor outcomes. Transformations of response variables can be found in Table 4.3. The physiognomic guild assignment of each taxon is given in parentheses; H = 'high profile', L = 'low profile', M = 'motile'.

			Periphyton response variables		
Stressor	Shape category	# of responses/category	Taxon density	Community structure	Community-level variables
Sediment	negative	13	*Synedra ulna* var. *biceps* (H)	% Filamentous greens	Algal cover
	(linear or curvilinear)		*Melosira varians* (H)	% High profile guild	Chlorophyll a
	⇩		*Synedra acus/ulna* (H)		Chl a:Phaeophytin
			Nitzschia amphibia (M)		Carotenoid:Chl a
			Encyonema minutum (H)		Algal cell density
			Cymbella tumida (H)		
	positive	6	*Navicula capitoradiata* (M)	% Non-filamentous greens	Algal evenness
	(linear or curvilinear)		*Navicula cryptotenella* (M)	% Cyanobacteria	
	⇧			% Motile guild	
	subsidy-stress	2	*Nitzschia dissipata* (M)		
	↷		*Nitzschia* sp.1 (M)		
Nutrients	negative	8	*Synedra ulna* var. *biceps* (H)	% Filamentous greens	Algal cover
	(linear or curvilinear)		*Synedra acus/ulna* (H)	% Diatoms	Carotenoid:Chl a
	⇩		*Cymbella tumida* (H)	% High profile guild	
	positive	7	*Scenedesmus* spp. (L)	% Non-filamentous greens	Chl a:Phaeophytin
	(linear or curvilinear)		*Ankistrodesmus* spp. (L)	% Cyanobacteria	Bacterial species richness
	⇧			% Low profile guild	
	subsidy-stress	11	*Nitzschia gracilis/palea* (M)	% Motile guild	Algal cell density
			Nitzschia amphibia (M)		Algal evenness
	↷		*Nitzschia dissipata* (M)		
			Encyonema minutum (H)		
			Fragilaria vaucheriae (H)		
			Nitzschia sp.1 (M)		
			Navicula capitoradiata (M)		
			Navicula cryptotenella (M)		

Pearson's correlation coefficients among the six algal community response variables showed high correlations between two pairs of variables (r = -0.80 for percent high profile and percent motile guild; r = 0.92 for percent non-filamentous green algae and percent low profile guild) (Table 4.5).

Table 4.5 Pearson's correlation coefficient (r) matrix of algal response variables (data transformation as for analysis and given in parenthesis).

	% Diatoms (arcsin)	% Filamentous greens (sqrt)	% Non-filamentous greens (sqrt)	% Cyanobacteria (log(x+1))	% High profile guild (sqrt)	% Low profile guild (sqrt)	% Motile guild (sqrt)
% Diatoms (arcsin)	1	-0.32	-0.62	-0.47	-0.05	-0.50	0.46
% Filamentous greens (sqrt)		1	-0.15	-0.05	0.43	-0.14	-0.38
% Non-filamentous greens (sqrt)			1	0.00	-0.49	0.92	-0.04
% Cyanobacteria (log(x+1))				1	0.20	-0.07	-0.24
% High profile guild (sqrt)					1	-0.52	-0.80
% Low profile guild (sqrt)						1	-0.05
% Motile guild (sqrt)							1

4.5 Discussion

4.5.1 Response shapes and subsidy-stress hypothesis

Fine sediment gradient. My subsidy-stress hypothesis for fine sediment was based on the assumption that sediment augmentation can initially increase surface heterogeneity and therefore provide additional microhabitats for species that are suppressed or excluded by stronger competitors in stable sediment-free substrates, while further augmentation decreases habitat heterogeneity by smothering substratum and hence reduces or eliminates habitat for sediment-sensitive taxa. I found only two subsidy-stress responses to sediment, involving *Nitzschia dissipata* and *Nitzschia* sp.1. Their tolerance of elevated fine sediment may be related to a motile growth form, while their reduced success at very high sediment levels may possibly be explained by increasing vulnerability of motile taxa to higher shear stresses that result when high profile taxa, which slow water movement within the mat, decline (Dodds &

Biggs, 2002). The sediment subsidy-stress hypothesis was not supported at the community level, with neither algal taxon richness nor any other periphyton community response variable responding in a unimodal fashion. Algal taxon richness was unaffected by fine sediment, conforming to results from my field survey (Chapter 2), but the pattern in algal community composition in the NMDS plot indicates that taxon identities changed across the fine sediment gradient, with sensitive taxa being replaced by other taxa that are able to grow on fine sediment. For example, *Melosira varians* and *Nitzschia amphibia* seemed particularly sensitive to fine sediment showing strong declines across the gradient, whereas *Nitzschia dissipata* and *Nitzschia* sp.1, both showing subsidy-stress responses, and *Navicula capitoradiata* and *N. cryptotenella*, both showing strictly positive responses, seemed to be more able to withstand sediment inputs.

Overall, augmented fine sediment produced predominantly negative responses. The densities of six common periphyton algae declined in a linear or curvilinear fashion with increasing sediment, all but one (*Nitzschia amphibia*) being in the high profile guild. Only two species, both in the genus *Navicula* and belonging to the motile guild, were positively affected by sediment. It seems, therefore, that growth form is an important determinant of whether a species is positively or negatively affected by sediment. At the community level, sediment effects on algal taxonomic community composition were also evident. Percent filamentous green algae declined from 17.8 to 2.4 % with increasing sediment (at ambient nutrient levels), a pattern likely to be related to the unstable nature of fine particles preventing extensive growth of filamentous forms. Percent non-filamentous green algae and cyanobacteria, on the other hand, increased with increasing sediment while diatoms did not respond. The cyanobacteria in my experiment, *Phormidium* and *Oscillatoria*, are filamentous taxa that can form well-developed mat on fine substrates because their filaments grow prostrate and can bind and stabilize the finer particles (Biggs, 1996). However, overall algal accrual (total algal cell densities, algal biomass as chl a and visually estimated algal cover) declined with increasing fine sediment in my experiment, in agreement with the expectation that fine particles should sustain lower algal biomass because of substrate instability and periodic disturbance that sets the community back to an early-successional stage (Peterson, 1996). Shading of periphyton by deposited fine sediment particles may be an additional potential mechanism explaining negative effects of sediment on algal accrual. Shade effects could also help explain the reduction in the carotenoid:chlorophyll ratio with increasing sediment. Carotenoids act as photo-protectants in algae, dissipating excess excitation energy and thereby protecting the cells' photosystems (Ferris & Christian, 1991), and shading experiments in streamside channels have shown low beta-carotene in algae under high-shade compared with unshaded conditions (Quinn *et al.*, 1997b).

Nutrient gradient. My subsidy-stress hypothesis for nutrients was based on the assumption that increasing nutrients stimulate primary production at the lower end of the gradient, with subsidy effects on individual algal taxa and the community as a whole, but may cause negative effects when concentrations are very high. This hypothesis was well supported at both the population and community levels. Strictly negative linear responses to nutrients were observed for the densities of only three diatom taxa (*Synedra ulna* var. *biceps*, *Synedra acus/ulna* and *Cymbella tumida*) and strictly positive linear or curvilinear responses for only two non-filamentous green algal taxa (*Ankistrodesmus* and *Scenedesmus*). Subsidy-stress responses, on the other hand, were found for eight of the 16 common algal taxa, including two in the high profile guild (*Fragilaria vaucheriae* and *Encyonema minutum)* and six in the motile guild (*Nitzschia gracilis/palea*, *N. dissipata*, *N.* sp.1, *N. amphibia*, *Navicula capitoradiata* and *N. cryptotenella*). Subsidy effects on *Nitzschia amphibia* and *N. dissipata* were particularly pronounced, with maximum densities at intermediate nutrient levels being, respectively, over six and four times higher than those at the lowest nutrient level (independent of fine sediment level). Subsidy effects on motile *Navicula* and *Nitzschia* species were expected since they have previously been recognised as nutrient-tolerant (eutrophic) on the basis of a large database of surveyed stream sites in the U.K. (Kelly & Whitton, 1995; Kelly *et al.*, 2008). Kelly *et al.* (2008), however, designated *Fragilaria vaucheriae* and *Encyonema minutum* as nutrient-sensitive and indicators of high water quality status, a classification which is contradicted by the initial subsidy effects I found for both species and the positive association of *F. vaucheriae* with intermediate resource supply reported in other studies (Biggs *et al.*, 1998; Lange *et al.*, 2011). Despite the well-known tolerance to high nutrient concentrations of most of the algal taxa that showed an initial subsidy effect in my experiment, a further increase in nutrient concentrations eventually produced a reduction in cell densities, supporting the subsidy-stress hypothesis at the taxon level.

At the community level, total algal cell density also followed a subsidy-stress pattern but both the subsidy and stress components were relatively weak. Algal biomass as chlorophyll a was not affected by nutrients at all and averaged 143 mg/m^2 in channels without added sediment, a value which is considered to be high and typical of enriched streams (Biggs, 1996). Algal cover was also high and only weakly negatively affected by nutrients. In contrast to these minor effects on overall algal accrual, augmented nutrient concentrations affected the taxonomic and physiognomic structure of the community quite strongly. All broad taxonomic groups responded to nutrients. Filamentous green algae were overall negatively affected but increased in relative abundance from intermediate nutrient levels, whereas non-filamentous greens and cyanobacteria showed strictly positive and diatoms strictly negative responses. Proliferation of filamentous green algae under enriched conditions is common (Biggs & Price, 1987; Biggs, 1995; Chetelat *et al.*, 1999); therefore, the reason for their initial decline in relative abundance with increasing nutrient concentrations in my experiment is not clear.

Neither of the two indices of physiological condition of the algal mat followed a subsidy-stress pattern. The chlorophyll:phaeophytin ratio, an index of the vitality of the algal mat (Peterson, 1996), responded strongly to nutrients in a positive curvilinear fashion where from levels 1 to 4 the ratio was close to 1.0 but then increased steeply with ratios of 1.3, 1.8, 2.7 and 4.6 for levels 5 to 8, respectively. The positive response of this ratio, coupled with a lack of response of chlorophyll standing stocks, may be an indication of faster algal turnover at higher nutrient levels (i.e., increased rates of primary production but at the same time increased rates of biomass loss through sloughing of senesced algal cells).

The carotenoid:chlorophyll ratio, an index of the nutrient status of the algal mat (Heath *et al.*, 1990), also responded in a monotonic fashion across the entire nutrient gradient with values decreasing (i.e., cells becoming increasingly nutrient-replete) from a ratio of 0.94 at the lowest to 0.83 at the highest nutrient level. This pigment ratio has, to my knowledge, not been used as an index for stress by inorganic nutrient pollution but rather as an index of stress by nutrient depletion. Heath *et al.* (1990) suggested a limit of 1.5 above which stress by nutrient depletion of marine phytoplankton is indicated, and all values in my experiment were well below 1.5. Consequently my findings indicate that, despite the already high nutrient status of the algal mat at the lowest nutrient level, an increase in nutrient supply positively affected the nutrient status of those taxa present. It seems that the algal community was able to assimilate further inorganic nutrients beyond the point where reduction of total algal cell densities, or of densities of nutrient-tolerant taxa, became apparent.

