

Ecological Integrity of Boreal Streams

**Assessing Impacts on Community
Structure and Function**

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Abstract

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Running waters provide a number of services for humans, such as drinking water and food resources and many freshwater animals are confined and specialised to this environment. However, this natural resource has become increasingly impacted by humans resulting in a substantial loss of biodiversity and services. To assess ecological integrity of streams a number of bioassessment schemes have been developed and most of these are based on community structure and composition. Although many of the biological metrics developed have been used successfully in bioassessment, it has been suggested that ecosystem functions, such as leaf-litter decomposition, should be incorporated in modern bioassessment schemes. In this thesis I compare a number of structural metrics with functional metrics along a nutrient gradient in nine boreal streams in south-central Sweden to assess the potential of ecosystem function as a biomonitoring tool. Leaf-litter breakdown (*Alnus glutinosa* (L.) Gaertner) was studied during four seasons and stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and stoichiometric ratios (C:N) of phytobenthos, CPOM, FPOM, invertebrates and fish were also analysed. My results indicate that leaf-litter breakdown is a relatively insensitive tool to assess ecosystem impairment compared to invertebrate metrics. However, $\delta^{15}\text{N}$ in organic matter has been suggested as a potential tool to assess ecological integrity of streams and my results support this conjecture. A strong response in $\delta^{15}\text{N}$ in organic matter with nutrient enrichment was revealed, suggesting that $\delta^{15}\text{N}$ could serve as a simple tool to assess nutrient enrichment effects in boreal streams. I also found that leaf-litter associated fungi and invertebrates were positively correlated with leaf-litter breakdown rates and a nutrient gradient. Moreover, I found that certain dominating species, e.g. waterlouse (*Asellus aquaticus* (L.)), can have a strong influence on ecosystem processes. In this thesis I show that leaf-litter breakdown is not a simple low-cost biomonitoring tool as several field trips were necessary to assure adequate litter-bag recovery. Also natural factors, such as fluctuating water levels and heavy snow fall, resulted in substantial loss of litter bags thereby confounding data interpretation.

Keywords: bioassessment, nutrient enrichment, biodiversity, leaf-litter breakdown, stable isotope ecology, ecological stoichiometry

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II. Bergfur, J., Johnson, R. K., Sandin, L. & Goedkoop, W. Assessing the ecological integrity of boreal streams: a comparison of functional and structural responses. *Fundamental and Applied Limnology (Archiv für Hydrobiologie)* 168 (2), 113-125.

III. Bergfur, J. Seasonal variation in leaf-litter breakdown in nine boreal streams: implications for assessing functional integrity. (Submitted manuscript).

IV. Bergfur, J., Johnson, R. K., Sandin, L., Goedkoop, W. & Nygren, K. Effects of nutrient enrichment on boreal streams: invertebrates, fungi and leaf-litter breakdown. *Freshwater Biology* (Accepted 2007).

V. Bergfur, J., Johnson, R. K., Sandin, L. and Goedkoop, W. Effects of nutrient enrichment on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N ratios in boreal streams. (Submitted manuscript).

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Introduction

Human induced impacts on running waters

Streams and rivers are fundamental to human existence, as well as global biodiversity (Hauer & Lamberti, 1996). They provide a number of services for humans such as water for domestic, industrial and agricultural purposes, power generation, waste disposal, navigational routes and locations for recreational activities (Malmqvist & Rundle, 2002). Many freshwater animals are confined and specialised to this dynamic environment, thus running waters have a rich, diverse and unique biota (Giller & Malmqvist, 1998; Downes *et al.*, 2002). However, this natural resource has become increasingly impacted by human activities and stress is increasing at an alarming rate resulting in a substantial loss of aquatic biodiversity (Vitousek *et al.*, 1997; Ricciardi & Rasmussen, 1999).

Examples of human-induced impacts on running waters include habitat alterations such as siltation and modification of riparian corridors; changes in water chemistry such as acidification, eutrophication and toxic metals; and species removal and addition (Malmqvist & Rundle, 2002). Stressed systems often show a reduction in species richness, a predominance of pollution-tolerant species, and a change in the number of individuals within a species (Johnson, Wiederholm & Rosenberg, 1993). There is also a growing concern that not only species diversity is lost, but that ecosystem services may become lost or impaired with anthropogenic stress (Daily *et al.*, 2000). Although the contribution of species diversity for ecosystem function is currently debated, several studies have shown nonetheless that biological communities regulate important ecological processes such as productivity, decomposition and elemental cycling, and that changes in community composition can alter the structure and functioning of ecosystems (Naeem *et al.*, 1994; Naeem, Hahn & Schuurman, 2000; Petchey *et al.*, 2004).

As awareness of these impacts grows, many industrialised countries have implemented conservation and restoration programmes that focus on retaining and improving the quality of running waters and their ecological integrity. Historically, the focus of human influence on freshwater habitats has been mostly on the chemical status and for water management purposes the assessment of water quality has for a long time been based on physical and chemical data. But as recognition increased that chemical controls were not sufficient to protect freshwater resources, biological criteria have been added to many monitoring schemes (Karr, 1991; Cairns & Pratt, 1993; Karr, 1993). Indeed, assessing the ecological integrity or ecological status of aquatic ecosystems has become an important management issue and has gained increasing focus, in particular through the Clean Water Act in the USA (passed by the Congress in 1972 and amended in 1977), focussing on “biological integrity” and in Europe through the recent ratification of two environmental directives, namely the European Water Framework Directive (European Commission 2000), which focuses on the “ecological status” of freshwaters and the Habitat Directive, which focuses on conservation and maintenance of biodiversity (Habitats Directive (92/43/EEC)).

Ecological integrity

Ecological integrity can be expressed as the maintenance of all internal and external community processes and attributes so that high ecological integrity corresponds to a natural state and where the natural community is preserved by regulation, resilience, and resistance to environmental stress (Moog, 1995), this definition is also similar to Karrs (1991) definition of ecological (or biological) integrity. By contrast, others refer to the concept of “ecosystem health” for describing an ecosystem that when healthy is “sustainable and resilient, maintaining its ecological structure and function over time while continuing to meet societal needs and expectations” (Meyer, 1997). Due to human activity it is now extremely difficult to find streams which have not been affected in some way (Hynes, 1970). In this thesis, I refer to high ecological integrity as a general term for good stream “health”, i.e. a minimally disturbed ecosystem (*sensu* Stoddard *et al.*, 2006). Ecological integrity can further be divided into structural integrity which refers to “spatiotemporal patterns, particularly of biological communities and their resources”, and functional integrity which can be referred to as “the ecosystem level processes” (Bunn & Davies, 2000; Gessner & Chauvet, 2002).

