Investigations of species richness effects on ecosystem functioning using stream-living macroinvertebrates as model organisms

Micael Jonsson

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Department of Ecology and Environmental Science
Umeå University
SE-901 87 Umeå
Sweden

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Author Micael Jonsson

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Abstract
The work in this thesis deals with effects of changed species richness on process rates among stream-living macroinvertebrates. Global biodiversity is decreasing rapidly and it is poorly known what the consequences of this loss may be for ecosystems and the services they provide. Hence, it is important to investigate the potential effects of losing species. In streams, deforestation, introduction of non-native species, pollution and channelization are examples of events that may affect species richness negatively. In this thesis emphasis is on changes in species richness within functional feeding groups (FFGs) of stream-living macroinvertebrates. The FFGs used were shredding detritivores, grazers, filter feeders and predators - all of which uphold important ecological processes in streams. Along with an observational field study, species richness was manipulated in laboratory and field experiments to investigate the effects of changed species richness on process rates and thus ecosystem functioning.

The results show that effects of changed species richness on process rates may be dramatic. Among the shredding detritivores there were negative effects on leaf mass loss, regardless whether fixed, random or predicted sequences of species loss was investigated. These effects could be attributed to either species richness per se or species composition. However, among the other FFGs the relationship between species richness and process rates was less consistent. In filter feeders, there was no or a negative effect of decreasing species richness while both grazers and predators showed positive effects of species loss.

The results also show that the most important interactions between species in an experiment, thus potentially in a natural community, are likely to determine what the effect of species loss on process rates will be. Facilitation and niche differentiation lead to reduced process rates if species are lost, while mechanisms, such as interspecific resource or interference competition, produce the opposite effect. Furthermore, in systems with a diminishing resource, the first two mechanisms may become more important over time enhancing the effect of species loss in the long term.

In conclusion, effects of species loss may be dramatically negative or positive even if lost species are classified as redundant. The effect in the short term most likely depends on which species are lost, on the original species composition and on the underlying mechanisms. Questions remaining to be answered are how important the observed effects are in more complex systems and if they are persistent over time? Future studies will tell.

Key words Species richness, species loss, process rates, ecosystem function, streams, macroinvertebrates.
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**Appendices (papers I-VI)**
List of papers

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


VI Jonsson, M. Effects of species richness on ecosystem function magnify with time. Submitted manuscript.

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Introduction

The value of biodiversity

Diversity of life on Earth is astonishing. Although there are only 1.4-1.8 million documented species, it is believed that up to 30 million species may exist (May 1990). Consequently, we are experiencing a peak in biodiversity - never before has life been so diverse – but we are also experiencing an unprecedented extinction of organisms (Chapin et al. 2000). Albeit extinction is natural - over 99% of all species that ever existed are today extinct (Leakey 1996) - the rate at which species currently are disappearing and the factors behind the loss are likely to be important for what the consequences on remaining life will be. We know that species are disappearing 100-1000 times faster now than before the existence of humans (Chapin et al. 1998) and that the majority of extinctions is caused by human activities such as cultivation, deforestation, habitat fragmentation, pollution and introduction of non-native species (Chapin et al. 1996), but we still know little about the consequences of this species loss for ecosystems and their remaining inhabitants - including humans.

Historically, when protecting species (and ecosystems), aesthetic reasons have been important. Species that are considered beautiful or that spark sympathy among the public are easy to preserve and protect - in terms of financial and moral support. It is, however, questionable if aestheticism is a reliable reason for preserving species since the ecological importance of the species not is considered. Furthermore, beauty is highly subjective. Animal rights and environmental movements have taken conservation one step further by pointing out that it is ethically wrong to drive species to extinction. Since humans have the ability to both preserve and exterminate, the former should be the virtuous thing to do. But even though ethics may be a strong argument it seldom takes the ecological effects of species loss into account and therefore discriminates between organisms just as aesthetic reasons do. For example, it is generally not regarded as morally wrong to kill a fish as it is to kill a fox although they may be equally important from an ecological perspective. Because both aesthetic and ethical reasons for preserving species are subjective and to a large extent disregard the ecological effects of losing species, other criteria for conservation of species are crucial if protection and restoration are to prevent potentially large, negative effects of species loss on the function of ecosystems around the world.

In the beginning of the 1990’s a more objective criterion for species conservation was introduced. If one could measure the effect of losing species by investigating any change in the function of ecosystems, the effect could be quantified and no aesthetic or ethical reason were needed to justify conservation. This argument sounds acceptable, but as research has progressed many obstacles have been encountered. Although some of these issues have been resolved it is still not obvious how to perform studies on species loss so that they may be relevant to effects of species extinction in natural systems (Bengtsson 1998, Loreau et al. 2001, 2002).
Biodiversity and Ecosystem Functioning (BD-EF)

A number of problems with experimental designs, statistics, interpretations of results and definitions of ambiguous terms have been brought to the surface in the field of BD-EF over the past decade (see Loreau et al. 2001, 2002, for reviews). I will discuss the problems later in this introduction, but feel that it is important to begin with clarifying a few commonly used terms.

- Biodiversity or diversity has often been used in titles and text of published work although it, in most cases, has been species richness or functional group richness that has been investigated and manipulated. Lately, however, some studies have also taken species evenness into account since, in natural systems, a few species tend to dominate in abundance while most species are fairly rare, whereas in most experiments manipulating species richness all species used have been approximately equally abundant.