The possible causes for these density reductions might include physiological stress caused by very high oxygen levels due to high photosynthetic activities or anoxic conditions as a consequence of decomposition processes. Competition with heterotrophic organisms for space or inorganic nutrients is unlikely to be responsible for the decline in algal cell densities (Rier & Stevenson, 2002; Carr *et al.*, 2005), but the positive relationship between bacterial taxon richness and nutrient enrichment in my experiment might be indicative of a high turnover of periphyton biomass at high nutrient concentrations (see above). Algal taxon richness, on the other hand, was not affected by nutrients, conforming to results from my field survey (Chapter 2) but contrasting with another nutrient enrichment experiment in the same mesocosm system looking at diatom species richness (Liess *et al.*, 2009) and results from another field survey (Stevenson *et al.*, 2008). The contrast with Liess et al.'s (2009) study may be due to her focus on diatoms alone (compared to my inclusion of both diatoms and soft-bodied algae) or to the difference in seasons when the experiments were conducted. The lack of a subsidy effect may also be related to the absence of nutrient limitation stress across all nutrient levels in my experiment, and therefore a lack of a subsidy effect due to release from nutrient limitation; this is the usual suggested mechanism for an increase in species richness with rising nutrient levels (Biggs & Smith, 2002). On the other hand, it may be that the oligotrophic water source for the mesocosms lacked algal species colonists that could exploit the small patches of eutrophic conditions provided by the treatments, potentially limiting the biomass response (chlorophyll a) to nutrients often seen in enriched rivers (Biggs, 2000). However, a distinct gradient pattern of algal community composition was apparent in the NMDS plot for my nutrient addition treatments, implying that tolerant taxa replaced those that were sensitive to augmented nutrient concentrations at higher nutrient levels.

Finally, the observed response of algal evenness to nutrient enrichment is consistent with a subsidy-stress hypothesis at the community level: augmented nutrients seemed to initially provide an increase in resource supply allowing for a more evenly-distributed community but as concentrations increased further, eutrophic taxa seemed to increasingly dominate the community.

4.5.2 Single-stressor and multiple-stressor responses

My expectation that periphyton variables would show a range of responses to sediment and nutrients, including no effect, single-stressor effect, simple multiple-stressor effect (no interaction) or complex multiple-stressor effect (interaction), was realised. The majority of response variables followed a single or simple multiple-stressor effect, with only two variables showing a complex multiple-stressor pattern and four variables being unresponsive to either stressor.

I found single-stressor responses for nine periphyton variables and in all but two cases the variables responded to nutrients only, so that overall nutrients affected more periphyton response variables than fine sediment. *Melosira varians* was the only species that responded to sediment but not to augmented nutrients, an unexpected result since this species has been described as being very tolerant of eutrophication (Kelly & Whitton, 1995; Kelly *et al.*, 2008) and typically dominates in streams with high resource supply (Biggs *et al.*, 1998). At the community level, algal biomass as chlorophyll a was also affected only by sediment. While the lack of a nutrient effect on algal biomass might be surprising considering that augmented nutrient concentrations probably stimulated primary production (see above), this is not an uncommon result (Pan & Lowe, 1994; Chapter 2) because an increase in primary production may be masked by increased rates of biomass loss via grazing or autogenic sloughing (Biggs, 1996; Hillebrand, 2002). Substantial sloughing occurred during most of my experiment (personal observations).

Seventeen of a total of 32 periphyton variables showed simple multiple-stressor responses where the variables were affected by both augmented sediment and nutrient concentrations but in a simple additive way (i.e. no interaction evident). All combinations of possible response shapes along the two stressor gradients were realised (negative, positive and hump-shaped). Moreover, there was no obvious coupling of the same response shapes along the sediment and nutrient gradients, indicating that the mechanisms underlying effects of augmented deposited fine sediment and nutrients are very different from one another. This difference might also help explain the scarcity of complex multiple-stressor effects on the periphyton in my experiment.

Only two algal response variables followed complex multiple-stressor patterns where fine sediment interacted with nutrients. The motile guild was strictly positively affected by increasing sediment, reflecting tolerance to fine sediment (compared to the other two growth form guilds) across all nutrient levels, but due to interactions the positive effect of sediment was slightly weaker when nutrient levels were high compared to when nutrients were low signifying antagonistic interactive effects. Further, interactive effects modulated the response of the motile guild to increasing nutrient concentrations. Although the response followed subsidy-stress patterns across all sediment levels, the inflection point was pushed toward lower nutrient levels as sediment levels increased. Given that more than one mechanistic pathway is likely to be involved in shaping the individual stressor response of the motile guild to nutrients, mechanisms underlying these complex interactive effects are far from clear.

The other complex multiple-stressor response was found for cyanobacteria, comprising species of the genera *Phormidium* and *Oscillatoria*. This result could be of particular interest to resource managers because proliferation of cyanobacterial species that are able to produce toxins, including species of these two genera, can be a concern for stream condition and human health. The individual effects of sediment and nutrients were both barely perceptible at low levels of the other stressor gradient, with an increase in percent cyanobacteria from 4.0 to 4.7 % and 4.0 to 4.3 %, respectively. Due to a synergistic interaction, however, the positive effects became stronger at high levels of the other stressor gradient with an increase in percent cyanobacteria from 4.3 to 16.5 % and from 4.7 to 16.5 % across the entire gradient, respectively. Hence, cyanobacteria made up just a small proportion of the algal community in sediment-free mesocosms with low nutrient concentrations and an increase in either sediment or nutrients to high levels still hardly affected their relative abundance if only a single stressor was operating. However, when both sediment and nutrient levels were high, the proportion of cyanobacteria in the community was four times higher indicating that these species benefitted from environmental conditions that were less suitable for species in the green algae and diatom group, which otherwise were the stronger competitors for resources. Filamentous cyanobacteria of the order Oscillatoriales, which includes the two genera recorded in my experiment, have been noted to be more prevalent in streams of high nutrient status (Douterelo *et al.*, 2004) although my results suggest that augmented inorganic nutrients in isolation may not be responsible for cyanobacterial proliferation.

4.5.3 Algal growth form and stressor gradients

My hypothesis regarding algal growth form representation across the fine sediment gradient was fully supported. Thus, although the high profile guild was dominant across all sediment levels, it declined from an average of 74 to 53 % while that of the motile guild increased from 12 to 30 % with rising sediment levels (at ambient nutrients). This opposing relationship was also apparent in the strongly negative correlation between the two response variables. The low profile guild (for which I had no specific hypothesis) did not change across the sediment gradient. These patterns partly agree with previous research, which showed that guild distribution can be habitat-specific (Passy, 2007). In Passy's study, the epilithon was dominated by the high profile guild, while two habitats with finer substrates, the epipsammon (attached to sand grains) and epipelon (on the surface of even smaller particles), were dominated by the low profile and motile guild, respectively. I conclude that the growth morphology traits defined by Passy (2007) are important determinants of algal survival of burial by fine sediment or establishment on the surface of deposited fine sediment. Therefore, they have utility for understanding substrate-related changes in algal community composition and potential bottom-up effects on invertebrates.

My final hypothesis that nutrient augmentation should produce proportional increases in both motile algae and high-profile algae was only partly supported. The high profile guild was the dominant group at all nutrient levels but the relative abundance of all three guilds responded to augmented nutrients, with motile and high profile guilds being negatively correlated and showing reverse response patterns. The motile guild, as predicted, increased in prevalence with increasing nutrients but only up to a maximum of 28 % (in mesocosms without added sediment) and at the expense of the high profile guild. At intermediate nutrient levels, however, the patterns reversed with representation of the high profile guild then increasing, also as predicted, with a further rise in nutrients. The low profile guild (for which I had no hypothesis) responded by increasing significantly across the nutrient gradient with a relative abundance of 15 % at the lowest and 30 % at the highest nutrient condition, mainly driven by the non-filamentous green algae *Scenedesmus* and *Ankistrodesmus*.

My hypothesis about motile and high profile guilds was based on results of previous studies that concerned only diatom species (Passy, 2007; Lange *et al.*, 2011). The authors of these studies argued that increasing nutrients should favour high profile over low profile forms because the former are more competitive in taking up inorganic nutrients, leading to nutrient depletion and shading in the lower layers of the multi-story algal mat. In contrast, the motile guild was hypothesised to increase because many motile taxa are eutrophic. The reason for the discrepancy between these studies and my own may be partly related to the fact that I included all algal taxa and not just diatoms. On the other hand, the point along the gradient where motility seemed to cease providing a competitive advantage might be an indication of nutrient saturation for algal growth. If so, other determinants of community structure might come to the fore, such as tolerance of high inorganic nutrient concentrations or other adverse physiological conditions. A final possibility is that the three guild classification is an oversimplification. For example, some species categorised as low profile can also grow epiphytically and hence escape resource limitation under the canopy of high profile species and this type of life form is known to become more prevalent under enriched conditions (Kelly *et al.*, 2008; Veraart *et al.*, 2008).

4.5.4 Management implications

Augmented deposited fine sediment was shown to be a pervasive stressor that markedly reduced algal standing stocks and altered the taxonomic and physiognomic structure of algal communities. These changes can be expected to have bottom-up effects on higher trophic levels due to changes in food quantity and quality. Although most taxa responded in a consistently negative way to increasing amounts of fine sediment, the inflection points along the sediment gradient where subsidy turned to stress for two motile *Nitzschia* species (between level 4.7 and 5.8) may be identified as potential stressor thresholds. Thus, quite marked changes in the periphyton community occurred when about three quarters of the bed surface was covered by fine sediment. Equally, potential stressor thresholds for augmented nutrient concentrations, which also had dramatic effects on algal taxa, may be identified where subsidy turned to stress. This occurred between nutrient level 2.4 and 5.0, thus spanning a range from about 145 μg DIN /L and 15 μg DRP /L (level 2) to 728 μg DIN /L and 70 μg DRP /L (level 5). The inflection points for community-level variables such as percent motile guild and taxon evenness were around nutrient level 4, which equates to 472 μg DIN /L and 34 μg DRP /L. Thresholds of inorganic nutrient concentrations based on relationships with algal response variables in field surveys are similar. Thus, thresholds of 438 μg DIN /L and 38 μg TP /L were defined based on change points across the nutrient-benthic chlorophyll relationship for streams in Ohio (Miltner, 2010), of between 10 and 20 μg TP /L for stream diatom diversity indices and attributes of taxonomic composition in the mid-Atlantic highlands of the USA (Stevenson *et al.*, 2008), and of 37 μg TP /L and 780 μg TN /L based on diatom community structure for large rivers in New York State (Smith & Tran, 2010).

Algal taxon richness was not related to deposited fine sediment or nutrient concentrations, which is in line with results from my field survey (Chapter 2). This biological response variable is thus likely to be less useful as an indicator when tracking impacts of land-use intensification or the consequences of remediation. On the other hand, the proportion of motile taxa in the algal community, which has been suggested as a potentially useful indicator for nutrient enrichment (Passy, 2007), may be suitable for detecting early signs of nutrient enrichment in streams because of a steep increase in motile taxa at the lower end of the tested gradient. However, this variable might have less utility for classifying differential impacts across a relatively large gradient of inorganic nutrient pollution because of its subsidy-stress pattern.

Managers also need to be aware of multiple stressors affecting periphyton response variables, as revealed in this experiment. Augmented deposited fine sediment and nutrients can affect algal responses in similar or opposing ways, yielding different outcomes than what would be expected if only single-stressor effects have been taken into account. Furthermore, complex multiple-stressor effects may occur, as shown for the proportion of cyanobacteria in the community in my experiment. The proportion of cyanobacteria was unaffected by increasing sediment and nutrients across their wide stressor gradients when operating in isolation but dramatically increased when both stressors acted in concert. Knowledge of such complex multiple-stressor responses of potentially harmful algal species may help resource managers to avoid unexpected negative outcomes.