Assessing ecological integrity

A large number of methods, ranging from subtle changes at the suborganism-level to changes in population, community and ecosystem-level structure are currently used to assess the ecological integrity of aquatic ecosystems (Karr, 1991; Johnson, Wiederholm & Rosenberg, 1993). In Table 1, I have listed a number of metrics that have been used to assess ecological integrity (adapted from papers by Karr (1993) and Giller *et al.* (2004)).

Table 1. Components of metrics for assessing ecological integrity; structure and function, adapted from Karr (1993) and Giller (2004).

Component of ecological integrity	Basic type of metric
Species composition	Identity of species
	Presence of rare or endangered species
	Presence of intolerant species
	Presence of tolerant species
Community structure	Species (taxa) richness
	Relative abundances
	Dominance
Individual health	Levels of parasitism or disease
	Biomarkers
	Skeletal anomalies, lesions and so on
	Hybridisation
Ecosystem process	Contaminant levels
	Population age structure
	Productivity: primary and secondary
	Organic matter transformation
	Ecosystem metabolism
	Elemental cycling
Physical structuring	

Human-induced disturbances strongly affects fish (e.g. Snyder *et al.*, 2003), invertebrate (e.g. Statzner *et al.*, 2001) and periphyton (e.g. Coring, 1999) communities and the most common approach today in biomonitoring of running waters is community level assessment using these organisms (Hunsaker & Carpenter, 1990; Cairns & Pratt, 1993; SEPA, 1999). Many of the biological metrics developed have successfully been used to aid legislators in protecting aquatic ecosystems from further degradation (Karr, 1993; Knobon, Roos & van Oirshot, 1995). Indeed, the Water Framework Directive states that fish, benthic invertebrates, macrophytes and benthic algae should form the basis for assessing ecological status in running waters (European Commission 2000).

Although assemblage structure and composition of benthic organisms have been successfully used in studies of impairment, there has been a renaissance in the use of ecosystem-level processes as a complementary approach to assessing ecological integrity (Gessner & Chauvet, 2002). Many ecosystem functions such as species interactions and mineralisation of organic matter are connected to water quality and therefore a more integrated approach to water management is needed if the full integrity of an ecosystem is to be assessed (Knobon, Roos & van Oirshot, 1995; Gessner & Chauvet, 2002). Although not addressed in this thesis, analyses of species traits such as functional feeding groups (FFG) (Cummins, 1974; Moog, 1995), is an indirect way of measuring the functional integrity of a stream. However, such information about ecosystem-level processes is a deduction from structural parameters (Gessner & Chauvet, 2002). Methods such as classifying sites according to the feeding behaviour of the taxa present have been used extensively in ecological studies (Johnson, 1999), although several studies have found that metrics such as ratios between functional groups were not better than measurements of community structure (Resh & Jackson, 1993).

Biodiversity has been placed on the political agenda in recent years, as maintaining biodiversity may be important for several reasons, for example, for ecological, commodity or moral reasons (Walker, 1992; Cardinale, Nelson & Palmer, 2000; Daily, *et al.*, 2000). The relationship between biodiversity and ecosystem processes has received much attention in recent years (Cardinale, Nelson & Palmer, 2000; Loreau, 2000; Loreau *et al.*, 2001). It has been argued that the maintenance of high biodiversity might provide a buffer against stress. Accordingly, loss of functionally redundant species would not be expected to have high impact on ecosystem processes (Loreau, *et al.*, 2001). On the other hand, loss of sensitive taxa might have dramatic implications for ecosystem function, in particular if the species is a dominant contributor to the ecosystem process and no redundancy exists. However, if processes are supported by dominant species and only rare species become extinct, then these processes would remain largely unaltered (Giller, *et al.*, 2004). It has also been argued that one of the best ways to maintain biodiversity in ecosystems is to preserve the integrity of ecosystem functions. In doing so, the chances of losing hitherto undescribed and unknown species would be minimised (Walker, 1992). As pattern determines process and vice versa, and since stressors clearly have an impact on ecosystem processes (Cardinale, Ives & Inchausti, 2004; Gessner *et al.*, 2004; Giller, *et al.*, 2004), there is a need to integrate functional responses in management schemes, thereby

maintaining ecosystem integrity more effectively (Bunn & Davies, 2000; Gessner & Chauvet, 2002).

Objectives

This thesis focuses on the assessment of ecological integrity of running waters. I compare a number of structural and functional metrics in boreal streams along a nutrient enrichment gradient. In particular, the main objectives were:

1. To test if there is a congruent pattern in taxon richness among different organism groups in boreal streams and consequently if any organism group can act as surrogate indicator for the other organism groups. Here I studied the taxon richness of fish, invertebrates, macrophytes and benthic algae in 28 boreal streams along a nutrient enrichment gradient (*Paper I*).
2. To assess the efficacy of functional and structural metrics for determining impairment of streams. Here I compared the response of leaf-litter breakdown rates (i.e. the exponential breakdown coefficient and percentage leaf mass remaining after a defined period of time) with the response of selected biotic indices (i.e. the ASPT index, DSFI index, number of EPT taxa and Simpson's Diversity) to nutrient enrichment in nine boreal streams in Sweden (*Paper II*).
3. To investigate seasonal patterns in leaf-litter breakdown. I investigated variations in leaf-litter breakdown during four seasons (i.e. autumn-winter 2003, spring and autumn-winter 2005 and spring 2006) and how this variability has implications for the use of leaf-litter breakdown in biomonitoring (*Paper III*).
4. To assess the influence of nutrients on leaf-litter bag associated invertebrates and fungi and their contribution to leaf-litter breakdown. I also examined the invertebrate community associated with the decaying leaves in relation to a number of site descriptors such as water chemistry, in-stream habitat and land use characteristics (*Paper IV*).
5. To examine changes in carbon and nitrogen elemental composition (i.e. stable isotope ratios, $^{12}\text{C}/^{13}\text{C}$ and $^{14}\text{N}/^{15}\text{N}$, and stoichiometric ratios, C:N) of a number of biological elements along a nutrient gradient. More specifically, isotope ratios and elemental ratios in fish, invertebrates, periphyton and coarse (CPOM) and fine (FPOM) particulate organic matter were analysed. I also used these metrics to investigate food preferences of invertebrates and fish at high- vs. low-impact streams (*Paper V*).