- Early in the BD-EF-era the term redundancy was introduced (Walker 1992) describing the seemingly redundant coexistence of ecologically very similar species. By some this was misinterpreted as if these species were dispensable and consequently many species could be lost without affecting ecosystem properties, which was not the intended use of the term (Walker 1995). Redundancy may instead be seen as an insurance for the long-term function of a system, since, even though species appear to be redundant under present environmental conditions, the species may have different environmental optima leading to a “redundant” species being of particular importance if the environment is changing (which we can assume it is) (Chapin et al. 1995). Moreover, species classified as redundant (i.e. members of same functional group) have been found to exhibit enough niche differentiation to produce positive effects on ecosystem function when co-existing (e.g. Naeem and Li 1997, Jonsson and Malmqvist 2000, Cardinale et al. 2001, Jonsson and Malmqvist 2003a). Thus, the usefulness of the term redundancy can be questioned (Gitay et al. 1996).

- Ecosystem functioning, or function, is also a term that has been widely used in published work. This term entails nearly everything that goes on in an ecosystem and that is mediated by the inhabiting organisms in concert with the physical environment (Costanza et al. 1997). Examples of variables that have been used to measure ecosystem functioning are process rates (i.e. leaf mass loss, grazing, predation, water uptake, CO2-emission, nitrogen uptake, biomass production), stability, invasibility, predictability, sustainability and resilience (Schwartz et al. 2000, Loreau et al. 2001, 2002). In this introduction and in the included papers, I deal mostly with processes and biomass production (growth) and how species richness may influence the rates of these.

- Lastly, ecosystem services is a term that considers the products a well functioning ecosystem can provide with (see Myers 1996, for a review). This may be seen as an extension of the term ecosystem functioning and represents values of biodiversity and healthy ecosystems that are relevant to human welfare (Luck et al. 2003).
Effects of species loss

Theory
Five major hypotheses concerning the effect of species loss on ecosystem functioning have been presented (see Johnson et al. 1996, for a review: 1) the Rivet hypothesis (Ehrlich and Ehrlich 1981), 2) the Idiosyncratic hypothesis (Lawton 1994), 3) the Redundancy hypothesis (Walker 1992), 4) the Stability-Productivity (or linear) hypothesis (MacArthur 1955) and 5) the Keystone species hypothesis. Some other hypotheses have been proposed, but the five listed above are the major ones and will be grounds for further discussion in this introduction.

The rivet hypothesis assumes that species make up important parts of an ecosystem like rivets holding an engine together. The effect of losing the parts is always negative, but when a critical number of lost parts is reached there is a large negative effect on the functioning. The idiosyncratic hypothesis may seem difficult to separate from a null hypothesis. However, while the null hypothesis predicts no effect of species loss on ecosystem functioning, the idiosyncratic hypothesis predicts that there is an effect, but its magnitude and direction differ depending on which species is lost and on the original species composition. The redundancy hypothesis predicts, as revealed by its name, that nothing will happen with ecosystem functioning as species are lost until species richness is very low and the remaining species are the last ones maintaining a function, and then ecosystem functioning will dwindle rapidly. The fourth hypothesis, the stability-productivity hypothesis, predicts a linear, positive relationship between species richness and ecosystem function with zero function with no species and the highest function at maximum species richness. Lastly, the keystone species hypothesis predicts a dramatic loss of functioning when an important, keystone species is lost, whereas the other species exhibit high levels of redundancy and produce insignificant effects on ecosystem properties if they are lost.

Empirical investigations
Pioneering experimental studies investigating effects of species loss on ecosystem functioning were performed in the Ecotron, England, on artificial ecosystems comprised of several trophic levels (Naeem et al. 1994, 1995), and on grassland ecosystems at Cedar Creek, USA (Tilman and Downing 1994). The results of these studies showed that species richness indeed had an effect on ecosystem properties and thus the functioning of the experimental systems. The study by Naeem et al. (1994, 1995), using low, medium and high diversity treatments, showed significant effects of changed diversity on several different ecosystem processes, although the change in diversity produced different responses across processes. In the grassland study, using treatments with one to 24 species (Tilman and Downing 1994), both productivity and retention of soil nutrients increased with plant diversity. The results of these studies received much attention, although later also criticism, and they provided good foundations for future research in BD-EF and aided in the development of experimental designs and analytical methods for subsequent studies.

The field (BD-EF) has thus far been dominated by studies on plant systems, probably because of the global importance of primary producers (i.e. grasslands) and since animal systems are more difficult to control, at least on large temporal and spatial scales. Although results from plant studies may be important and generally applicable (but see Giller et al. in press), the apparent differences between plants and animals are likely to be essential for what the consequences of species loss may be. Even differences among
plants or animals are probably large enough to render different results depending on what species is lost, on the original species composition and what system they inhabit. Therefore, to understand the general impact of species loss studies of all types of systems may contribute importantly to the field of BD-EF. Lately, several studies on various organisms in different types of systems have been published (see Schwartz et al. 2000, Loreau et al. 2001, for reviews). Examples of non-plant systems where diversity effects on process rates have been investigated are terrestrial decomposer food webs (Mikola and Setälä 1998), streams (Jonsson and Malmqvist 2000, Jonsson et al. 2001, Ruesink and Srivastava 2001, Cardinale et al. 2002, Cardinale and Palmer 2002, Dangles et al. 2002, Huryn et al. 2002, Jonsson et al. 2002, Jonsson and Malmqvist 2003a, b), seagrass beds (Duffy et al. 2001), intertidal invertebrate systems (Emmerson et al. 2001), pond food webs (Norberg 2000, Downing and Leibold 2002) and pollinators (Klein et al. 2003).