CHAPTER 5 Multiple-stressor effects on organic matter breakdown: A mesocosm experiment

5.1 Summary

Changes to land use have induced increases in deposited fine sediment and inorganic nutrient concentrations in streams and rivers, and sometimes the two stressors interact to affect macroinvertebrate and periphyton community structure. However, little is known about multiple-stressor effects on functional aspects of stream ecosystems such as organic matter processing. Knowledge of multiple-stressor effects on organic matter breakdown is crucial for predicting future stream condition based on land-use and climate change and for accurate assessment of stream ecological condition using functional indicators. Using the same replicated regression design as in the previous two chapters, I determined the shapes of responses concerning breakdown rates of organic matter. Breakdown of mahoe leaves (an evergreen shrub common in riparian forests in New Zealand) and cotton strips was assessed across both broad stressor gradients after 21 and 7 days of exposure, respectively. I tested (1) the subsidy-stress hypothesis for each stressor and (2) whether sediment and nutrients operated as single or multiple stressors and whether they interacted. Subsidy-stress patterns were found neither across the sediment nor the nutrient gradient. Augmented fine sediment acted as a single stressor on leaf breakdown, accelerating rates measured as loss of leaf mass and strength. This positive sediment effect runs counter to the negative effects regularly reported in the literature, possibly because I used fresh, living leaves (to realistically simulate leaf fall from mahoe shrubs) instead of the dried leaves that have been used most often before. The positive sediment effect in this experiment may be a result of burial by fine sediment causing leaf cell death followed by leaching and microbial attack. The lack of a positive nutrient effect on leaf breakdown, which has also been commonly reported in the literature, is probably also related to the leaves in my experiment being still alive. On the other hand, responses of the cotton strips conformed to the expected stimulating nutrient effects on breakdown rates, measured as loss of tensile strength, and the negative effects of sediment. Interactive multiple-stressor effects were also found for cotton strips. Nutrients strongly positively affected breakdown rates when sediment levels were low but were not influential when cotton strips were buried under a layer of fine sediment. In addition, augmented fine sediment was not influential at low levels of nutrients but had strong negative effects when nutrient concentrations were high. Thus, managers need to be aware that multiple-stressor effects of deposited fine sediment and nutrient enrichment on organic matter processing are likely to occur, sometimes with complex interactions. Cotton strips hold promise for use as a general indicator of ecosystem function although resource management decisions need to be tailored to specific circumstances because some streams will be subject to just one stressor,

other streams just to a second stressor, and yet other streams will be affected by both. Fresh mahoe leaves, on the other hand, seem to be unsuitable as a functional indicator because they responded only weakly to nutrient enrichment and their response to sediment addition contrasted with those of most other leaf materials used as decomposition assays in running waters.

5.2 Introduction

Changes to land use have induced increases in deposited fine sediment and inorganic nutrient concentrations in streams and rivers, and sometimes the two stressors interact to affect macroinvertebrate and periphyton community structure (Townsend *et al.*, 2008; Matthaei *et al.*, 2010; Chapters 2-4). Knowledge of multiple-stressor effects is crucial when assessing ecological stream condition or predicting future condition based on land-use and climate change and when choosing effective resource management strategies that will avoid ecological surprises (Paine *et al.*, 1998). In order to fully assess ecosystem health and integrity, functional aspects need to be incorporated into monitoring programmes to augment the more commonly used structural aspects (Bunn & Davies, 2000; Gessner & Chauvet, 2002). However, current knowledge of the effects of multiple stressors on ecosystem function is limited, with the few recent studies indicating synergistic interactions (Matthaei *et al.*, 2010; Ferreira & Chauvet, 2011).

Much research effort has gone into finding suitable functional indicators for stream ecosystems, and most of these indicators relate primarily to organic matter processing (Gessner & Chauvet, 2002; Young *et al.*, 2008; Sandin & Solimini, 2009; Tiegs *et al.*, 2009; Clapcott *et al.*, 2010; Imberger *et al.*, 2010). Leaf litter breakdown is the best studied process, mainly because it is an important ecosystem function in detritus-based streams where leaves are the principal basal energy source for organisms at higher trophic levels (Cummins *et al.*, 1989; Wallace *et al.*, 1997). Furthermore, leaf litter breakdown is an integrative process (involving bacteria, fungi and shredding invertebrates), is responsive to environmental gradients and can be relatively easily measured, making it a suitable functional indicator for anthropogenic pollution (Gessner & Chauvet, 2002). Because breakdown rates of natural leaves can be highly variable even within tree species (Webster & Benfield, 1986), alternative approaches using standard organic materials such as cotton strips have also been suggested (Tiegs *et al.*, 2007; Young & Collier, 2009; Clapcott *et al.*, 2010). These materials provide a measure of cellulose decomposition potential. Although cotton strips do not necessarily provide a perfect surrogate for leaf breakdown, they may give a good measure of organic matter breakdown and thus be a suitable functional indicator for the assessment of stream ecological condition (Tiegs *et al.*, 2007).

Functional indicators need to be sensitive and behave predictably across anthropogenic stressor gradients (Norris & Hawkins, 2000). When Young & Collier (2009) investigated a suite of functional measures related to organic matter processing and ecosystem metabolism across a land-use stress gradient in New Zealand, they found that several of these measures showed quantifiable yet contrasting and often nonlinear response shapes. In contrast, other studies failed to find predictable response patterns of functional indicators across gradients of urbanisation (Imberger *et al.*, 2010) or agricultural land-use intensity (Hagen *et al.*, 2006; Magbanua *et al.*, 2010), despite profound variations in physicochemical properties (such as nutrient concentrations, sedimentation and temperature) across the land-use gradients. Magbanua *et al*. (2010) noted in a survey of sheep/beef farming streams that the interplay of multiple stressors might be responsible for the failure to observe net effects of agricultural intensity on leaf breakdown. Different stressors are known to have opposing effects on functional measures when they act individually (Young *et al.*, 2008), or to interact with each other in complex ways, as demonstrated experimentally (Matthaei *et al.*, 2010; Ferreira & Chauvet, 2011).

Both augmented deposited fine sediment and nutrients might be expected to have opposing effects as individually acting stressors on organic matter breakdown (Niyogi *et al.*, 2003; Young *et al.*, 2008) and to interact with each other when acting as multiple stressors (Matthaei *et al.*, 2010). Augmented deposited fine sediment is generally expected to slow breakdown because buried material is less accessible to colonisation by microbes and invertebrates, low dissolved oxygen concentrations might deter invertebrates, and potentially anaerobic conditions might hamper microbial processes (Webster & Benfield, 1986; Benfield *et al.*, 2001). Breakdown may also be slowed because of indirect negative effects of fine sediment on most invertebrates (Chapters 2 & 3). On the other hand, increasing amounts of deposited fine sediment have also been reported to positively affect leaf breakdown, running counter to most published studies (Matthaei *et al.*, 2010). Mechanisms explaining such results could be related to the increased physical breakdown due to abrasion of bed-moving fine particles and softening of the material, which can make the material more accessible to microbes (Webster & Benfield, 1986; Benfield *et al.*, 2001). Increasing concentrations of dissolved inorganic nutrients generally accelerate organic matter breakdown because they stimulate microbial activity (Webster & Benfield, 1986; Gulis & Suberkropp, 2003), which in turn can stimulate invertebrate production and feeding (Robinson & Gessner, 2000; Graça, 2001; Sponseller & Benfield, 2001). It is conceivable that a stimulatory effect on breakdown processes ceases at high nutrient concentrations when decomposition processes are no longer nutrient-limited. Thus, further nutrient increases may lead to negative effects on breakdown processes if shredding invertebrates are negatively affected by eutrophication, producing the subsidy-stress response shape (Odum *et al.*, 1979) shown by results from a field survey (Baldy *et al.*, 2007). However, as with other survey studies of breakdown rates along broad nutrient gradients associated with increasing agricultural intensity (Niyogi *et al.*, 2003; Lecerf *et al.*, 2006; Hladyz *et al.*, 2010), confounding factors might have augmented or masked any individual nutrient effects. Finally, most studies that have experimentally tested nutrient effects on organic matter breakdown in the field (Grattan & Suberkropp, 2001; Gulis & Suberkropp, 2003; Ferreira *et al.*, 2006) or in streamside channels (Matthaei *et al.*, 2010) have increased nutrient concentrations only to levels no greater than 1000 µg nitrate-N /L and 300 µg DRP /L. Much higher concentrations have been recorded in streams with intensively farmed catchments in New Zealand and other parts of the world (Hamill & McBride, 2003; Wilcock *et al.*, 2006; Jarvie *et al.*, 2008; Zheng *et al.*, 2008; Friberg *et al.*, 2010; Yuan, 2010).

The aim of this mescocosm study was to investigate the individual and combined effects of augmented nutrient and surface sediment levels on ecosystem response variables related to the breakdown of organic matter (riparian leaves and cotton strips). I used a broad range of stressor levels along both gradients to permit precise definitions of stressor response patterns and tested the following hypotheses that are based on the findings of previous multiple-stressor research in New Zealand streams (Townsend *et al.*, 2008; Matthaei *et al.*, 2010; Chapter 2-4 of this thesis): (1) augmented sediment and nutrients can act as a subsidy to organic matter breakdown variables at low levels but as a stressor at high levels, and (2) organic matter breakdown variables show a range of responses to sediment and nutrients including no effect, single-stressor effect, simple multiple-stressor effect (no interaction) or complex multiple-stressor effect (interaction).

5.3 Methods

5.3.1 Study site and experimental design

The study site and experimental design of the mesocosm experiment to test measures of organic matter breakdown across gradients of deposited fine sediment and nutrient concentrations are described in detail in Chapter 3 and illustrated in Figure 3.1.

5.3.2 Measures of organic matter breakdown

Leaf breakdown. Leaf packs (10 g fresh weight of mahoe leaves - *Melicytus ramiflorus* Forster - a common native riparian shrub in New Zealand; see Matthaei *et al.*, 2010) were prepared two days before imposition of the stressor treatments (day -2), stored overnight in ziplock bags in a cool room and deployed one in each mesocosm on day -1. The leaves of the evergreen mahoe shrub fall intermittently throughout the year (rather than predominantly during a specific season) and do not change colour or shrivel before falling into streams (C.D. Matthaei, personal observations); therefore, mahoe leaves were not dried or pre-conditioned before assembling them in leaf packs. Each pack was held together by a staple and kept in position on the mesocosm bed by surface stones. Ten leaf packs were frozen directly after preparation for later determination of baseline values. The experimental leaf packs were retrieved on the final sampling day (day 21), three weeks being an appropriate deployment period for the fast-decaying mahoe leaves (Matthaei *et al.*, 2010). After retrieval, leaf packs were immediately placed on ice in individual ziplock bags and frozen at -20°C in the laboratory for later analysis. Leaf packs were defrosted and carefully rinsed to remove invertebrates, algae and sediments. I determined two measures of leaf breakdown, namely (1) ash-free dry mass (AFDM) loss and (2) leaf strength loss, by subtracting the measured values of each leaf pack after retrieval from the corresponding baseline values and calculating percentages lost. Leaf strength was determined using a ‘penetrometer’ that involved measuring the mass required to force a blunt pin through the leaf (Young *et al.*, 2008). For each leaf pack, the force was averaged for a single random location (avoiding the middle vein) on each of five leaves. AFDM was determined by weighing and subtracting the oven-dried (at 60°C overnight) and ashed weights (after 550°C for 2.5 hours in a muffle furnace) for each complete leaf pack. Leaf mass and strength loss were expressed as breakdown rate coefficients (k per degree-days) using a formula assuming exponential breakdown and degree-days (332 dd; the duration of the experiment (22 days) multiplied by the average daily water temperature (15.1°C) in the channels determined by data loggers, see Chapter 3) as the time variable (Petersen & Cummins, 1974).