Leaf-litter breakdown

Streams are, as all other ecosystems, dependent on a constant input of energy obtained from autochthonous pathways, i.e. in-stream primary production, and/or allochthonous pathways, i.e. organic matter that is produced elsewhere (Giller & Malmqvist, 1998). One of the most important components of allochthonous input to streams are leaves from the surrounding vegetation (Webster *et al.*, 1999). Many in-stream factors affect the processing of terrestrial leaf-litter, such as physical fragmentation, leaching and decomposition mediated by invertebrates, aquatic fungi and bacteria (Webster & Benfield, 1986; Royer & Minshall, 2003).

Because leaf-litter breakdown rates are sensitive to physical and chemical environmental changes, leaf-litter breakdown rates have been used to study patterns and mechanisms driving decomposition in streams (Webster & Benfield, 1986; Graça, 1993; Gessner & Chauvet, 2002). For instance, nutrient enrichment of streams is usually expected to increase leaf-litter breakdown rates by increasing microbial processing (Meyer & Johnson, 1983; Benfield *et al.*, 2001; Pascoal, Cassio & Gomes, 2001). Moreover, effects on invertebrates associated with decomposing leaves have also been found. For example, invertebrate richness, densities and biomass associated with the decaying leaves have been shown to increase with nutrient additions (Elwood *et al.*, 1981; Pascoal *et al.*, 2003; Gulis, Ferreira & Graça, 2006).

As human-induced impacts such as nutrient enrichment (nitrogen and phosphorous) have been shown to be important regulators of leaf-litter breakdown (Elwood, *et al.*, 1981; Meyer & Johnson, 1983; Pascoal, *et al.*, 2003) and as leaf-litter breakdown is relatively easy to measure, usually by constructing artificial leaf-bags (Boulton & Boon, 1991), leaf-litter breakdown rates have been suggested as a tool to detect human-induced changes on ecosystem function (Gessner & Chauvet, 2002; Pascoal, *et al.*, 2003).

Ecological stoichiometry and stable isotope ecology

Carbon (C) and nitrogen (N) are two of the most important elements regulating biotic processes in aquatic ecosystems (Suberkropp & Chauvet, 1995; Dodds *et al.*, 2004). The balance of these elements in different organisms and organic matter and their biological transformation are considered in ecological stoichiometry (Frost *et al.*, 2002; Sterner & Elser, 2002; Cross *et al.*, 2003). For example, ratios of these elements have often been used to assess how elemental balance in ecosystems affect and are affected by organisms and how these processes in turn affect ecosystem function (Cross *et al.*, 2005; Frost, Cross & Benstead, 2005). To date, most studies on ecological stoichiometry have focused on understanding nutrient cycling and food web interactions (Elser *et al.*, 1996; Elser & Urabe, 1999; Frost *et al.*, 2003), and stable isotope analysis (i.e. ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ in biota) is now a common tool used to study nutrient cycling and food web relationships (Ehleringer, Rundel & Nagy, 1986; Peterson & Fry, 1987; Finlay, 2001).

Anthropogenic effects at local (point source), catchment (e.g. land use) and regional (climate change) scales can alter important ecosystem processes by influencing stoichiometric relationships in aquatic ecosystems (Daufresne & Loreau, 2001; Cross, *et al.*, 2003; Bowman, Chambers & Schindler, 2005). Elemental composition of organic matter and invertebrate consumers are also likely to have strong effects on ecosystem processes such as leaf-litter breakdown rates, ratios of nutrients recycled by consumers and trophic interactions (Frost, Cross & Benstead, 2005). For example, increased nutrient concentrations in detritus have been shown to affect breakdown rates positively (Enríquez, Duarte & Sand-Jensen, 1993).

Stable isotope ratios have also been used to follow changes in ecosystems and show which processes or components are most sensitive to perturbation (Peterson & Fry, 1987). Indeed, stable isotopes have been suggested as an important component in stream health assessments (Bunn, Davies & Mosisch, 1999; Udy *et al.*, 2006). For instance, enriched $\delta^{15}\text{N}$ values in aquatic environments have been found along nutrient and agricultural gradients (Harrington *et al.*, 1998; Vander Zanden *et al.*, 2005; Udy, *et al.*, 2006). Although stable isotope ratios is a structural aspect of streams, Udy & Bunn (2001) suggest that $\delta^{15}\text{N}$ values also represents an integrated signature of N cycling processes. Moreover, indicators of nutrient processes could serve as indicators of ecological integrity, as these processes are influenced by disturbances in the catchments (Udy, *et al.*, 2006).

Materials and Methods

Below I give a brief description of the methods used in the five papers included in this thesis. For more detailed information for methods and standards see the individual papers referred to by their roman numerals.

Study sites

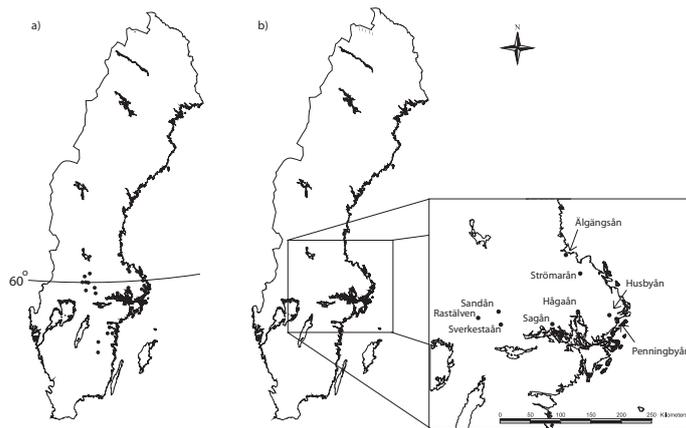


Figure 1. (a) Over-view map of the 28 study sites in Sweden and (b) the nine sites selected for studying ecosystem function.

Twenty-eight sites (in different streams and catchments) were chosen to study stream ecological integrity. The sites were all situated in southern Sweden (Fig. 1a). Stream order varied between three and six and the upstream catchments ranged from 45 to 1139 km² (with all but two sites having catchment areas < 500 km²) (Table 2). The streams were chosen to constitute a nutrient enrichment gradient; using existing data of stream chemistry as well as expert opinion from Local County Board managers. From these 28 sites, nine were chosen to study ecosystem functions (Fig. 1b) (*Paper II-V*). These nine sites were also selected to represent a nutrient enrichment gradient. The sites were selected based on the stream characteristics of the 28 sites sampled in autumn 2002 and additional sampling during 2003.