Studies using very low levels of species richness have many times found positively linear effects of species richness supporting the linear hypothesis. Where several species (3+) have been used, the effect has mostly been found to saturate somewhere along the species richness gradient. Such results support either the rivot or redundancy hypothesis depending on at how low richness the functioning saturates. In studies where several trophic levels have been used, the results have been complex and therefore lend support to the idiosyncratic hypothesis. Thus, none of the above hypotheses have been confirmed, or, in fact, all of them have, the variation in results probably depending on types of organism and systems studied, and the experimental design - including number of species, complexity of the system and the duration of the experiment.

**Mechanisms**

*Niche complementarity* has often been used as an explanation for positive effects of species richness, especially when including both niche differentiation and facilitation in the definition (e.g. Loreau and Hector 2001). Facilitation between pairs of species has been found in several studies (e.g. Soluk and Collins 1988, Kotler et al. 1992, Soluk 1993, Soluk and Richardson 1997, Cardinale et al. 2002, Jonsson and Malmqvist 2003), but it is not well known how important such interactions may be in more species-rich assemblages. It is, however, well known that species mostly experience stronger competition from conspecifics than from individuals of other species due to inter-specific niche differentiation (e.g. Volterra 1926, Lotka 1932). Thus, *niche complementarity* as a mechanism behind positive species richness effects on ecosystem function has much support from other ecological studies.

Another frequently used and debated explanation for diversity effects is the *sampling effect* (Aarsen 1997, Huston 1997, Tilman et al. 1997). The *sampling effect* implies that the probability of including species with disproportionate effects on ecosystem functioning increases with increasing species richness, and it has been debated whether this is a confounding factor in experimental studies (Huston 1997) or an important property of natural communities (e.g. Tilman et al. 1997). A related and more recently suggested mechanism is the *selection effect* (Loreau 2000) that takes into account selective pressures determining extreme trait values in a community. The effect of changed species richness may therefore be either negative or positive, depending on what traits in the community are favoured.

Even though positive effects of diversity are found in experiments, and although the proposed mechanisms responsible for such effects are few, it is difficult to determine
which ones are responsible for the effects obtained since they produce very similar results. However, there are methods to statistically separate the effects of particular species (i.e. the sampling effect) from the other possible mechanisms, limited, however, by the design of the study (i.e. Jonsson and Malmqvist 2000, Loreau and Hector 2001). Despite the importance of investigating mechanisms behind diversity effects and feasibility of statistically separating the mechanisms from each other using the proper experimental designs, there have so far been few empirical studies that have performed such investigations (however, see Tilman et al. 2001, Cardinale et al. 2002, Jonsson and Malmqvist 2003a).

**Spatial and temporal scales**

Published studies on the effect of species loss have varied greatly in complexity and duration, which has caused a great deal of discussion among ecologists (see Loreau et al. 2002, for a review). Seemingly, there is a trade-off between complexity of the experimental set-up and interpretability of the results - the simpler the system, the more straightforward are the results, but at the cost of lost resemblance to natural systems. However, would it be favourable to perform complex experiments to better resemble natural systems if the results are very difficult, maybe even impossible, to interpret? Furthermore, how long should an experimental study have to be to be applicable to the effects of species loss in natural systems?

The scales at which experiments are performed of course depend on the types of organisms used - their habitat, size and generation time. Also, laboratory studies tend to be performed at smaller spatial scales than field experiments. Scale may be an important, confounding factor in experiments investigating the effects of species loss, especially since both positive and negative interactions between species seem to be the dominant mechanisms behind any effects. Furthermore, although similar, positive relationships between species richness and ecosystem functioning have been found across large regional scales (e.g. Hector et al. 1999), the environmental context, including mechanisms promoting species coexistence (Fox 2003, Monquet et al 2002, Nijs and Impens 2000) and environmental factors such as heterogeneity and disturbance (Biles et al. 2003, Cardinale et al. 2000, Naeem et al. 2000, Cardinale and Palmer 2002), are probably important for what effect species richness will have on ecosystem functioning (see Fridley 2001, for a review).

As mentioned above, the persistence of species richness effects observed in controlled, short-term experiment has been questioned. The duration of a study is important when extrapolating experimental results to natural systems and even if systems are of low complexity it should be preferable to make them as long-term as possible. However, very few studies have performed long-term investigations on effect of species loss on ecosystem functioning (Symstad et al. 2003, but see Tilman et al. 2001). Thus, it is not well known if initial (or short-term) effects are transient or persistent over time since changing species composition and resource levels over time may affect competitive interactions within and between species leading to increased importance of other mechanisms affecting function differently. Consequently, one of the major questions in BD-EF-research is if effects of species loss observed in short-term experiments are relevant to long-term effects in natural systems. The critical point is that sufficient time should be allowed so that possible temporal dynamics of the species richness effects can be appreciated. In a long-term grassland study it was found that the initial effects of species
richness persisted over time the fact that underlying mechanisms changed (Tilman et al. 2001). These results suggest that the effects of species loss found in short-term experimental studies importantly indicate what the long-term consequences of species loss may be. However, in systems other than grasslands the effects may change in another fashion over time. Therefore, future investigations on the temporal dynamics of species richness effects across different types of systems are crucial if we are to understand the meaning of short-term effects both in the laboratory and in natural systems.