Cellulose decomposition potential. Unbleached cotton test material (Product no. 222; EMPA, St. Gallen, Switzerland) was cut into strips of 240 × 40 mm that were double-folded to obtain a lower and an upper sample per cotton strip, with the expectation of faster breakdown of the lower sample because it was in direct contact with the biofilm. On day 0, just prior to the imposition of the stressor treatments, the double-folded strips were deployed on the streambed of each mesocosm and kept in position by a surface stone. A fishing line attached to each strip led to the outside of the mesocosm to allow retrieval on day 7 with minimal disturbance to the mesocosms. Exposure of cotton samples for seven days has previously been shown to reduce tensile strength to about 50 %, which maximises the power to detect experimental effects (Young *et al.*, 2008; Clapcott *et al.*, 2010). Samples were placed on ice immediately after retrieval and frozen at -20°C shortly after for later analysis. In the laboratory the strips were defrosted, gently rinsed and oven-dried at 40°C for 24 hours. Each strip was reduced to a standard width of 32 mm (100 threads) by carefully fraying away individual outer threads (Clapcott *et al.*, 2010), before cutting to separate the lower and upper samples. Each sample was further cut in half to yield two 6 × 32 mm replicates (subsamples) per lower or upper cotton strip sample. Tensile strength (kg) of each subsample was measured on a motorised tensometer (Sundoo, Whenzhou, China) and the two measurements averaged, resulting in one tensile strength value for each upper or lower cotton strip sample. Baseline tensile strength (58.3 ± 0.56 kg, mean ± SE) was determined using a set of eight control strips that had not been subject to any breakdown. The loss of tensile strength was expressed as a breakdown rate coefficient (k per degree-days), as for leaf mass and strength loss, but using 112 dd as the time variable (because of the shorter exposure period of 7 days and an average daily temperature of 16.0°C for the period).

5.3.3 Data analysis

To determine the individual and combined effects of the two manipulated stressors (deposited fine sediment and nutrients) on organic matter breakdown, I used experimental regression analysis and a model selection approach (see Chapter 3 for details). Transformations of the response variables (breakdown rate coefficients expressed as $k_{dd} \times 10^4$) were performed to meet the assumptions of linear regression analysis as follows: rates of loss of leaf mass, tensile strength (lower cotton strip) and tensile strength (upper cotton strip) were log-transformed (natural logarithm) while rates of loss of leaf strength were square-root transformed. All response variables were also scaled by dividing each transformed value by one standard deviation of the sample population. Predictor variables were both scaled and centred, to allow calculation of standardized partial regression coefficients as described in more detail in Chapter 3. Analyses were performed using the statistical software package R.

5.4 Results

Breakdown rates (k) of leaf mass and strength ranged overall from 3.4 to 43 × 10^4 dd^{-1} and 0.0 to 71 × 10^4 dd^{-1} and rates of cellulose decomposition potential (k) for the upper and lower cotton strips from 5.4 to 68 × 10^4 dd^{-1} and 6.6 to 85 × 10^4 dd^{-1}, respectively. The corresponding values for percentage loss of leaf mass and strength after the 22-day incubation period spanned 10.8 to 76 % and 0.0 to 91 %, and percentage loss of cotton tensile strength after the 7-day incubation period ranged from 5.9 to 53 % for the upper and 7.2 to 61 % for the lower cotton strips. For both leaves and cotton strips, percentage loss spanned a wide range of values as required to maximise the power of detecting treatment effects (Young *et al.*, 2008). Summary statistics of all breakdown variables are shown in Table 5.1.

Table 5.1 Summary statistics of the organic matter breakdown variables expressed each as breakdown rate coefficients and percent loss.

Organic matter breakdown variables	Mean	Min	Max
Breakdown rate coefficient (k_{dd} × 10^4):			
Leaf mass	15	3.4	43
Leaf strength	22	0.0	71
Cotton tensile strength (upper)	18	5.4	68
Cotton tensile strength (lower)	34	6.6	85
% Loss:			
Leaf mass	37	10.8	76
Leaf strength	45	0.0	91
Cotton tensile strength (upper)	18	5.9	53
Cotton tensile strength (lower)	30	7.2	61

For all four breakdown response variables, the global model (1) was a statistically significantly better fit than the null model (13) and I proceeded with the model selection approach. Table 5.2 presents the results of steps 2 and 3 of the model fitting procedure (Chapter 3, section 3.3.4). Only a single top model was selected for ‘cotton tensile strength (upper)' and two or three top models for the other response variables. Akaike weights of the 13 models sum to 1 and the weight of a model can be interpreted as the probability of this model, given the data, being the best model in the candidate set of models (Johnson & Omland, 2004). The Akaike weights of the selected models in Table 5.2 ranged from 0.03 to 0.49, the highest value being ascribed to the top model for 'cotton tensile strength (upper)'. R^2 values were not used in the model selection but are shown for each regression model in Table 5.2 to provide a general measure of model fit. R^2 values of the top models were 0.63 (leaf mass), 0.40 (leaf strength), 0.50 (cotton strength upper) and 0.77 (cotton strength lower). For each response variable, Table 5.3 shows the final model (determined by model averaging, see Step 4 of the model fitting procedure in section 3.3.4) which can be used to determine the average value of the response for any sediment and nutrient level of interest. Figure 5.1 presents the standardized partial regression coefficients (plus 95% CIs) as standardized effect sizes (left hand panels) and three-dimensional response surfaces (right hand panels). For ease of comprehension, these figures show the surfaces of the non-scaled response variables along the non-centred and non-scaled levels of nutrients and sediment. Several of the final models included a term whose effect size was very small. If the partial regression coefficient of a predictor was less than 0.1, I considered it to be of no ecological significance.

Table 5.2 AIC_c values, Akaike weights and R^2 values of models in the candidate set of 13 linear regression models calculated for each of the four breakdown response variables reported as breakdown rate coefficients ($k_{dd} \times 10^4$). The predictor variables in the models are: S = level of deposited fine sediment (1-8, centred and scaled), N = nutrient level (1-8, centred and scaled), SxN = interaction term, SxS and NxN = 2nd-order polynomial terms. The response variables (also scaled) comprise breakdown rate coefficients $k_{dd} \times 10^4$ of leaf mass and strength and of the strength of an upper and lower cotton strip. For data transformations see Methods or Table 5.3. AIC_c values and Akaike weights are results of Step 2 of the model selection procedure. Bold values indicate all models that were in the top model set identified in Step 3 of the model selection procedure. The underlined model is the best top model. For more details see Chapter 3, section 3.3.4.

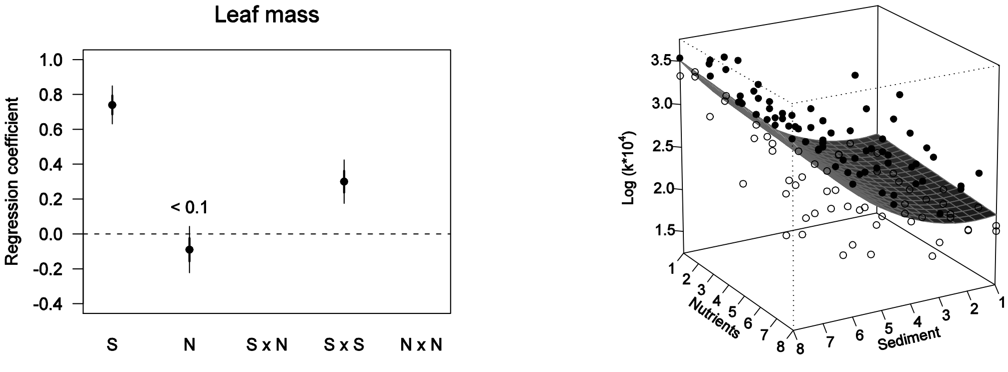
		Organic matter breakdown response variables			
		Leaf mass	Leaf strength	Cotton tensile strength (upper)	Cotton tensile strength (lower)
Model	Predictor terms	AIC$_c$ of models in candidate model set			
1	S+ N+ SxN+ SxS+ NxN	249.1	308.2	287.9	187.1
2	S+ N+ SxN+ SxS	248.0	309.1	286.5	192.1
3	S+ N+ SxN+ NxN	268.6	309.9	285.8	**187.1**
4	S+ N+ SxS+ NxN	246.9	306.0	312.9	190.7
5	S+ N+ SxN	267.3	310.8	**284.5**	**192.0**
6	S+ N+ SxS	**245.8**	307.0	311.4	195.4
7	S+ N+ NxN	266.4	307.8	310.8	**190.6**
8	S+ N	265.2	308.7	309.4	195.2
9	S+ SxS	**248.3**	**305.3**	350.5	362.6
10	N+ NxN	368.4	368.4	335.6	215.2
11	S	266.9	**307.0**	348.5	361.0
12	N	366.7	368.1	334.0	218.6
13	Null model	366.3	366.3	366.3	366.3
Model	Predictor terms	Akaike weights of models in candidate model set			
1	S+ N+ SxN+ SxS+ NxN	0.08	0.07	0.09	0.39
2	S+ N+ SxN+ SxS	0.14	0.04	0.17	0.03
3	S+ N+ SxN+ NxN	0.00	0.03	0.25	**0.40**
4	S+ N+ SxS+ NxN	0.24	0.19	0.00	0.07
5	S+ N+ SxN	0.00	0.02	**0.49**	**0.03**
6	S+ N+ SxS	**0.42**	0.12	0.00	0.01
7	S+ N+ NxN	0.00	0.08	0.00	**0.07**
8	S+ N	0.00	0.05	0.00	0.01
9	S+ SxS	**0.12**	**0.28**	0.00	0.00
10	N+ NxN	0.00	0.00	0.00	0.00
11	S	0.00	**0.12**	0.00	0.00
12	N	0.00	0.00	0.00	0.00
13	Null model	0.00	0.00	0.00	0.00
Model	Predictor terms	R^2 of models in candidate model set			
1	S+ N+ SxN+ SxS+ NxN	0.63	0.42	0.50	0.77
2	S+ N+ SxN+ SxS	0.63	0.40	0.50	0.76
3	S+ N+ SxN+ NxN	0.56	0.40	0.50	**0.77**
4	S+ N+ SxS+ NxN	0.63	0.42	0.38	0.76
5	S+ N+ SxN	0.56	0.38	**0.50**	**0.76**
6	S+ N+ SxS	**0.63**	0.40	0.38	0.75
7	S+ N+ NxN	0.56	0.40	0.38	**0.76**
8	S+ N	0.56	0.38	0.38	0.75
9	S+ SxS	**0.62**	**0.40**	0.14	0.06
10	N+ NxN	0.02	0.02	0.24	0.70
11	S	0.55	**0.38**	0.14	0.06
12	N	0.01	0.00	0.24	0.69
13	Null model	0.00	0.00	0.00	0.00

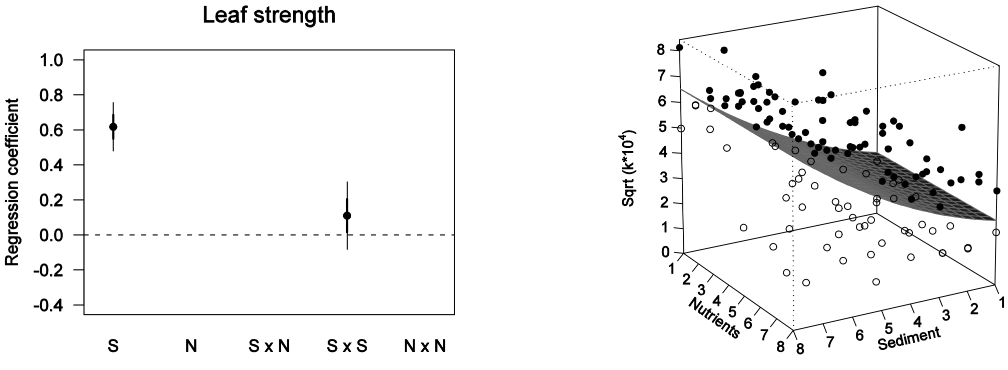
Table 5.3 Intercepts and regression coefficients for the final model (the top model, or averaged for the top model set) for each of the four breakdown response variables. Transformations of response variables are shown in parentheses and all of them are scaled. The predictor variables in the models are S = level of deposited fine sediment (1-8, centred and scaled), N = nutrient level (1-8, centred and scaled), SxN = interaction term, SxS and NxN = 2nd-order polynomial terms. The average of levels 1+2+3 etc. up to level 8 plus the corresponding standard deviation of both predictor variables are as follows: mean = 4.50, SD = 2.30. Sample size is n = 128.