Land-use within the catchments was obtained by GIS, using a general over-view map (1:100 000). Riparian land use and vegetation cover were assessed in field using a standardised field protocol (Wilander, Johnson & Goedkoop, 2003). At each site, habitat composition (e.g. mineral substratum and biotic substratum classification) was obtained according to Hering *et al.* (2003). In autumn 2003, coarse particulate organic matter (CPOM) was quantified (g m⁻²) at the nine study streams, the CPOM was freeze-dried and weighed to nearest 0.1 g.

Water chemistry sampling was conducted twice in autumn 2002 (*Paper I – II and IV - V*), in spring, summer and autumn 2003 (*Paper II - V*) and in spring and autumn 2005 and spring 2006 (*Paper III*). Water samples were analysed for nutrients (for example: NO₂+NO₃-N, total nitrogen, PO₄-P and total phosphorous)

(*Paper I-V*) and other relevant variables (e.g. pH, conductivity, alkalinity, water colour and TOC content) (*Paper II-V*). All chemical analyses were performed according to standardised protocols outlined in Wilander, Johnson & Goedkoop (2003).

Table 2. Selected stream characteristics for the 28 study streams. Water chemistry (mean±SD) samples were taken on four occasions from autumn 2002 to summer 2003. *sites selected to study ecosystem functions (*Paper II-V*)

Stream name	Longitude	Latitude	Catchment area (km ²)	Conductivity (mS m ⁻¹)	TP (µg L ⁻¹)	Water colour (absorbance)	Forest (%)	Arable land (%)
Älgängsån*	60 37'	17 17'	66	8.0±4.5	18±3.6	0.19±0.13	74	0.6
Borkhultsån	58 16'	16 11'	116	12±0.2	12±3.6	0.05±0.01	53	10
Broströmmen	59 46'	18 46'	226	32±2.6	48±38	0.09±0.05	50	18
Forsmarksån	60 20'	18 12'	373	18±1.2	18±3.0	0.21±0.02	62	4.6
Gusumån	58 16'	16 30'	280	10±0.5	26±11	0.06±0.03	53	8.6
Hågaån*	59 48'	17 36'	122	46±9.8	86±33	0.17±0.10	49	24
Hamrångeån	60 55'	17 01'	381	3.7±0.2	11±3.0	0.16±0.02	60	1.4
Hörksälven	59 59'	14 55'	110	3.6±0.9	6.3±4.3	0.10±0.01	67	0.3
Husbyån*	59 44'	18 29'	156	34±3.7	50±16	0.10±0.03	48	19
Järsöströmmen	59 51'	18 40'	138	27±0.8	30±9.7	0.07±0.02	55	7.3
Jonsbergsån	58 31'	16 49'	106	28±6.0	150±48	0.28±0.10	50	26
Kagghamraån	59 06'	17 48'	79	21.1±2.3	53±18	0.12±0.02	48	14
Kisaån	57 58'	15 36'	231	11±0.2	82±142	0.07±0.02	75	3.4
Muskån	58 59'	17 56'	84	15±1.0	39±6.7	0.17±0.03	51	16
Nittälven	59 59'	14 45'	76	6.6±3.0	7±1.4	0.23±0.06	67	0.3
Pajsoån	60 11'	14 40'	62	3.0±0.3	6.5±0.6	0.23±0.05	73	0.1
Penningbyån*	59 40'	18 41'	99	25±1.9	27±6.2	0.11±0.04	53	13
Rastälven*	59 43'	14 46'	247	3.9±0.2	8.5±2.6	0.12±0.01	71	1
Sagån*	59 37'	16 52'	843	32±8.3	77±29	0.17±0.06	46	36
Sandån*	59 48'	15 21'	45	2.6±0.1	11±2.6	0.29±0.06	78	0.1
Sävälven	59 59'	14 32'	57	2.6±0.1	10±2.7	0.22±0.04	66	0.1
Saxhyttån	60 17'	15 00'	50	4.0±0.7	16±15	0.22±0.05	78	0.3
Silverån	57 37'	15 35'	109	6.7±0.5	8.0±2.2	0.14±0.05	81	3
Skeboån	60 01'	18 36'	476	26±1.2	37±5.4	0.15±0.02	65	6.8
Storån	58 28'	16 19'	328	20±2.9	156±27	0.21±0.04	49	28
Strömarån*	60 20'	17 41'	55	26±1.1	26±5.6	0.19±0.03	72	6.3
Sverkestaån*	59 37'	15 25'	386	3.8±0.3	13±4.4	0.13±0.01	70	3.1
Tämnarån	60 25'	17 36'	1139	30±5.6	53±17	0.15±0.03	58	19

Biological sampling (*Paper I, II and IV*)

Fish, macrophytes, benthic invertebrates and diatoms were sampled at all 28 sites once in autumn 2002 within a stream section of 500 metres (*Paper I*). Fish was sampled using electro-fishing and identified to lowest taxonomic unit possible. Macrophytes were sampled in late summer 2002 using a slightly adapted form of the Mean Trophic Rank (MTR) sampling protocol. Diatoms were sampled from five fist-sized cobbles at each site by brushing the stones, rinsing the phytobenthos with distilled water into plastic bottles and thereafter preserved in ethanol. Benthic invertebrates were sampled using the standardised Swedish kick-sampling method. Accordingly, using a 25 cm wide hand-net (mesh size 500 µm), five 1m x 1 minute kick-samples were preferably taken from hard bottom substratum (*Paper I*). Additionally, 20 replicate kick-samples (25 x 25 cm) were taken at each site, distributed according to the substratum distribution at each site (*Paper II and IV*).

A number of structural metrics were calculated using the species x site matrix: taxon richness, Simpson's Diversity (Simpson, 1949), Average Score per Taxon (ASPT, Armitage *et al.*, 1983), Danish stream fauna index (DSFI, Skriver, Friberg & Kirkegaard, 2000), number of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa (Lenat, 1988), and invertebrate density (ind. m⁻²) (*Paper II and IV*).

Leaf-litter breakdown (Paper II-IV)

Leaf-litter breakdown studies were performed in autumn-winter 2003 (*Paper II - IV*), spring 2005, autumn-winter 2005 and spring 2006 (*Paper III*). Leaf-litter bags (15 x 15 cm) were constructed, coarse (5 mm) and fine plastic-mesh (0.3 mm) were used, and filled with air-dried alder leaves (*Alnus glutinosa* (L.) Gaertner). The fine mesh bags were constructed to exclude the effect of invertebrates on leaf-litter breakdown.