**Problems with empirical investigations**

Although several studies on different kinds of systems have shown that species richness matters for process rates (and other ecosystem properties) and thus functioning, the results have been questioned. One part of the critique is on experimental designs (e.g. Huston 1997, Naeem and Li 1998, Wardle 1998, Allison 1999) and what actually is tested when the number of species is manipulated - is it species richness *per se*, effects of particular species, or something in between? One could argue that using non-random assemblages merely tests for species richness effects among the organisms used, but at the same time, if all possible combinations are investigated, it allows for statistical separation of species identity and species richness effects (e.g. Jonsson and Malmqvist 2000). Also, when comparing observed and expected values in multi-species treatments one may obtain useful indications of interactions between species, thus mechanisms underlying the observed species richness effects (e.g. Jonsson and Malmqvist 2003b). However, since non-randomness does limit the generalisation of results obtained, one may argue for studies performed on randomly composed assemblages leading to more generally applicable results (Huston 1997), but there are several drawbacks from doing so. First, such an approach limits the possibility to separate compositional effects from those of species richness (but see Loreau and Hector 2001). Second, it is difficult to form assemblages completely at random. Instead, semi-random assemblages are achieved since the randomness is limited by the species pool from which species are drawn. Third, to obtain information on all monocultures and on several levels of species richness replication has to be quite large. This may, however, be an even greater problem if all possible combinations are to be used, depending on how many species there are in the study. Lastly, and maybe most importantly, communities are seldom randomly assembled in natural systems and when species loss occurs it often does so in a particular fashion depending on the species and type of perturbation. Therefore, the most relevant way to test the effects of species loss would possibly be to use a predicted order of species loss as it occurs in natural systems (e.g. Petchey et al. 1999, Jonsson et al. 2002). This, of course, limits the general applicability of the results, but at the same time it gives more realistic results and specific knowledge of the effect of species loss in the system studied.

There has also been intense correspondence about what the results so far tell us about effects of species loss in natural systems (Naeem et al. 1999, Naem 2000, Tilman 2000, Wardle et al. 2000), and more surprisingly there has been discussion about what lack of results may tell us (Hector et al. 2000, Huston et al. 2000). However, one important point that has been discussed is that since it is not always clear what response variables to measure in experiments manipulating species richness, non-significant results may simply be explained by stating that the wrong variables to detect an effect of species
loss were measured. It is, however, in my opinion even more wrong to accept the null hypothesis if no significant results are obtained!

Macroinvertebrates and processes in streams

Although streams are among the most threatened ecosystems on Earth (Vinson and Hawkins 1998) very few empirical studies on the effects of species loss have been performed. Already in 1996 did Covich point out the lack of studies investigating the importance of invertebrate diversity for stream ecosystem functioning, but although a number of studies have been published these systems are still underrepresented in the field of BD-EF.

Streams vary in species composition according to the major sources of available energy, which in turn are related to stream size, or stream order (“The River Continuum Concept”, Vannote et al. 1980). Small streams often depend on allochthonous input from surrounding riparian vegetation and have little autochthonous production due to shading from canopies. With increasing stream size allochthonous input makes up a smaller proportion of the energy in the system and autochthonous production becomes more important. Along with increasing stream size, also the amount of suspended particles increases as a result of upstream resource processing producing faecal particles, flocculation of dissolved matter, physical and biological fragmentation of detritus, and terrestrial run-off (Petersen and Cummins 1974, Suberkropp and Klug 1980, Ward 1984). As the characteristics of a stream change so does the community of the stream inhabitants due to their preferred food resource and habitat. Thus, shredding detritivores (hereafter, called ‘shredders’) are proportionally more common in smaller streams while suspension feeders, collectors and grazers are relatively more numerous downstream in larger streams and rivers, where particulate matter is more abundant and in-stream primary production is higher. Predators are about equally common along the stream size continuum although the species composition may change (Vannote et al. 1980).

Stream macroinvertebrates are classified to functional feeding groups (FFGs) according to their mouthpart morphology that reflects their feeding behaviour (e.g. Cummins 1973). Although this classification may be helpful to group organisms, hence simplifying studies of highly complex stream system, it has been shown that some of these organisms exhibit a high degree of feeding plasticity (e.g. Ledger and Hildrew 2000a, 2000b, Dangles 2002). Nonetheless, the FFG concept may be used in studies demonstrating the opposite - that species assumed to be redundant are not so, even though they feed on the same resource (e.g. Jonsson and Malmqvist 2000, Jonsson et al. 2001, Cardinale et al. 2002, Jonsson et al. 2002, Jonsson and Malmqvist 2003a, b). Furthermore, despite the observed plasticity in resource use, shredders feed readily and grow well on leaves in laboratory environments when no other resource is available.

There are several factors that may affect streams negatively. The perturbation probably causing the most dramatic effects is acidification. Following acidification a stream may lose up to 60% of its original species number (Otto and Svensson 1983, Guérold et al. 2000) with potentially large consequences for the function of the stream. Furthermore, macroinvertebrate taxa in streams are differently sensitive to decreasing pH leading to a selective loss of species and therefore also a loss of higher taxonomic levels (e.g. Raddum and Fjellheim 1984, Lien et al. 1996). Eutrophication of streams - a
common problem in agricultural regions - leads to oxygen depletion also affecting species selectively so that only species that tolerate low levels of oxygen remain in the system (e.g. Kolkwitz and Marsson 1909, Woodiwiss 1964, Chandler 1970).