		Standardized partial regression coefficients					
Organic matter breakdown response variables	SD	Intercept	S	N	SxN	SxS	NxN
Leaf mass $k_{dd} \times 10^4$ (log)	0.58	4.136	0.740	-0.090		0.300	
Leaf strength $k_{dd} \times 10^4$ (sqrt)	2.14	1.864	0.617			0.110	
Cotton tensile strength $k_{dd} \times 10^4$ (upper) (log)	0.56	4.828	-0.380	0.486	-0.345		
Cotton tensile strength $k_{dd} \times 10^4$ (lower) (log)	0.72	4.706	-0.237	0.831	-0.089		-0.123

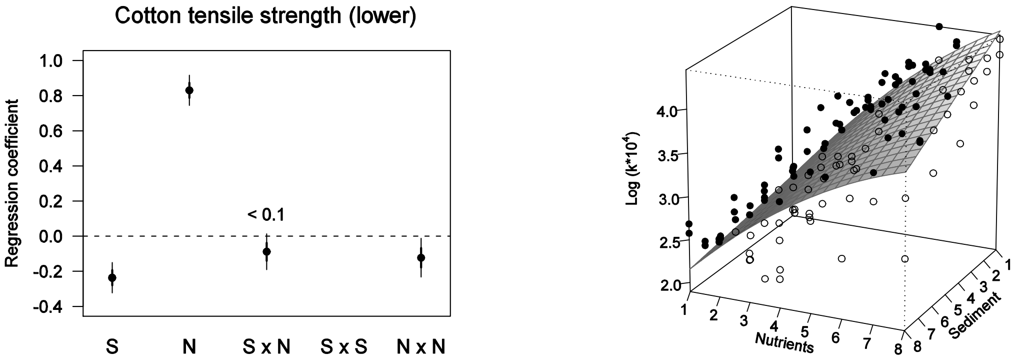
Figure 5.1 (next page) Relationships between stressor levels and breakdown response variables for which the model selection procedure revealed a single-stressor response (no nutrient term in the final model or effect size of nutrient term less than 0.1), simple multiple-stressor response (no interaction term in the final model or effect size of interaction term less than 0.1) or complex multiple-stressor response (with interaction term and its effect size 0.1 or greater in the final model). Standardized partial regression coefficients (plus 95% CIs) of the terms in the final model are shown on the left and three-dimensional response surfaces on the right. Note that these coefficients are also standardized effect sizes of the predictors (for example, a negative or positive effect size of sediment (S) is the reduction or increase in the response in unit standard deviations due to the main effect of sediment at the mean level of nutrients when sediment increases from the mean by one standard deviation). See Table 5.3 for details of transformations used for response variables, all of which were scaled. The predictor variables in the models are S = level of deposited fine sediment (levels 1-8, centred and scaled), N = nutrient level (levels 1-8, centred and scaled), SxN = interaction term, SxS and NxN = 2nd-order polynomial terms. The response surfaces are plotted for the entire range of stressor levels. For ease of comprehension, all three axes of the response surfaces have been plotted with non-centred and non-scaled values. Please note that the highest nutrient and sediment levels are in the front corner of each 3D plot. The data points are the observed values in the 128 mesocosms with data points that lie above the surface shown as solid circles and those below it as open circles.

Single-stressor responses

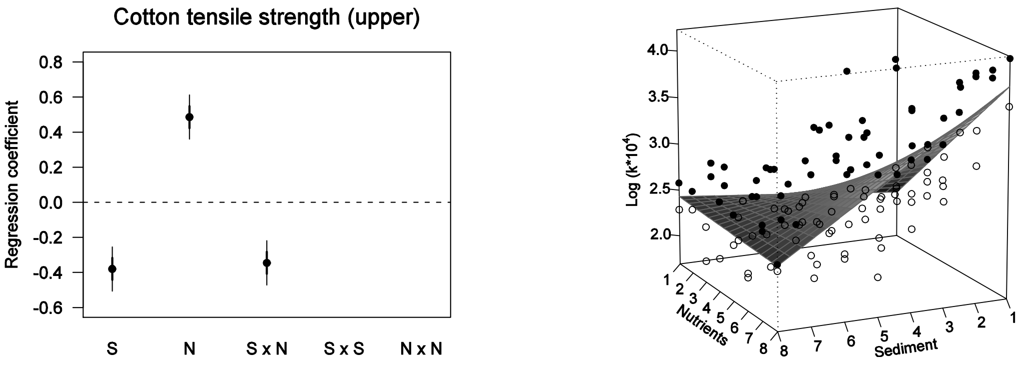




Simple multiple-stressor response



Complex multiple-stressor response



The final models for leaf mass and strength were single-stressor models where only terms with sediment were included or, in the case of leaf mass, the effect size for the nutrient term was less than 0.1. The breakdown rates of both leaf mass and strength showed curvilinear increases with increasing levels of fine sediment, with a more pronounced shape for leaf mass. Leaf mass breakdown changed little at lower sediment levels but then increased steeply from intermediate sediment levels.

The final models for loss of tensile strength of both the lower and upper cotton strips included sediment and nutrient terms and also an interaction term. For the lower strip, however, the effect size of the interaction was smaller than 0.1. Thus, I classified the final lower cotton strip model as a simple multiple-stressor response and the final upper cotton strip model (with a large interaction term) as a complex multiple-stressor response. Both upper and lower strips showed generally similar responses, being positive in relation to nutrients and negative to fine sediment, and with the highest breakdown rates when high nutrient levels coincided with low sediment levels. However, due to the strong interaction in the response of the upper cotton strip the positive effect of nutrients was only prevalent at low to intermediate sediment levels and there was hardly any effect across the nutrient gradient when sediment levels were high. Equally, the negative effect of sediment was only pronounced at high nutrient levels and sediment had hardly any effect on the breakdown rate of the upper cotton strip when nutrient levels were low.

5.5 Discussion

5.5.1 Response shapes along individual stressor gradients

Nutrient concentrations. My hypothesis that increasing nutrient concentrations would cause a subsidy-stress response of organic matter breakdown was only partly supported and the results differed according to the type of organic material used. Rates of cellulose decomposition potential (cotton) were strongly positively affected by nutrients across the entire gradient tested, with breakdown rates at the highest nutrient concentration being 2.3 and 5.7 times as fast compared to those at ambient concentrations (at the lowest fine sediment level) for the lower and upper cotton strips, respectively. While the subsidy effect seemed to be most pronounced as nutrient concentrations increased from low levels and was less marked as nutrient concentrations increased further, there was no evidence of a subsidy-stress response across the broad gradient tested. Leaf breakdown rates (measured as loss of leaf mass and strength) did not respond across the nutrient gradient, running counter to the generally positive effects of increasing inorganic nutrients reported from field surveys (Niyogi *et al.*, 2003; Pascoal *et al.*, 2005; Gulis *et al.*, 2006) and previous field and mesocosm experiments (Robinson & Gessner, 2000; Grattan & Suberkropp, 2001; Gulis & Suberkropp, 2003; Ferreira *et al.*, 2006; Matthaei *et al.*, 2010; Ferreira & Chauvet, 2011). Shredding invertebrates are unlikely to be playing much role in the observed patterns of breakdown, as reported in another study (Hladyz *et al.*, 2010), because they made up a very small proportion (1.6 – 6.2 %) of the macroinvertebrate community (Chapter 3). Generally, acceleration of leaf breakdown rates under enriched conditions is attributed to the stimulation of fungal activity (Suberkropp & Chauvet, 1995; Grattan & Suberkropp, 2001; Pascoal *et al.*, 2005; Gulis *et al.*, 2006) because fungi take up a significant proportion of their nutrient requirements from inorganic nutrients in the water column (Suberkropp & Chauvet, 1995). Fungal biomass measured as ergosterol in the mahoe leaves in my experiment, however, was extremely low (so near the detection limit that data are not worthy of presentation) indicating that fungi were not important colonists of the leaves (D. Niyogi, personal communication). Fungal colonisation might have been inhibited by defensive secondary leaf compounds such as tannins (Bärlocher, 1992), which are known to maintain their effects even after senescence (Schweitzer *et al.*, 2005). It is likely that the mahoe leaves, which were picked from the shrub two days before their application as fresh material (for justification see Methods), were still alive and with defensive mechanisms intact. The different response patterns of cotton strips and leaves may also be related to the difference in the nutritional quality of the organic material. The mahoe leaves, in comparison to cotton material, are of high nutritional value (Quinn *et al.*, 2000) and microbial decomposition may have not been a nutrient-limited process, an explanation that was also offered by the authors of another study where leaf breakdown was unresponsive to nutrient enrichment (Royer & Minshall, 2001). Similarly, differences in the nutritional quality of fresh compared to the pre-conditioned dried leaves that are often used in leaf breakdown studies may also account for the discrepancy between my study and most studies reporting nutrient enrichment effects. Fresh leaves are generally assumed to be of higher nutritional value because they do not lose their soluble and readily available compounds through the purely physical process known as leaching, which accounts for a recorded mass loss of up to 33 % of dry mass within the first 24 hours after immersion when leaves have been dried before application (Gessner & Schwoerbel, 1989). While these explanations for a lack of nutrient effect on leaf breakdown in my experiment may be plausible, however, they cannot be further confirmed because ergosterol was not measured on the cotton strips. It will be interesting to compare in a future study the responses of freshly picked leaves (as used here) and dead leaves subjected to a period of in-stream conditioning.

Deposited fine sediment. My expectation that increasing amounts of deposited fine sediment should have subsidy-stress effects on organic matter breakdown was only partly supported, as well, and also depended on the type of organic material used. As for nutrients, the observed effects of increasing sediment on leaf breakdown rates (measured as loss of leaf mass and strength) ran counter to previous studies. Increasing deposited sediment accelerated the rates of leaf breakdown, which were 3.7 and 6.7 times as fast at the highest sediment level compared to the lowest level for leaf mass and strength, respectively. Strongly positive effects of elevated fine sediment levels on mass loss of mahoe leaves, applied as fresh material, were also observed in an experimental streamside study involving larger channels and with water supplied from the same river (Matthaei *et al.*, 2010). It is unlikely that the underlying mechanism of positive sediment effects on leaf breakdown rates in Matthaei *et al.* (2010) or in my own experiment was mediated by increased abrasion and softening of the leaf material by the movement of unstable fine sediment particles, leading to increases in surface area and rates of breakdown by bacteria (Benfield *et al.*, 2001), because added fine sediment particles did not move much in either study (A. Wagenhoff and C.D. Matthaei, personal observations). Again, the different response patterns of cotton strips and leaves may be partly related to the living nature of the latter. Leaf mass breakdown showed a pronounced positive curvilinear response to burial by fine sediment: up to sediment level 4, breakdown was hardly noticeable but it increased steeply from level 5 onwards, when the surface of the mesocosm was 88 % covered by fine sediment on average. Leaves in mesocosms with no or very little added fine sediment seemed intact and alive (personal observations) and were hardly subject to breakdown, probably because microbial-inhibitory secondary leaf compounds were still intact and soluble compounds were probably retained by the living plant cells (Gessner & Schwoerbel, 1989; Bärlocher, 1992). On the other hand, leaves buried by increasing amounts of fine sediment might have been subject to faster breakdown because burial accelerated leaf cell death, followed by leaching and microbial attack. In addition, extracellular enzymatic activities might also be higher in fine sediment habitat than on coarser substrata (Romani & Sabater, 2001). In contrast to the strong positive sediment effects on leaf breakdown rates, fine sediment caused a more moderate, linear negative response in the breakdown rates of lower cotton strips. Upper strips followed the same response shape as lower strips but only when nutrient concentrations were high.