After 6, 13, 20, 34, 55 and 118 days of incubation three replicates of each mesh size were collected from the streams and brought back to the lab. Invertebrates from the coarse mesh bags were preserved in 70% ethanol. The remaining leaf material was frozen, freeze-dried and the percentage remaining leaf mass was calculated. All weights were corrected for mass loss due to leaching and handling. During spring 2005 and spring 2006 the leaves were only incubated for 34 days and in the autumn-winter 2005 leaves were incubated for 34 and 55 days, from these three additional studies no invertebrates were collected. During the autumn-winter study in 2003 I had to deviate from the sampling protocol at many sites due to the loss of leaf-litter bags and the heavy snow fall and ice-coverage (for more details see *Paper II*). Also in spring 2006 some replicate bags were lost (for more details see *Paper III*).

The decay rate coefficient (the k-value) was calculated for three lengths of incubation (34, 55 and 118 days of incubation) by regressing percentage leaf mass remaining (ln(x)-transformed) against days of incubation according to Benfield (1996). As suggested by Gessner & Chauvet (2002), the ratio between breakdown rates in coarse mesh bags (k_c) and fine mesh bags (k_f) was calculated. This ratio has been suggested as the basis for a functional index to describe the degree of functional impairment in a stream. Also the mean percentage leaf mass remaining on day 34, day 55 and day 118 was calculated as an alternative to the decay rate coefficient (as suggested by Maltby *et al.*, 1995; Jonsson, Malmqvist & Hoffsten, 2001; Gessner & Chauvet, 2002).

Invertebrates and fungi (Paper IV)

Invertebrates collected from the litter bags were identified to the lowest taxonomic level possible (usually species) and metrics, such as taxon richness (number of taxa litter bag⁻¹), abundance (ind. litterbag⁻¹) and density (ind. g⁻¹ DW), were calculated from the invertebrates collected from the coarse mesh bags (*Paper IV*). The animals were also assigned to different functional feeding groups according to Moog (1995), Schmedtje & Colling (1996) and the AQEM Consortium (2005). In addition to sampling invertebrates from the litter bags, fungal biomass was

estimated from ergosterol extraction. Analyses of invertebrates and fungal biomass were only performed in the autumn-winter 2003 study.

Sampling for stable isotope and stoichiometric analyses (Paper V)

CPOM was quantified (g m^{-2}) at each site as described above under “*Study sites*”. Water samples were collected and the seston (FPOM) was retained on pre-combusted ($550\text{ }^{\circ}\text{C}$) GF/C glass-filters (47 mm). Periphyton was sampled in autumn 2003 from three arbitrarily selected cobbles taken from each site. Invertebrates were collected using hand-nets (mesh size $500\text{ }\mu\text{m}$) in autumn 2003. Identification was to the lowest taxonomic unit possible, usually to species. Species were assigned to functional feeding groups as described above (see Table 3). Fish was sampled by electro-fishing in autumn 2005. Species were identified to lowest taxonomical unit possible, usually to species, and frozen before processing. Trophic levels were assigned according to Sundbom *et al.* (2003) and FishBase.se (http://artedi.nrm.se/fishbase_se).

All samples were freeze-dried and ground in a mortar and weighed to nearest 0.001 mg in tin capsules for C and N content (mg) and stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analyses. Isotopic $\delta^{13}\text{C}$ data are reported relative to the Peedee Belemnite carbonate (CO_2) standard and $\delta^{15}\text{N}$ data relative to the atmospheric N (N_2) standard.

Table 3. Trophic level classification of invertebrates and fish.

Trophic level	Feeding mode/food preferences	Species
2	Shredder, Grazer, Filtraters, Detritivores	<i>Asellus aquaticus</i> (Linnaeus 1758), <i>Baetis</i> spp., <i>Heptagenia</i> spp., <i>Heptagenia sulphurea</i> (Müller 1776), <i>Oligochaeta</i> spp, Sphaeriidae spp. <i>Taeniopteryx nebulosa</i> (Linnaeus 1758)
3	Shredder, Grazer, Filtraters, Detritivores, and Predators	<i>Gammarus pulex</i> (Linnaeus 1758), <i>Hydropsyche angustipennis</i> (Curtis 1834), <i>Hydropsyche pellucidula</i> (Curtis 1834), <i>Hydropsyche siltalai</i> Döhler 1963, <i>Hydropsyche</i> spp.,
	Omnivores; benthic macro fauna/periphyton/macrophytes	Roach (<i>Rutilus rutilus</i> (Linnaeus)), Minnow (<i>Phoxinus phoxinus</i> (Linnaeus))
4A	Predator; zooplankton/benthic meiofauna	Lamprey (<i>Lampetra</i> sp.)
4B	Predators; benthic macro fauna	<i>Rhyacophila nubila</i> (Zetterstedt 1840)
		Ruffe (<i>Gymnocephalus cernuus</i> (Linnaeus)), Bullhead (<i>Cottus gobio</i> Linnaeus)
5A	Predators; benthic macrofauna (turns to piscivores at later life stages)	Perch (<15 cm) (<i>Perca fluviatilis</i> Linnaeus), Trout (<i>Salmo trutta fario</i> Linnaeus)
5B	Predators; piscivores	Bulbot (<i>Lota lota</i> (Linnaeus)) Perch (>15-20 cm), Eel (<i>Anguilla anguilla</i> (Linnaeus)), Pike (<i>Esox lucius</i> Linnaeus)

Results and Discussion

Biodiversity patterns (Paper I)

Principal components analysis (PCA) on selected chemistry variables (e.g. different fractions of N and P) showed a clear nutrient gradient among the 28 study streams (Fig. 2), hence the first PC axis was interpreted as a nutrient gradient. Spearman's rank correlation of taxon richness of fish, benthic macroinvertebrates, macrophytes and diatoms against the first PC axis resulted in few significant relationships between nutrient enrichment and taxon richness. Only macrophyte richness was correlated to the nutrient gradient (Spearman's $\rho = 0.41$, $p < 0.05$), where higher taxon richness was related to higher nutrient contents.