Another large-scale modification of streams is the building of dams resulting in reservoirs upstream the dams, a physical barrier (the dam) and changes in flow downstream the dam (see Petts 1984, for a review). The reservoir dramatically changes the original stream habitat with changes in species composition as a consequence. The dam creates a barrier for migrating fish and drifting macroinvertebrates, and the regulation of the flow downstream the dam leads to changed flow conditions that may have positive effects on strong competitors and negative effects on species that depend on the natural (seasonal) regulation of flow and water levels for their life cycles.

The introduction of non-indigenous species is yet another factor that has shown to negatively affect native species in streams (e.g. Ross 1991). Introduction of new species can be either deliberate or accidental, but the consequences may nonetheless be dramatic. Other factors affecting stream systems are stream channelization, remaining from days when streams were used for timber floating, and clearance of riparian vegetation. Both these perturbations change the habitat structure of streams probably affecting species composition of stream organisms, leading to the loss of certain species and potentially negative effects on stream functioning.

As can be seen in this short review, the threats to stream habitats and their organisms are many, common and current. All these factors that may have negative effects on stream biodiversity and functioning may in the long run disable streams to provide us with their important ecosystem services such as fresh water, food and recreation. Hence, it is crucial to be able to assess the effects of species loss, restore perturbed systems and prevent, or limit the number and magnitude of, events having negative effects on biodiversity and ecosystem functioning from taking place.
Aims of the study

1. To investigate the relationship between species richness and process rates within different stream functional feeding groups both in the laboratory and in the field (I, II, III, V).
2. To find underlying mechanisms behind observed, positive species richness effects (IV).
3. To investigate the effects of non-random, random and predicted species loss (I, IV, V).
4. To investigate the effects of species richness on fitness-related life history traits such as growth (IV, VI).
5. To investigate species richness effects in the long term in a detritus-based stream community (VI).
Materials and methods

The observational field study (paper I) was performed in 23 streams of various sizes in northern Sweden in two different years (1997 and 1998). In each stream, 10 cages with pre-weighed alder (Alnus incana L.) leaves were introduced and retrieved after one month. A number of variables, such as standing stock of detritus, water temperature, water depth, current velocity, pH, latitude, longitude, stream order and stream width were measured at the start (and some at the end) of the study. From the leaf cages, shredder abundance, biomass and species richness and leaf mass loss were estimated. Partial least squares (PLS) analysis was performed on the data since many of the independent variables co-varied.

All laboratory studies (papers II, III, IV and VI) were set up in temperature-controlled rooms with natural light regimes. As mesocosms, either 1.0- or 1.5-L (paper II) aquaria were used. Each aquarium was provided with aeration that also created water current. The experimental period was 46 days in paper II. In paper IV, the experimental period was 32, 35 and 48 days for the facilitation, density and six-species experiments, respectively. In paper III, the experimental period was 24 hrs for the predators, 20 hrs for the filter feeders and 4 days for the grazers. The experiment in paper VI lasted for 20 weeks. An experimental design with all possible species identities or combinations nested under each species richness treatment allowed for statistical separation of species richness and identity. This design was used in all laboratory experiments testing the effect of species richness on process rates except for in the six-species experiment (paper IV), where species were drawn randomly disallowing the same statistical analysis. Also, in the long-term study (paper VI), a statistical separation of species richness and identity was not possible, although the nested design was used, since replication of species identity within each species richness treatment was low and replication over time was favoured.

The animals were always kept at constant abundance across the species richness treatments. However, since the abundance was not always dividable by two in the predator study only close to constant abundances were used in that experiment. Also, in the density study, where 12, 6 and 4 individuals from each of the three species were used, the abundance obviously differed between the treatments. In the facilitation experiment, the animals were switched between aquaria after half the experimental period so that species ‘A’ switched place with species ‘B’ and vice versa. There was also switching within species in the control treatments so that all animals were subjected to the same treatment.

For the shredders, alder leaves were used as food resource. These leaves were picked from trees prior to abscission and dried at 50°C for 48 hours before they were weighed and introduced into the aquaria. Along with tap water, an amount of sieved stream water was used to provide with microbes that conditioned the leaves before the introduction of the animals.

The filter feeders were provided with yeast particles that were mixed with water into a solution used in all aquaria. Thus, we obtained a quantified starting value, and blackfly larval feeding could be estimated by comparing the starting value with values of yeast particles at the end of the experiments. The yeast particle numbers were quantified by counting sub-samples on microscopic slides after that the particles had been stained with Bengal Red dye.

In the grazer study, ceramic tiles were submerged into the aquaria and an amount of algal solution was added. The solution was left in standstill water for a period of time to
allow for algal growth on the tiles. At the introduction of the grazers, aeration was turned on. The amount grazed was determined by comparing amount of chlorophyll a in the controls without animals with the remaining amount of chlorophyll a in the treatments with animals.

For the predator experiment, the inside of the aquaria were lined with plastic netting creating a substrate for the prey (the blackfly larvae) to attach to and enabling the predators to move around. The prey was introduced one day before the introduction of the predators. Since size differences were large between the three predator species, half the abundance was used for the two large species compared to the small one. Predation was measured as number of lost prey items in addition to the loss, or mortality, in controls without prey.