5.5.2 Single-stressor and multiple-stressor responses

Leaf breakdown rates in my experiment were affected by increasing fine sediment but not by increasing nutrient concentrations; hence, the breakdown rates of both leaf mass and strength can be categorised as single-stressor responses. In contrast, cellulose decomposition potential showed multiple-stressor responses with both nutrients and fine sediment affecting breakdown rates. Patterns for both lower and upper cotton strips were generally similar but the response of the lower strips can be classified as a simple multiple-stressor response while that of the upper strips showed a strong complex interaction. There were also pronounced differences in breakdown rates, with lower strips decomposing faster, most likely because of their proximity to the biofilm growing on the surface of the mesocosm substrata. This is probably due to higher extracellular enzymatic activity in the biofilm matrix as compared to the water column, and the stimulating effect that photoautotrophic activities of the algal community can have on detritus decomposition (Rier *et al.*, 2007). On the faster-decomposing lower strips the two stressors had opposing effects, a strong positive effect of nutrients and a weaker negative of sediment but without interaction. By contrast, strong interactions between stressors were apparent for the more slowly decomposing upper strips, a pattern that might have also occurred in the unrecorded earlier breakdown stages of the lower strips. For the upper strips, increasing nutrients stimulated breakdown at low levels of fine sediment but breakdown seemed unaffected by increasing nutrients when sediment levels were high and the cotton was buried. It is possible that the observed patterns were mediated by the physical barrier provided by the added fine sediment and by associated changes to environmental conditions such as dissolved oxygen when both sediment and nutrients were high. For example, Niyogi *et al.* (2003) suggested that negative effects of sedimentation strongly counteracted the positive effects of nutrients on breakdown rates of tussock leaves in agricultural streams in New Zealand. Equally, Pascoal *et al.* (2005) found faster leaf breakdown rates at more polluted, nutrient-enriched downstream sites except for those that were also affected by sedimentation. On the other hand, experimental results revealed a positive synergistic interaction where nutrient enrichment had the strongest positive effect on leaf mass loss when fine sediment levels were high (Matthaei *et al.*, 2010) while leaf strength loss was unaffected by either sediment addition or an interaction between the two stressors.

5.5.3 Management implications

Managers need to be aware that multiple-stressor effects of deposited fine sediment and nutrient enrichment on organic matter processing rates are likely to occur, sometimes with complex interactions between stressors such as the one I found for cellulose decomposition potential in the present experiment. Thus, resource management decisions need to be tailored to specific circumstances because some streams will be subject to just one stressor, other streams just to a second stressor, and yet other streams will be affected by both. Equally, interactions between multiple stressors may confound the accurate assessment of current stream ecological condition when using organic matter processing rates as indicators. Nevertheless, these rates can be useful indicators of inorganic nutrient enrichment as they have been shown to be responsive in most studies reported in the literature as they were in my experiment utilising cotton strips. Indeed, cotton strips hold promise for use as a general indicator of ecosystem function. Fresh mahoe leaves, on the other hand, seem to be unsuitable as a functional indicator because they responded only weakly to nutrient enrichment and their response to sediment addition contrasted with those of most other leaf materials used as decomposition assays in running waters.

CHAPTER 6 General Discussion

6.1 Ecological response shapes across multiple-stressor gradients

My thesis presents ecological response shapes across multiple stressor gradients that can be visualised as response surfaces in three-dimensional space. I quantified these response surfaces for ecological variables measured in both experimental mesocosms and real streams, using multiple linear regression models that include polynomial terms and the interaction term between the two focal stressors, augmented nutrient concentrations and deposited fine sediment. Hence, this quantitative approach allowed for description of a suite of different possible response shapes across each individual stressor gradient, including linear and curvilinear shapes, some of the latter potentially with a maximum or minimum. The shape of a response across a given stressor gradient could also vary across the second stressor gradient, thereby accounting for potential interactions. For the purpose of my thesis and the tested hypotheses, I distinguished between three general response shapes of ecological variables across individual stressor gradients: (1) strictly negative (could be either linear or curvilinear), 2) strictly positive (linear or curvilinear) and (3) unimodal ‘subsidy-stress’ shapes. I also distinguished between single- and multiple-stressor responses, depending on whether an ecological variable responded to only a single stressor gradient or to both. Multiple-stressor responses were further assigned to either simple (additive) or complex (synergistic and antagonistic) multiple-stressor responses. Because definition of a complex multiple-stressor effect as synergistic or antagonistic can depend on the precise location across the stressor gradients, I classified the interaction according to the effect observed at the centres of the gradient. However, while these classifications were useful for hypothesis testing, summarising and communicating multiple stressor effects, the nature of the observed individual and combined multiple stressor effects can be understood best either from the visually presented response surfaces or my descriptions in words in the results sections of Chapters 2-5.

6.2 Single-stressor and multiple-stressor responses

Table 6.1 presents a summary of the multiple-stressor responses of benthic macroinvertebrate and periphyton variables to augmented levels of nutrients and deposited fine sediment including the categories ‘no response’, ‘single-stressor response’, ‘simple multiple-stressor response’ and ‘complex multiple-stressor response’ for comparisons between the organism groups (invertebrates versus periphyton (mainly algae)), between levels or biological organisation (population versus community), and between experimental and observational approaches.

Table 6.1. Summary of multiple-stressor responses of invertebrate and periphyton response variables to augmented levels of nutrients and deposited fine sediment including the categories ‘no response’, ‘single-stressor response’, ‘simple multiple-stressor response’ and ‘complex multiple-stressor response’. See the Data Analysis and Results sections in Chapters 2-4 for details on the statistical analyses and categorisation.

Invertebrate and algal response variables in field survey (Chapter 2)

Response	# of responses per category	Taxon relative abundance	Relative trait representation	Community-level variables
No response	10	*Aoteapsyche*	Burrower	Invertebrate taxon richness
		Orthocladiinae	Low body flexibility	Algal taxon richness
			Grazer	
			Shredder	
			Filter-feeder	
			Respires using gills	
Single stressor (sediment)	2	*Potamopyrgus antipodarum*		Chlorophyll a
Simple multiple stressor	4	*Pycnocentrodes*	Lays eggs at water surface	EPT richness
				MCI
Complex multiple stressor (all synergistic)	6	*Deleatidium*	> 2 reprod. cycles/individual	% EPT
		Oligochaeta	Single individual reproduction	
		Elmidae		

Invertebrate response variables in mesocosm experiment (Chapter 3)

Response	# of responses per category	Taxon density	Relative trait representation	Community-level variables
No response	1	*Potamopyrgus antipodarum*		
Single stressor (sediment)	10	Nematoda	Burrower	
		Copepoda	Clinger	
		Hydora	Grazer	
		Temnocephalus	Deposit-feeder	
		Oxyethira	Predator	
Simple multiple stressor	19	Oligochaeta	> 2 reprod. cycles/ind.	Invertebrate density
		Cladocera	Single ind. reproduction	EPT density
		Deleatidium	Lays eggs at water surface	% EPT
		Tanypodinae	Low body flexibility	Invertebrate richness
		Ostracoda	Spherical body shape	Evenness
		Psilochorema	Filter-feeder	Average body size
			Respires using gills	
Complex multiple stressor (all synergistic)	2	Chironomidae		EPT richness

Periphyton response variables in mesocosm experiment (Chapter 4)

Response	# of responses per category	Taxon density	Relative representation of trait or broad taxonomic group	Community-level variables
No response	4	*Navicula cryptocephala*		Algal taxon richness
		Gomphonema minutum		Bacterial evenness
Single stressor (sediment)	2	*Melosira varians*		Chlorophyll a
Single stressor (nutrients)	7	*Scenedesmus* spp.	Diatoms	Bacterial species richness
		Nitzschia gracilis/palea	Low profile guild	
		Fragilaria vaucheriae		
		Ankistrodesmus spp.		
Simple multiple stressor	17	*Synedra ulna* var. *biceps*	Filamentous greens	Algal cover
		Synedra acus/ulna	Non-filamentous greens	Chl a:Phaeophytin
		Nitzschia amphibia	High profile guild	Carotenoid:Chl a
		Nitzschia dissipata		Algal cell density
		Encyonema minutum		Algal evenness
		Nitzschia sp.1		
		Navicula capitoradiata		
		Cymbella tumida		
		Navicula cryptotenella		
Complex multiple stressor	2		Cyanobacteria (synergistic)	
			Motile guild (antagonistic)	

Overall, I found that nutrients and fine sediment acted predominantly as multiple stressors, while single-stressor outcomes (where invertebrate or periphyton response variables were only affected by either sediment or nutrients) were less common. In my field survey, ten variables showed multiple-stressor responses and two were affected by a single stressor only. In my experiment, the ratio of multiple to single stressor responses was similar across organism groups, 21:10 for invertebrate and 19:9 for periphyton variables. Across the field survey and experiment, few community-level variables responded only to a single stressor. For example, all of the community-level macroinvertebrate indices commonly used in biomonitoring were affected by both nutrients and sediment.

Amongst the multiple-stressor responses, the ratio of 'complex' to 'simple' responses differed between the field survey and experimental approaches. While in the experiment only two invertebrate variables showed complex multiple-stressor responses (both synergistic) and 19 were simply additive, complex outcomes of invertebrate variables (all six synergistic) were more frequently found than simple outcomes (four) in my field survey. Periphyton algal response variables, which were extensively investigated only in my experiment, similarly showed complex multiple-stressor outcomes in only two cases (one synergistic, one antagonistic), while 17 algal variables conformed to the simple multiple-stressor response type. This discrepancy between the observed frequencies of complex multiple-stressor responses in my survey and experiment is likely to be related to spatial and temporal limitations inherent in the experimental approach. For example, the experimental period of 21 days was probably too short for sublethal stressor effects to be reflected in invertebrate densities and community structure. Most invertebrate taxa would have not gone through their full life cycle and through recruitment processes, which are known to be particularly sensitive to multiple stressors (Hughes & Connell, 1999). The stressor effects I observed in my experiment probably reflected mainly fairly immediate consequences, such as those related to invertebrate mortality and drift. In comparison to invertebrates, the small sizes and shorter life cycles of algae mean that spatial and temporal constraints of experiments are less problematic. Nevertheless, multiple-stressor outcomes in my experiment may also have been compromised by keeping the overall stressor load consistent throughout the experiment, instead of applying sediment and nutrient inputs in pulses as they are more likely to occur in nature (whether regular or temporally variable; Riddle *et al.*, 2009; Molinos & Donohue, 2010). Finally, the effects of individual and multiple stressors observed in my experiment might have been somewhat muted in comparison to real streams because of continuous delivery to the mesocosms of organisms from a stream that is little impacted by anthropogenic stressors.