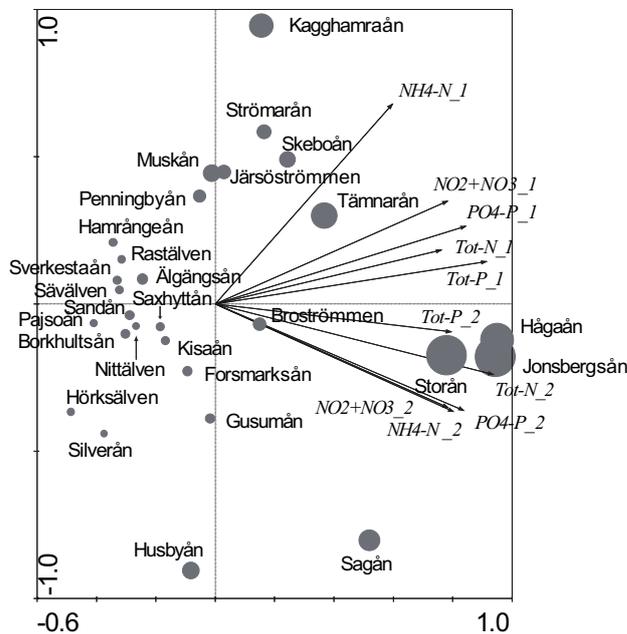


Figure 2. Standardised principal components analysis (PCA) on nutrient variables from the 28 study streams, sampled twice in autumn 2002. The size of each circle representing a stream is proportional to the total phosphorous level measured on the first sampling occasion. Letters 1 and 2 indicate sampling occasion.

Identification of streams and rivers worthy of protection would greatly improve if there are suitable taxon groups that can be used as biodiversity indicators in these systems (Brennan *et al.*, 2006). A few studies have compared or analyzed different groups of benthic macroinvertebrates as possible biodiversity surrogate species for other macroinvertebrate groups (Heino *et al.*, 2003; Sanchez-Fernandez *et al.*, 2006). In this study, we found that fish taxa richness was correlated to macrophytes richness (Spearman's $\rho = 0.50$, $p < 0.05$) and macroinvertebrate richness (Spearman's $\rho = 0.43$, $p < 0.05$); all other among-organism group comparisons were not significant ($p > 0.05$). Others have also found similar patterns, for instance, Heino *et al.* (2005) found a strong relationship between macroinvertebrate richness and bryophytes. They also found a relationship between fish richness and bryophyte richness and macroinvertebrates.

However, as our results showed only weak relationships for the four different organism groups and between nutrient enrichment and taxon richness, it remains unclear whether one organism group (i.e. fish) could act as a surrogate for high taxon richness in boreal streams. Further studies on large-scale, homogeneous datasets could further elucidate if fish is a possible indicator for general taxon richness.

Defining the nutrient gradient (Papers II-V)

Using Principal components analysis (PCA) on water chemistry, land-use and habitat characteristics of the nine sites chosen to study ecosystem functions, the environmental variables could be reduced to two principal components (Fig. 3). The first two principal components explained 50% of the among-stream variance. Several variables indicative of nutrient enrichment were correlated with loadings of the 1st PC axis (for example; TP, TN and arable land in catchment area), thus this axis was interpreted as a nutrient gradient. The 2nd PC axis was interpreted as representing habitat quality (i.e. correlated to estimated CPOM, wood and coarse gravel). Hereafter, the 1st and 2nd PC axes are referred to as nutrient and habitat gradients, respectively.

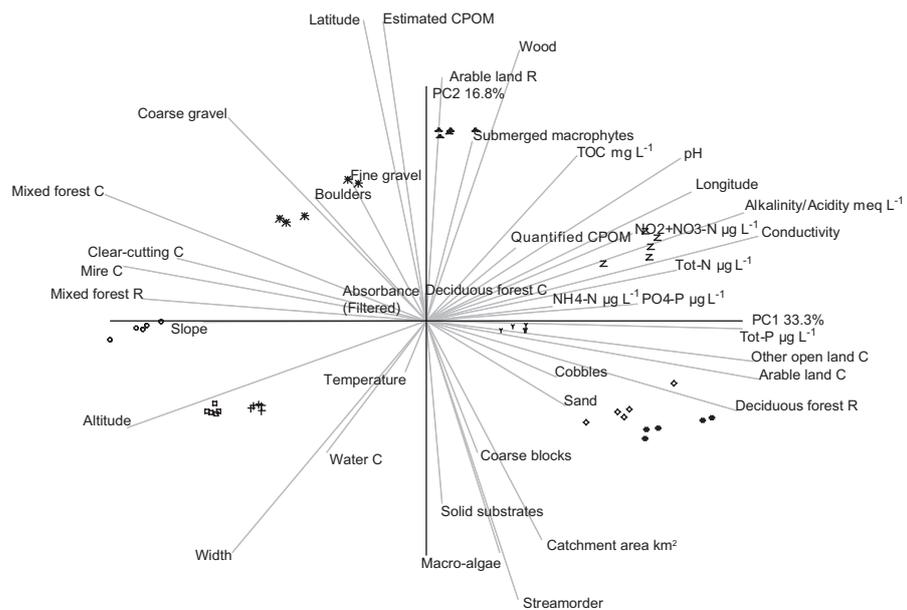


Figure 3. Principal components analysis (PCA) on water chemistry, land use and habitat variables. Eigenvalue axis 1: 13.3, axis 2: 6.78. Sandån:○, Rastälven:□, Sverkestaån:†, Älgängså:✱, Strömarån:△, Penningbyån:Y, Husbyån:◇, Hågaån: Z, Sagån ●. Abbreviation C = catchment area, R = riparian zone.

Due to the high loss of litter-bags in my study in 2003, complete leaf-litter breakdown data were only available from all nine sites during a 34 day period, from eight sites during a 55 day period and from five sites for the whole 118 day period. Three PCAs were constructed to compare the effects of nutrient

enrichment on leaf-litter breakdown from different sampling protocols (*Paper II*). One of the PCAs included all nine sites (*used in Paper II and V*), the second included eight sites (*used in Paper II and IV*) and the third included five sites (*used in Paper II*). In Paper III, water chemistry sampled in autumn-winter 2003, spring and autumn-winter 2005 and spring 2006, and the land-use and substratum classifications described above was used in the PCA. The first PC axes were always interpreted as nutrient gradients and the second PC axes as habitat gradients in all PCAs, and hence referred to as such throughout this thesis.