The field experiment (paper V) was performed in a concrete dam through which water from a nearby stream was flowing. Fine-mesh bags were filled with leaf discs of either alder (in this case, *Alnus glutinosa* (L.) Gaertn.) or beech (*Fagus sylvatica* L.). The same number of discs, thus the same leaf area, of each leaf species was used. The experiment lasted for 14 days. In this experiment a wider phylogenetic variety of shredders was used than in the other studies: one crustacean, one caddisfly and one stonefly species. The wide phylogenetic variety was used to be able to mimic the way in which shredder species disappear from a stream following both acidification and organic pollution. If pH drops in a stream, crustaceans disappear first, second to disappear are caddisflies and the acid tolerant stonefly species are finally the only remaining. The order of extinction is reversed when a stream is exhibited to organic pollution. The same nested design as in the laboratory studies was used enabling separation of species identity and richness when all species identities were pooled in each species richness treatment. However, since only certain one- and two-species treatments were used when assessing the effect of the predicted loss, it was not possible to investigate the effects of species identity and species richness separately.
Results and Discussion

Effects of shredder species richness on leaf mass loss (paper I, II, IV, V and VI)

The results obtained in the field study (paper I) indicated an importance of shredder species richness that was higher than that of abundance and biomass. The only two factors that were more important than shredder species richness were year and temperature. It is not surprising that year explained much of the variance since the two years the study was carried out were extremes in precipitation and thus autumn water levels probably affecting the shredders. Furthermore, temperature has previously been shown to strongly affect biological processes in streams (e.g. Petersen and Cummins 1974, Irons et al. 1994). It was somewhat surprising, however, that both abundance and biomass of shredders were ranked lower than species richness. This suggests that neither abundance nor biomass compensated fully for species loss so that functioning remain unchanged, as it has been suggested (e.g. Ruesink and Srivastava 2001).

With increasing stream size both the standing stock of leaf detritus and shredder abundance decreased linearly. Shredder species richness, however, showed a hump-shaped pattern with the highest species richness in mid-sized streams. The low species richness in small streams, in spite of the high abundance, may be caused by frequent disturbances such as drought, spates or bottom freezing, setting the system back to zero. Thus, quickly re-colonising species existing in high numbers, but being represented only by a few species, would be characteristic of such streams (e.g. Hurlbert 1975, Minshall et al. 1983, While and Wallace 1992). Larger streams are more resistant to natural disturbances and therefore represent more stable environments. Thus, their low shredder species richness and abundance probably rather reflects a low availability of food.

The laboratory study with three species of shredders (paper II) empirically tested the results from the field study. We found that from three to one species, leaf mass loss decreased by approximately 50% although abundance was kept constant and small differences in biomass were accounted for. The fact that we found this effect of species richness among species within a FFG shows that there is some niche differentiation between the species although they feed on the same resource. The nested design used in this experiment allowed us to separate the effect of species identity, or composition, from that of species richness per se. Species identity was of no importance while species richness significantly affected leaf mass loss meaning that the variance was higher between than within different species richness treatments. There was a positive, linear relationship between species richness and leaf mass loss suggesting that process rates could continue to increase beyond three species. However, in another experiment, using up to six shredder species (paper IV), the effect of species richness saturated around the three-species level. In this experiment the species were drawn at random in the three- and four-species richness treatments from a species pool of six species allowing for more general results, but the random design prevented separate statistical analysis of the effect of species identity and richness. Therefore, to obtain results on the underlying factors of observed diversity effects the complete nested design with all possible combinations of species
nested under the species richness treatments is to prefer, but at the same time this design limits the generality of the results.

Although it may be important both to separate the effects of particular species from those of species richness per se and to be able to draw general conclusions using random species assemblages, it is also important to investigate the effects of species loss as it occurs in nature. Many times species loss occurs in a predicted order depending on characteristics of the existing species and on the type of perturbation they are exhibited to (Patterson and Atmar 2000, Tilman and Lehman 2001). Hence, in yet another study we investigated the effect of shredder species loss both following acidification and organic pollution (paper V). This study was performed outdoors in the Vosges Mountains, France, where acidification of streams due to acid precipitation is a major problem. Using macroinvertebrates that we know are differently sensitive to both acidification and organic pollution we were able to simulate the loss of species following both these perturbations. Furthermore, we performed the experiment with two different types of leaf detritus, one of high (alder) and one of low quality (beech) to also investigate the effect of different resources. Leaf mass loss was significantly affected by shredder species identity, but not by species richness, when all treatments were included in the analysis. This suggests that when taxonomically distinct species are used, interactions between species may differ more within than across species richness treatments. When only the treatments applying to the predicted loss following acidification or organic pollution were used the effect of species richness was positive, though the effect of species identity could not be analysed separately. Furthermore, when beech leaves were used as the resource the effects of species richness was stronger than in the case of alder implying that species richness effects may be enhanced if resources are of poor quality, either because intra-specific competition is intensified due to lower resource levels or, for the same reason, that facilitative interactions become more important.

All our experimental studies thus far had been very short-term, nonetheless with strong effects from species richness on leaf mass loss. However, in natural systems, as the detrital resource diminishes over the season, underlying mechanisms may change causing a change in diversity effects. Therefore, the results observed in short-term laboratory studies might merely have represented transient effects. To study this, I performed a study over a relatively long period of time (paper VI), 20 weeks, which is comparable to the ≤ 30 weeks that leaf detritus is present in north Swedish streams and shredders are important consumers on this resource (Malmqvist and Oberle 1995). The results showed an increasing leaf mass loss with time due to time (of course), shredder biomass and shredder species richness. Up to week 16 species richness was still significantly important. However, at week 20 the interaction between time and species richness was significant while species richness per se was no longer. This shows that the effect of species richness may change with time and in this case it was magnified with increasingly different rates of leaf mass loss between the one-, two- and three-species treatments. The increase in leaf mass loss in the one-species treatment started to level out after approximately half the experimental period while the two-species treatment slowly levelled out throughout the experiment starting around week 12. The three-species treatment showed a linear increase throughout the whole experimental period. This implies that as resources are diminishing, species assemblages experience increasingly stronger negative intra-specific interactions that inhibit process rates, but the more species there are in the assemblage
the lower resource levels do they tolerate before the negative interactions become strong enough to affect process rates.