Turning now to the field survey, observed interactive patterns might have been a consequence of co-variation of nutrients or sediment with unmeasured environmental variables in the surveyed streams such as for example shading, water temperature, pesticides or other pollutants, which could not be accounted for in my analysis. Moreover, such potential additional stressors (of anthropogenic and/or natural origin) might themselves have interacted with nutrients and sediment, obscuring or changing the nature of the combined effect that nutrients and sediment appeared to produce. These shortcomings reflect the main limitation of survey approaches, namely their uncontrolled nature. Certainly, experimental manipulations of an additional, third stressor have been shown to produce complex three-way interactions in two thirds of marine cases reviewed, and also to double the frequency of synergistic interactive effects (Crain *et al.*, 2008).

The two focal stressors of my thesis, nutrients and fine sediment, have also been shown to affect ecosystem functioning. Thus, I found evidence for complex multiple-stressor effects on cellulose decomposition potential (cotton strips), while the breakdown rate of a natural substrate, fresh mahoe leaves, was affected by fine sediment as a single stressor only.

In summary, there is evidence from my field survey and experiment that nutrients and fine sediment act as multiple stressors on ecological response variables, sometimes interacting in complex and mostly synergistic ways, adding to similar evidence from previous studies using field survey and experimental approaches (Townsend *et al.*, 2008; Matthaei *et al.*, 2010). My investigation of multiple stressor gradients, however, has provided extra insights into the nature of the observed synergistic interactions. For example, in my field survey, nutrients had a strictly positive effect on the relative abundance of larval *Deleatidium* mayflies and an initial subsidy effect on individuals in the EPT insect orders (% EPT) at low levels of deposited fine sediment, but a strictly negative effect beyond intermediate sediment levels that became stronger as the level of sediment increased further. While this interaction changed the individual response shape to nutrients of *Deleatidium* and % EPT from a strictly or initially positive to a strictly negative shape, the direction of the sediment effect remained negative across the entire nutrient gradient and this effect simply became stronger (indicated by a steeper negative slope) at increasing levels of nutrients. Hence, augmented nutrients can have an opposing effect to the negative impact of fine sediment when nutrients act in isolation or when sediment levels are reasonably low, but when fine sediment levels are also augmented both stressors can have a similarly negative effect.

By contrast, in my experiment complex multiple-stressor interactions on invertebrate variables seemed more subtle because they did not change the overall response shape, a result which is in agreement with the generally lower frequency of complex multiple-stressor responses in the experiment compared to the survey. EPT richness, for example, showed a subsidy-stress response to nutrients across the entire sediment gradient but, due to interactions, the initial subsidy effect became weaker and the point of inflection, where the positive effect turned into a negative effect, was pushed towards a lower level of nutrients as the level of fine sediment increased. Finally, the nature of the synergistic interaction observed for one of the algal variables (cyanobacteria) was different from all other response variables. Fine sediment and nutrients seemed to not influence the relative abundance of cyanobacteria when each stressor acted in isolation or when levels of the second stressor were low, but the proportion of the algal community made up of cyanobacteria increased dramatically when both stressors acted in combination.

6.3 Response shapes and the subsidy-stress hypothesis

Nutrient gradient. Experimental addition of dissolved inorganic nutrients (N and P) to my streamside mesocosms affected most ecological response variables, confirming that exposure for a maximum of 21 days changed both structural and functional aspects of the simulated stream ecosystem. The subsidy-stress hypothesis across the nutrient gradient was well supported at the population level for both benthic algae and macroinvertebrates. The majority of the affected common benthic algal taxa (eight of 13) followed subsidy-stress patterns, while the cell densities of three taxa declined consistently and those of two taxa increased. The points of inflection, where the individual algal taxa reached their maximum densities across the gradient and the subsidy effect of augmented nutrients turned into a negative effect, were situated between my experimental nutrient levels 2.4 and 5.0, equating to a range from about 145 (level 2) to 728 µg (level 5) DIN /L and 15 to 70 µg DRP/ L. Subsidy-stress patterns were also the predominant response shape (four of seven responses) of common macroinvertebrate taxa to increasing nutrient concentration, while three taxa responded in a consistently negative fashion and none followed a consistently positive pattern across the nutrient gradient. The points of inflection for invertebrate taxa showing unimodal patterns were situated between nutrient levels 3.7 and 5.2, equating to a range of 282 (level 3) to 1776 (level 6) µg DIN /L, and 20 to 132 µg DRP /L. Hence the lowest and highest points of inflection of invertebrate taxon responses were slightly higher than those observed for algal taxa. This difference may indicate that algae, which are more directly influenced by nutrients than invertebrates and have shorter growth cycles, respond faster and more sensitively to the onset of eutrophication and associated physicochemical changes in the water that might include extreme diel oxygen concentrations due to high photosynthetic and respiration activities and toxins released by cyanobacteria (Miltner & Rankin, 1998; Allan, 2004; Camargo & Alonso, 2006; Miltner, 2010). Because invertebrates mainly respond to these secondary causes of nutrient enrichment, some researchers have turned to developing nutrient criteria based on clear associations between nutrients, chlorophyll a, daily oxygen concentrations and the condition of macroinvertebrate and fish communities (Miltner, 2010). The potentially higher sensitivity of stream algae might also make them suited for use as early signs of eutrophication (Dodds, 2007). Direct toxic effects of elevated nutrient concentrations on invertebrates can be ruled out as a cause for subsidy effects turning into negative effects in my experiment because only levels 7 and 8 exceeded the maximum level of 2000 μg NO_3-N /L that is assumed to protect most sensitive invertebrate taxa from toxic effects (Camargo *et al.*, 2005).

The subsidy-stress hypothesis was also supported at the community level for both organism groups in my experiment. Algal evenness and total algal cell densities both showed a subsidy-stress pattern, albeit a rather weak one for the latter. Algal taxon richness, on the other hand, was unaffected by nutrients. Invertebrate community subsidy-stress patterns were found for densities of total invertebrates and those of individuals in the EPT orders, as well as for total invertebrate and EPT taxon richness. My field survey also provided some evidence for subsidy-stress relationships between increased nutrients and invertebrate response variables, including the relative abundance of individuals in the EPT orders and of a grazing caddis fly (*Pycnocentrodes*). However, interaction with fine sediment appeared to overwhelm the initial positive effect of nutrients on % EPT so that this subsidy-stress pattern across the nutrient gradient was only apparent when sediment levels were low. In the absence of fine sediment, the point of inflection was at 144 μg DIN /L (equivalent to level 2 in my experiment), hence at a lower value than those identified from my experimental data (see above).

Finally, there was no support for the subsidy-stress hypothesis at the level of ecosystem functioning, measured as breakdown of organic matter in my experiment. Across the nutrient gradient, increasing concentrations strongly accelerated the breakdown of cotton strips, confirming that the decomposer microbial community responded quickly within the 7-day exposure period to nutrients. However, the breakdown of a type of natural organic matter commonly occurring in New Zealand streams, fresh mahoe leaves, remained unaffected by my experimental nutrient additions.

Sediment gradient. Experimental addition of fine sediment to the mesocosms also affected most ecological response variables, confirming that exposure for 21 days to different amounts of fine sediment, which were introduced as a single event, changed both structural and functional aspects of the simulated stream ecosystem. The subsidy-stress hypothesis across the sediment gradient was supported at the population level for both benthic algae and macroinvertebrates, but consistently negative response shapes were more prevalent. The densities of only two algal taxa showed subsidy-stress patterns, whereas the remaining common taxa either declined (six taxa) or increased (two) across the entire sediment gradient. Similarly, four invertebrate taxa showed subsidy-stress patterns (but none in the sensitive EPT orders), eight were consistently negatively affected by sediment and none responded in a consistently positive way. While fine sediment probably provided additional habitat for some taxa (e.g. Oligochaeta), other invertebrate and algal taxa are likely to have been indirectly affected via access to resources (food for invertebrates; light, nutrients and space for algae) whose availability might have been increased if more competitive but sediment-sensitive taxa declined. Tolerance to fine sediment seemed, at least partly, to have been related to the interaction of tolerant taxa with the substratum. Thus, a burrowing life-style of invertebrates and motile growth form of algae may be advantageous compared to a clinging invertebrate life-style or a high-profile algal growth form. Fine sediment also probably influenced invertebrate food quality and quantity, given substantial changes to algal community structure (growth form and broad taxonomy) and declines in algal biomass (chlorophyll a) as well as changes in the representation of invertebrate functional feeding groups. The relative representation of grazing and filter-feeding invertebrates decreased whereas deposit-feeding and predatory species increased with rising sediment levels. However, the mechanistic causes underlying the observed effects on stream invertebrates may also have been related to factors other than food quality and quantity. Filter-feeders, for example, might have been perturbed by direct effects of fine sediment clogging their filtering apparatus.

At the community level, all of the commonly-used invertebrate indicators for stream condition (including EPT density, % EPT, total invertebrate and EPT taxon richness) were consistently negatively affected by increasing amounts of deposited fine sediment. Only total invertebrate density and, more subtly, invertebrate evenness showed subsidy-stress patterns to sediment with points of inflection at sediment level 3.3 for density and 2.6 for evenness (level 2 equating to 5 % and level 3 to 26 % fine sediment cover). The subsidy-stress hypothesis was also not supported at the community level for benthic algae because total algal cell densities as well as algal biomass were consistently negatively affected by augmented fine sediment levels.

Finally, my experiment failed to reveal subsidy-stress patterns for organic matter breakdown, my chosen ecosystem function variable. Burial by fine sediment consistently negatively affected cellulose decomposition potential determined using standardised cotton strips, but had strong and consistently positive effects on the breakdown rates of fresh mahoe leaves.

6.4 Nutrients vs. fine sediment

In most cases dissolved inorganic nutrients and deposited fine sediment acted as multiple stressors, although some ecological variables responded to a single stressor only. Amongst the periphyton variables tested in my experiment, the majority of single stressor responses were those across the nutrient gradient with few variables being solely affected by fine sediment. By contrast, when macroinvertebrate variables were affected by only a single stressor, this was always sediment, in both my experiment and field survey. Furthermore, quantification of standardized effect sizes at the centres of each of the stressor gradients allowed comparison of the relative strengths of each stressor. For algal response variables at both the population and community levels, the relative strength of nutrient effects was larger than that of sediment effects in the majority of cases. Nevertheless, effect sizes of the two stressors averaged across these algal variables were similar at 0.30 for nutrients and 0.31 for sediment. By contrast, fine sediment was the more influential stressor for almost all invertebrate response variables, and the average effect size of sediment across all population and community-level variables (0.48) was also much larger than that of nutrients (0.15). My field survey provided similar results for invertebrates, in all but one case showing sediment (average effect size 0.50) to be a more influential stressor than nutrients (0.17).

The two stressors can be further compared in terms of the shapes of observed ecological responses across the two stressor gradients. Experimental addition of both stressors to streamside mesocosms and exposure for a maximum of 21 days revealed that nutrients and fine sediment differed in terms of the frequency of response shapes (strictly positive, strictly negative and subsidy-stress) for population-level algal and invertebrate variables. Across the gradient of increasing nutrient concentrations, the majority of the algal and invertebrate taxa conformed to a subsidy-stress pattern, while most of the algal and invertebrate taxa followed consistently negative patterns with increasing fine sediment. Subsidy-stress patterns across the sediment gradient were observed for some algal and invertebrate taxa but far less commonly than strictly negative shapes. Similarly, at the community level, the majority of invertebrate and algal variables responded in a consistently negative fashion to the sediment gradient, whereas across the nutrient gradient subsidy-stress responses were most frequent amongst invertebrate community variables but ranked equally with other shapes amongst algal community variables. These experimental results suggest that fine sediment is a stressor with negative ecological consequences even at low levels of augmentation, and that negative effects are realised quickly (in 21 days or less) by sensitive algal and invertebrate taxa, resulting in changes to community structure and potentially the functioning of the stream ecosystem. Augmentation of nutrient concentrations, on the other hand, seemed less pervasive at low concentrations, often providing a stimulating 'subsidy' effect on algal and invertebrate taxa and communities. Although negative ecological consequences of augmented nutrient concentrations could be realised within my 21-day experiment by sensitive algal and invertebrate taxa, resulting in changes to community structure, more detrimental effects on invertebrates can be expected when this stressor operates over longer time scales that includes recruitment stages. Furthermore, in a multiple-stressor situation, detrimental effects on invertebrates may also occur even at low levels of augmentation of nutrients when nutrients interact with other stressors. Results from my field survey suggest that the initial subsidy response of stream invertebrate communities to enhanced nutrients when they act in isolation fails to occur when fine sediment is augmented as well.