Effects of nutrient enrichment on leaf-litter breakdown (Paper II)

The results showed that four of the five selected invertebrate metrics responded as predicted to increased nutrient enrichment, indicating changes at the community level (Table 4). Consequently, we anticipated that ecosystem processes might also be affected and our results of increased leaf-litter breakdown rates (k-values) with increased nutrient enrichment supported this conjecture (Table 5). These results also support the findings of earlier studies, namely that nutrients enhance leaf-litter breakdown in streams (Elwood, *et al.*, 1981; Meyer & Johnson, 1983; Pascoal, *et al.*, 2003). Also the percentage leaf mass remaining changed significantly with increased nutrients (i.e. decreased). However, comparisons of the effect of nutrients on leaf-litter breakdown rates from the different incubations showed that incubation length influenced our results. For instance, comparisons of coarse and fine mesh bags showed that as incubation period increased, the relationship between the breakdown rate in the coarse mesh bags and the nutrient gradient became stronger. However, for fine mesh bags we found no such pattern. The strongest relationship between the nutrient gradient and leaf-litter breakdown rates in the coarse mesh bags was found after 118 days of incubation, even though number of study sites decreased. However, the relationship between the nutrient gradient and percentage of leaf mass remaining after 118 days was stronger than between the nutrient gradient and breakdown rates after 118 days.

Table 4. Linear regression of biotic indices against the first (PC1) and second (PC2) axes from PCA on characteristics of nine study streams. $p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$. R^2 = coefficient of determination adjusted for degrees of freedom, RMSE = root means square error.

Biotic indices	PC1			PC2		
	R ²	Slope	RMSE	R ²	Slope	RMSE
Number of taxa	0.233***	-	8.520	0.111*	+	9.173
Simpson's diversity	-0.020	-	0.059	-0.020	+	0.059
ASPT	0.851***	-	0.299	-0.022	+	0.782
DSFI	0.672***	-	0.723	0.061	+	1.222
EPT	0.629***	-	4.997	-0.020	+	8.285

Table 5. Linear regressions of functional metrics (34, 55 and 118 days of incubation) against the first and second PCA axes of the nine (34 days), eight (55 days) and five (118 days) sites, $p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$. R^2 = coefficient of determination adjusted for degrees of freedom, RMSE = root mean square error.

Functional measures	34 days (9 sites)				55 days (8 sites)				118 days (5 sites)									
	PC1		PC2		PC1		PC2		PC1		PC2							
	R ²	Slope	RMSE	R ²	Slope	RMSE	R ²	Slope	RMSE	R ²	Slope	RMSE						
K-value coarse mesh bags (k _c)	0.364***	+	0.006	0.055	-	0.007	0.386***	+	0.013	0.231**	-	0.015	0.556***	+	0.009	0.138*	-	0.013
K-value fine mesh bags (k _f)	0.569***	+	0.001	0.009	-	0.002	0.187**	+	0.002	0.005	+	0.002	0.486***	+	0.003	-0.040	-	0.004
k _c :k _f	0.171**	+	0.532	0.030	-	0.575	0.296***	+	2.068	0.301***	-	2.061	0.168*	+	0.678	0.654***	-	0.437
% leaf mass remaining coarse mesh bags	0.359***	-	9.773	0.034	+	12.003	0.598***	-	13.841	0.033	+	21.458	0.602***	-	9.196	0.171*	+	13.277
% leaf mass remaining fine mesh bags	0.504***	-	2.992	-0.016	+	4.283	0.171**	-	8.188	-0.009	-	9.033	0.519***	-	11.396	-0.042	-	16.773

In this study we found that structural indices were found to be better predictors of nutrient enrichment than leaf-litter breakdown rates. Several studies have, however, shown that leaf-litter breakdown rates do work as indicators of nutrient enrichment in streams (e.g. Portugal, Pascoal, *et al.*, 2003; Gulis, Ferreira & Graça, 2006; and France, Lecerf *et al.*, 2006). The poor performance of breakdown rates as indicators of stream integrity in our study is likely attributed to slow rates of decomposition in boreal streams, resulting in the need for relatively long incubation times to increase power to detect change if/when it occurs. This conjecture was supported by the finding that the strongest correlation between leaf-litter breakdown in coarse mesh bags and degree of impairment was noted after 118 days of incubation. These results indicate that longer incubation periods are required to detect changes in ecosystem function in boreal streams. But, on the other hand, longer incubation time increases the risk of not recovering the leaf-litter bags.

Gessner & Chauvet (2002) suggested that the ratio between breakdown rates in coarse and fine mesh bags ($k_c:k_f$) could serve as a basis for a functional metric. The basis for this assumption was that the $k_c:k_f$ ratio would indicate a shifting balance in the contribution of different organism groups to leaf-litter breakdown. Although the biotic indices and breakdown rates showed predicted responses, albeit of different strengths depending on incubation length, site classification using the cut levels proposed by Gessner & Chauvet (2002) varied markedly with incubation time (Table 6). For instance, Sverkestaån, one of the low impact sites, changed from score 1 (compromised) after 34 days to score 2 (not compromised) after 55 days to score 0 (severely compromised) after 118 days. Our finding that the proposed scoring system does not accurately indicate functional integrity agrees with recent studies (Hagen, Webster & Benfield, 2006; Lecerf, *et al.*, 2006), whereas others have found that these ratios do serve as indicators of functional integrity (e.g. Pascoal, *et al.*, 2003).

Table 6. Functional scores assigned to each site after 34, 55 and 118 days of incubation as suggested by Gessner & Chauvet (2002), score 2 ($k_c:k_f = 1.2-1.5$), score 1 ($k_c:k_f = 1.5-2.0$ or <1.2) and score 0 ($k_c:k_f > 2.0$) where score 2 indicates "no compromised functional integrity", score 1 indicates "compromised functional integrity" and score 0 indicates "severely compromised functional integrity". Sites are ordered from low to high impact.

Days of incubation	34	55	118
	Functional scores		
Sandån	2	1	
Rastälven	1	1	0
Sverkestaån	1	2	0
Älgängså	1	0	
Strömarån	1	1	1
Penningbyån	2	0	0
Husbyån	0	0	0
Hågaån	1	0	
Sagån	0		

Seasonal variations in leaf-litter breakdown (Paper III)

Many studies on leaf-litter breakdown as a tool in bioassessment have only focused on the breakdown rates within one season, usually in autumn after abscission (Pascoal, *et al.*, 2003; Hagen, Webster & Benfield, 2006; Lecerf, *et al.*, 2006). However, studies on leaf-litter breakdown indicate that natural fluctuating factors such changes in invertebrate community structure, nutrients and temperature can be responsible for seasonal effects on leaf-litter breakdown (Garden & Davies, 1988; Menéndez, Hernández & Comín, 2003).