In this study (paper V), I also found an effect of species richness on the growth of shredders. Such effects of species richness has not been found in the previous studies, probably because of their short duration, high within-species variability in initial size and the low assimilation efficiencies of detritivores (Cummins et al. 1989). The effect of species richness on growth is important for the long-term consequences of species richness effects. Among macroinvertebrates with one-year life cycles, the larval size at emergence affects the reproductive success of adult individuals (e.g. Prout and McChesney 1985), thus the subsequent generation. However, it is not obvious what the consequence of diversity effects on growth in seasonal systems, such as the detritus-based shredder community, would be in the long term. Decreasing reproductive success as a consequence of species loss, lowered process rates and growth, could lead to more per capita resources in the following larval generation and therefore lower competitive pressures within species. This, in turn, may lead to competitive release, higher growth rates of larvae and higher fecundity of adults with positive effects on the subsequent generation. The effect of this would be higher competitive pressures in the following larval generation affecting the subsequent generation negatively. Hence, cycles of low levels of competition and high growth and high levels of competition and low growth would take turns over the years. However, since other studies have indicated that compensation in number or biomass cannot make up for the loss of species, the process rates should decrease over a long period of time if the system is not re-colonised by lost or new species, even though higher per capita resource levels prevent competition and promote growth.

Another scenario, if fecundity is negatively affected by the loss of species in the larval stage, suggests that already rare species might become even rarer or extinct in the following generation. This would decrease the biological insurance of the system leading to lowered functioning in the event of environmental change, perturbation or disturbance. To investigate the effect of changed adult fecundity, long-term studies would have to be conducted, but unfortunately the habitat switching from aquatic to terrestrial within generations make such studies very difficult to perform.

Effects of species richness among filter feeders, grazers and predators (paper III)

The effect of species loss seems fairly general among shredders, but what about other stream functional feeding groups? Three studies were performed to investigate if changed species richness would have negative effects on process rates and if species richness per se or species identity was important among filter feeders, grazers and predators. The results showed that among these three groups species identity was the only significant factor, indicating that variance between species identity treatments within each species richness treatment was higher then the variance between the species richness treatments. This also suggests that the factors producing the observed effects are less general than if species richness per se would have been the important factor.

The results in the two filter feeder trials were different – one showing a negative effect of species loss and one showing no effect, but both had significant effects of species identity. Thus, the effect of losing filter-feeding species would depend on the species used
in the study, or the species inhabiting the system, and the effect could vary from none to negative. The importance of species identity suggests that among blackfly larvae there are only few, certain species that compete harder with conspecifics than with larvae of other species, or that positive species interactions are rare. For the other species, interference was equal within and between species and positive interactions are non-existing producing no effect on process rate when species were combined.

Among grazers there were indications of strong inter-specific interference between the snail and the two mayfly species, probably mediated by the limited resource. This resulted in a strongly decreasing process rate with increasing species richness. In a natural system the mayflies probably would have left the patch to avoid the competitively superior snail (e.g. MacAuliffe 1984, Kohler and Wiley 1997). Depending on the interferences within the snail species, the resource levels and disturbance frequency, this system may possibly remain of low species richness and high functioning or be in a transient stage that will eventually lead to a population crash, re-colonisation of species, higher species richness and some “new” functioning depending on the interactions within that community.

The predators showed, as with the grazers, strong negative interactions between species, probably due to inferiority in size of one predator species compared to the other two. In every combination with the small and one of the large predators, the predation rate was much lower than expected leading to an on average lower predation rate with higher species richness. Therefore, the long-term scenarios in this predator system may be similar to what was suggested for the grazer system. Alternatively, although the species coexist in natural streams, they inhabit different microhabitats limiting encounters between the small and the large predators, whereas the heterogeneity in the laboratory study was too low to allow for such non-negative coexistence.

Mechanisms behind decreased function due to species loss (paper IV)

Since inclusion of particularly efficient species only in some assemblages was avoided in the first study (paper I) and since we found that species richness per se was the important factor behind the effect of species richness we could only think of two possible mechanisms behind the observed effects: facilitation and intra-specific interference. We were unable to investigate these mechanisms in the first study and since both mechanisms may produce similar results two separate studies were needed to find out if any one of them could be responsible for the observed effects.

The facilitation study, with sequential introduction and removal of species, showed a one-directional, positive interaction between the two species used. This indicates that if such mechanisms are present when species are lost, the effect of species loss would be highly dependent on which species is lost and how it interacted with other species - if the species was involved in facilitation, if it was the facilitator or the species being facilitated, or if it had no significant interactions with other species.

The density study showed, not surprisingly, that density is important for process rates in agreement with earlier studies (e.g. Hassel and Varley 1969). However, there seemed to be different intensity levels of intra-specific interference since one species
showed a significant effect of decreasing abundance, another species a non-significant, but strong, trend and a third species showed no trend at all. The differences in response to decreasing abundance may be explained by size in relation to the resource, or size differences or activity within species. If species are large, the resource will be relatively small leading to increased interference or resource competition. If there is a large intra-specific size difference, large individuals may be more aggressive towards smaller ones affecting process rates negatively. High activity may cause more encounters between individuals leading to increased negative interference. The largest species in this study showed a strong, though non-significant, trend of increasing process rate with decreasing abundance, whereas the smallest species – with the largest intra-specific size difference and highest activity – showed a significantly increasing process rate with decreasing abundance. Thus, also intra-specific interference, although a general phenomenon among all types of species, seems to be somewhat species specific probably due to the factors discussed above. However, it is still likely that negative interactions within species is a more general mechanisms behind species richness effects than positive interactions are, especially in a system with a diminishing resource where eventually during the season the resource will be so small that individuals within a species start to interact negatively with one another competing either for food or space.