6.5 Management implications

My combined results imply that augmented levels of fine sediment and dissolved inorganic nutrient concentrations need to be managed together because they mostly act as multiple stressors in their effects on algal, invertebrate and ecosystem response variables in streams and rivers.

Community-level macroinvertebrate indices such as the MCI, EPT richness and % EPT were a function of land-use related deposited fine sediment and nutrient status in a regional set of streams (routinely monitored by water resource managers in the province of Southland). Therefore, these measures should be useful in tracking long-term changes in stream health related to ongoing land-use intensification or remediation efforts.

When deciding upon measures to prevent degradation or mitigate adverse land-use effects, it is important to know not just the state of stream-health indicators, but also the cause-effect relationships between stressors and stream health together with the current state of the stressors. For example, if an agricultural stream is of poor health as indicated by macroinvertebrate indices and the nutrient status is consistently below a recommended threshold value, whereas sedimentation is apparent, fine sediment will probably be the main cause of impairment (provided these are the only two major stressors) and should receive attention by managers. On the other hand, if an ecologically-degraded stream site is subject to little sedimentation but nutrient concentrations are above a recommended threshold, managers should focus on measures that reduce the amount of nutrient inputs to the stream. However, if the current states of both nutrients and fine sediment are elevated, these two stressors most likely act in combination to cause stream-health impairment and hence a combination of managing both will be needed to achieve good stream condition.

Because my research has shown deposited fine sediment to be a pervasive stressor that, in most cases, exerted more influence on stream invertebrate communities than augmented levels of nutrients, focussing on the reduction of sedimentation is likely to be a particularly effective measure for improving stream condition. However, since cause-effect relationships, established by combining survey and experimental results, have been shown to sometimes be complex due to interactive multiple-stressor effects, the ecological outcome might not be proportional to the relative increase or reduction in deposited sediment and/or nutrients as expected from effects observed in single-stressor studies. Thus, for example, a small increase in nutrients at a site that is subject to high sedimentation might lead to a dramatic decline in stream health, while a similarly small increase in nutrients at a low-sedimentation site might only slightly change the condition of the stream. So mitigation of impairment might require a larger or smaller reduction of the stressor loads than expected. While nutrient concentrations are most commonly measured at stream-health monitoring sites in New Zealand and other countries worldwide, the status of deposited fine sediment is rarely recorded in a quantitative way. In order to best assess the likely causes of decline in stream health, it would be highly desirable to measure both nutrients and fine sediment routinely in the future.

Threshold values that can be adopted by resource managers should ideally be scientifically sound and based on ecological responses. I used my experimental invertebrate data and, for nutrients, defined potential thresholds of harm at the inflection point of the response variable ‘EPT density’, which is generally accepted to be indicative of stream ecological condition and which showed a subsidy-stress relationship with augmented nutrient concentrations. These potential thresholds of harm were at nutrient concentrations of 728 µg DIN /L and 70 µg DRP /L, higher than the estimated inflection point at 144 µg DIN /L of the response variable '% EPT' from my field survey, which only showed a subsidy-stress responses at low sediment levels. This indicates that in real streams, where multiple stressors are at play and operate over longer time scales, thresholds can be expected to be lower than those defined in my experiment. While the inflection point of an indicator of stream ecological condition in my survey was found to be lower than the current water quality 'trigger' values for ecosystem protection in New Zealand as listed in the ANZECC guidelines (ANZECC & ARMCANZ, 2000), the experimentally-defined thresholds of harm were higher. The ANZECC 'trigger' values, however, were not based on ecological responses and hence are not effects-based thresholds, but were derived from the 80th percentile of nutrient concentrations found in a set of baseline or pseudo-baseline sites and simply indicate whether values are within the normal range of variation of such sites. In contrast to augmented nutrient concentrations, fine sediment seemed to produce negative effects on most invertebrate variables in my experiment and I defined thresholds according to benchmarks of a proportional reduction of the mean expected EPT density of 5 % and 10 %, equating to a range of sediment covers between 5 % and 26 %. These cannot be compared to my field survey results because I used a different measure of deposited fine sediment but another study using a survey approach has defined a comparable threshold of 10 % fine sediment cover for protection of sensitive EPT taxa (Bryce *et al.*, 2010).

Finally, ecological response variables other than macroinvertebrates might have potential for defining stressor thresholds and in monitoring. For example, benthic algae are more directly affected by nutrients than macroinvertebrates and, in my experiment, algal population and community-level variables seemed to be more sensitive to eutrophication than their invertebrate counterparts, indicating their potential as early signs of eutrophication (Dodds, 2007). However, my experiment revealed that simple and complex multiple-stressor effects with fine sediment are likely to occur and more research will be needed before arriving at definitive conclusions about the potential of algal indicators such as the representation of motile species in the algal community. More specifically, the frequency of multiple-stressor responses that I found, even if mainly simple and additive, suggests that algal composition cannot be used as an indicator of eutrophication without any corroborating data on the nutrient status of the stream because the algal assemblage might be responding to some other stressor such as deposited fine sediment. Equally, potential multiple-stressor effects of the two focal stressors on functional indicators were apparent in my experimental results and more research is needed before these indicators can be fully incorporated into biomonitoring programmes.

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Appendix

List of all algal taxa that occurred in the stream mesocosms. Each taxon was assigned to one of the ecological guilds (EG) defined by Passy (2007); H = ‘high profile’, L = ‘low profile’, M = ‘motile’.

Division	Taxon	Authority	Year	EG
Bacillariophyta	*Achnanthidium minutissimum*	(Kützing) Czarnecki	1994	L
Bacillariophyta	*Cocconeis placentula*	Ehrenberg	1938	L
Bacillariophyta	*Cymbella aspera*	(Ehrenberg) Cleve	1894	H
Bacillariophyta	*Cymbella kappii*	(Cholnoky) Cholnoky	1956	H
Bacillariophyta	*Cymbella tumida*	(Brébisson) van Heurck	1880	H
Bacillariophyta	*Diatoma hyemale* var. *mesodon*	(Ehrenberg) Kirchner	1878	H
Bacillariophyta	*Encyonema minutum*	(Hilse) Mann	1990	H
Bacillariophyta	*Epithemia adnata*	(Kützing) Brébisson	1838	L
Bacillariophyta	*Epithemia sorex*	Kützing	1844	L
Bacillariophyta	*Fragilaria ungeriana*	Grunow	1863	H
Bacillariophyta	*Fragilaria vaucheriae*	(Kützing) Petersen	1938	H
Bacillariophyta	*Frustulia rhomboides*	(Ehrenberg) de Toni	1891	L
Bacillariophyta	*Frustulia vulgaris*	(Thwaites) de Toni	1891	L
Bacillariophyta	*Gomphoneis minuta* var. *cassieae*	Kociolek & Stoermer	1988	H
Bacillariophyta	*Gomphonema acuminatum*	Ehrenberg	1832	H
Bacillariophyta	*Gomphonema berggrenii*	Cleve	1894	H
Bacillariophyta	*Gomphonema clavatum*	Ehrenberg	1832	H
Bacillariophyta	*Gomphonema parvulum*	(Kützing) Kützing	1849	L
Bacillariophyta	*Gomphonema truncatum*	Ehrenberg	1832	H
Bacillariophyta	*Gomphonema minutum*	(Agardh) Agardh	1831	L
Bacillariophyta	*Hantzschia amphioxys*	(Ehrenberg) Grunow	1877	L
Bacillariophyta	*Melosira varians*	Agardh	1827	H
Bacillariophyta	*Navicula capitoradiata*	Germain	1981	M
Bacillariophyta	*Navicula cryptocephala*	Kützing	1844	M
Bacillariophyta	*Navicula cryptotenella*	Lange-Bertalot	1985	M
Bacillariophyta	*Navicula radiosa*	Kützing	1844	M
Bacillariophyta	*Navicula* sp. 1	Bory de Saint-Vincent	1822	M
Bacillariophyta	*Navicula* sp. 2	Bory de Saint-Vincent	1822	M
Bacillariophyta	*Nitzschia amphibia*	Grunow	1862	M
Bacillariophyta	*Nitzschia* cf. *acicularis*	(Kützing) Smith	1853	M
Bacillariophyta	*Nitzschia dissipata*	(Kützing) Grunow	1862	M
Bacillariophyta	*Nitzschia gracilis/palea*	Hassall	1845	M
Bacillariophyta	*Nitzschia intermedia*	(Hantzsch) Cleve & Grunow	1880	M
Bacillariophyta	*Nitzschia linearis*	(Agardh) Smith	1853	M
Bacillariophyta	*Nitzschia* sp. 1	Hassall	1845	M

Division	Taxon	Authority	Year	EG
Bacillariophyta	*Pinnularia* cf. *gibba*	Ehrenberg	1843	M
Bacillariophyta	*Pinnularia viridis*	(Nitzsch) Ehrenberg	1843	M
Bacillariophyta	*Planothidium lanceolatum*	(Brébisson ex Kützing) Round & Bukhtiyarova	1996	L
Bacillariophyta	*Rhoicosphenia abbreviata*	(Agardh) Lange-Bertalot	1980	L
Bacillariophyta	*Rhopalodia novae-zelandiae*	Hustedt	1913	L
Bacillariophyta	*Rossithidium petersenii*	(Hustedt) Round & Bukhtiyarova	1996	L
Bacillariophyta	*Stauroneis* cf. *anceps*	Ehrenberg	1843	L
Bacillariophyta	*Surirella angusta*	Kützing	1844	M
Bacillariophyta	*Surirella* sp. 1	Turpin	1828	M
Bacillariophyta	*Synedra acus/ulna*	Ehrenberg	1830	H
Bacillariophyta	*Synedra ulna* var. *biceps*	(Kützing) Schönfeldt	1913	H
Bacillariophyta	*Tabellaria flocculosa*	(Roth) Kützing	1844	H
Chlorophyta	*Ankistrodesmus* spp.	Corda	1838	L
Chlorophyta	Chlorococcales sp. 1	Pascher	1915	M
Chlorophyta	*Closterium* spp.	(Nitzsch) Ralfs	1848	L
Chlorophyta	*Cosmarium* spp.	(Corda) Ralfs	1848	L
Chlorophyta	Filamentous green algae sp. 1			H
Chlorophyta	*Gloeocystis* spp.	Nägeli	1849	L
Chlorophyta	*Mougeotia* spp.	Agardh	1824	H
Chlorophyta	*Oedogonium* spp.	(Link) Hirn	1900	H
Chlorophyta	*Scenedesmus* spp.	Meyen	1829	L
Chlorophyta	Chlorococcales sp. 2	Pascher	1915	M
Chlorophyta	*Spirogyra* spp.	Link	1820	H
Chlorophyta	*Staurastrum* spp.	(Meyen) Ralfs	1848	L
Chlorophyta	*Stigeoclonium* spp.	Kützing	1843	H
Cyanobacteria	*Oscillatoria* spp.	(Vaucher) Gomont	1892	H
Cyanobacteria	*Phormidium* spp.	(Kützing) Gomont	1892	H