The differences in results, depending on what macroinvertebrates were used and what the interactions between the species were, may be due to different levels of spatial and resource heterogeneity in the experiments. Shredders feed on a resource of leaves that is heterogeneous - in the laboratory due to variation in flow conditions and microbial conditioning, and in the field the presence of other leaf species further increases the variability of the resource. Thus, there is an incitement for niche differentiation between shredder species. Furthermore, leaves are both food and habitat for the shredders and they form spatially heterogeneous environment. For the predators the resource was of low heterogeneity because the prey (blackfly larvae) did not differ much from one another, and in the field prey items probably vary less in terms of quality for a specialist predator than detritus does for shredders. Thus, for niche differentiation to take place between predators, spatial heterogeneity may be more important than resource heterogeneity. This may also be the case in the grazer study where the system was of low heterogeneity, both spatially and resource-wise. Algae may, however, be highly heterogeneous in natural systems. Thus, when experiments are set up to detect effects of changed species richness important spatial and resource factors should be taken into account. For predators it is probably important to design a spatially heterogeneous system if the resource does not vary much and since negative inter-specific interactions may be strong creating a need for refugia. For grazers both the resource and possibly the space should be heterogeneous to resemble species interactions in natural systems. Shredder systems seem to be the most easily created with both high spatial and resource heterogeneity creating situations with effects of changed species richness that are more applicable to natural systems.
Conclusions

The results in this thesis show that species richness matters for process rates among stream macroinvertebrates in a variety of ways. The type of organisms used, their interactions with other species, and type of resource they feed on all contribute to non-trivial and interesting findings. Among shredders the results were consistent showing decreased process rate with lowered species richness in all studies. Either species richness \textit{per se} or species identity/composition caused the diversity effects, apparently depending on which the most important species interactions were. As found in the paper on mechanisms (paper IV), either positive interactions or intra-specific interference, or both were responsible for the effects of species loss among shredders. Since positive intra-specific interactions probably are species specific they will depend more on species identity than species richness \textit{per se}, unless all species interact positively. Distantly related species are more likely to exhibit positive interactions than closely related ones (paper V, Morse 1977, May 1982). Accordingly, the effects of species richness found in this study (paper V) were due to species identity. Closely related species may experience strong intra-specific interference while the importance of positive interactions is low, rendering an effect of species richness \textit{per se}, i.e. negative effects of losing any species (paper II). However, positive interactions may exist also in systems of closely related species (e.g. Cardinale et al. 2002, Jonsson and Malmqvist 2003a) adding importantly to a general effect of species loss on ecosystem function. If these positive interactions are strong enough, i.e. stronger than the negative effects of intra-specific interference, the effect of species loss should still be attributed to species identity or composition rather than species richness \textit{per se}.

Since both intra-specific interference and positive interactions are mediated by the resource they should also show increasing importance as the resource diminishes over time leading to an increasing difference in process rates across systems of different diversities (paper VI). Such time-magnified effects may be relevant to long-term effects of species loss in other types of systems with ephemeral resources (e.g. Finn 2001). Furthermore, these two mechanisms are of the type that promotes coexistence between species. Species rich systems may therefore contain both important levels of intra-specific interference and many positively interacting species, and therefore be affected by species loss just the way it was found in most of the studies of this thesis.

The negative relationship between species richness and process rates among grazers and predators is interesting, but probably of less importance for long-term effects in natural systems. The effects were probably caused by strong resource dominance among grazers and intense inter-specific interference among predators. Since these mechanisms are less likely to promote coexistence in natural systems than the previously mentioned ones, the observed effects would probably not remain over a longer period of time (e.g. Vandermeer et al. 2002). Natural systems containing species dominating a resource or exhibiting inter-specific interference may therefore, due to competitive exclusion of inferior species, have low diversity and be in a successional stage, depending, however, on the intensity of the competition (e.g. Vandermeer et al. 2002). The lack of ecological insurance in such species-poor systems may, in the event of disturbance or perturbation, further induce transient dynamics (Yachi and Loreau 1999).
Many ecologists would probably agree that mechanisms such as the ones we found being responsible for the effects of species richness are the ones that in theory should be responsible for any effects of changed species composition or richness in most types of systems. Thus, in terms of explaining the diversity effects, the results in the studies of this thesis are of general importance. Moreover, several experimental designs were used to test general and specific hypotheses on the effects of species loss on ecosystem function. There is still little, if any, evidence for general effects of species loss across systems and within systems across types of organisms. Therefore, the results in this thesis underpins the belief that effects of species loss often depend on the species identities involved. Hence, in future investigations of effects of species loss one of the most relevant courses of action would be to use predicted orders of species loss thereby obtaining specific, nevertheless important, information. It should still be possible to assess the general effects of species loss across systems by compiling data from several specific studies. Although the field of BD-EF still is in its infancy much has been accomplished so far, but expansion of studies in time, space and complexity, along with specific studies, is definitely needed if we are to find answers to the question of what the effects of the global extinction of species will be.

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