In order to investigate temporal differences in leaf-litter breakdown, the 2003 study was repeated in spring 2005, autumn-winter 2005 and spring 2006 at a smaller scale. Using the loadings on the first PC axis and one way ANOVA followed by Tukey's HSD, the nine streams were divided into three impact groups; low (Sandån, Sverkestaån and Rastälven), medium (Älgängsån, Strömarån and Penningbyån) and high (Hågaån, Sagån and Husbyån) impact sites. I found few differences in percentage leaf mass remaining among low, medium and high impacts sites during the four different seasons studied here (Fig. 4a-d). A significant difference between high and low impact sites in the coarse mesh bag was found only during spring 2006 after 34 days of incubation. However, precaution is advised when drawing conclusions about these results as during spring 2006 many of the bags were occasionally dried-up and had to be moved around in the streams to avoid desiccation. Also, many bags were lost due to the retreating water levels; thus the number of replicates used varied among sites. In the fine mesh bags significant differences between the high and low-impact sites were only found in autumn-winter 2005 after 34 days of incubation

I also observed a significant effect of season on leaf mass remaining in both coarse and fine mesh bags. For instance, after 34 days of incubation a higher percentage of leaf mass remained in the coarse mesh bags in autumn-winter 2003 than in the other three seasons ($F_{df3} = 10.12$, $p = 0.0002$). In the fine mesh bags there was also a significant effect of season on the percentage leaf mass remaining ($F_{df3} = 3.78$, $p = 0.02$). However, these findings were less obvious after 55 days of incubation. Here, seasonal differences (autumn-winter studies) were found for the coarse ($F_{df1} = 4.49$, $p < 0.05$) but not for the fine mesh bags. Seasonal variations were attributed to variations in temperature.

Although several studies have shown that leaf-litter breakdown rates are good indicators of stream functional integrity (Pascoal, *et al.*, 2003; Gulis, Ferreira & Graça, 2006; Lecerf, *et al.*, 2006), other recent studies have questioned their usefulness (Nelson, 2000; Hagen, Webster & Benfield, 2006). My studies showed that although linear regressions indicated a significant increase in breakdown rates (or decrease in percentage leaf mass remaining) (*Paper II*), comparisons of high and low impact groups using ANOVA showed the differences to be small (*Paper III*). The reason for this was most likely due to high variation in leaf mass loss in the different impact categories studied. This conclusion is supported by the findings of Hagen, Webster & Benfield (2006), who attributed similar findings to high variation within impact categories and many confounding factors acting on

leaf-litter breakdown rates. For instance, the antagonistic influence of nutrients on shredding macroinvertebrates, i.e. positive effects of low to moderate agricultural land use and the negative effects of intensive agricultural land use (Huryn *et al.*, 2002; Hagen, Webster & Benfield, 2006).

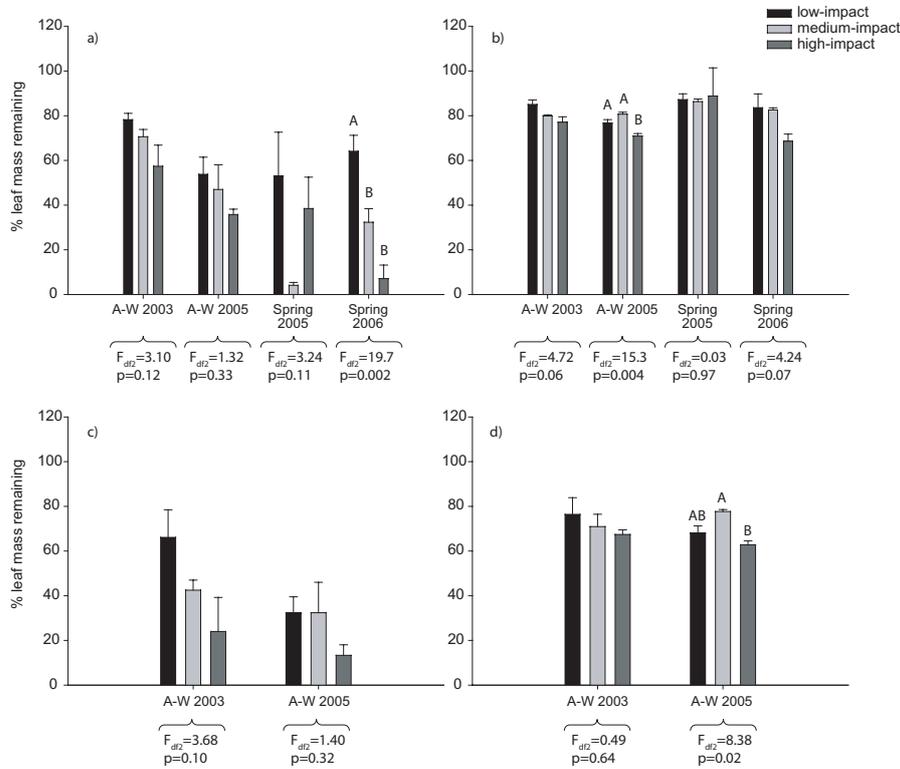


Figure 4. Percentage leaf mass remaining after 34 days of incubation in (a) coarse mesh bags (5mm) and (b) fine mesh bags (0.3mm) and after 55 days of incubation in (c) coarse mesh bags and (d) fine mesh bags. One-way ANOVA (results below each group) followed by Tukey's HSD, different letters indicate significantly different means ($\alpha = 0.05$). $n = 3$ except for the high impact group in autumn-winter 2003 where $n = 2$. Abbreviation A-W = Autumn-Winter.

A biomonitoring tool should be relatively insensitive to natural variations in physical and biological environments, but sensitive to anthropogenic-induced stress (Karr, 1991). That variables reflecting ecosystem function are relatively poor, early-warning indicators of human-generated stress was argued by Schindler (1987). Another desirable quality of bioassessment schemes is that they should be cost-effective (Resh & Jackson, 1993). In my studies, high flows in autumn-winter 2003 and retreating water levels in spring 2006 resulted in the loss of many litter bags. The problem of loss of leaf-litter bags in stream studies has also been reported elsewhere, e.g. McKie, Petrin & Malmqvist (2006) reported a 66% loss of litterbags. Thus, the success of leaf-litter breakdown studies depends in part on a number of factors such as water level fluctuations. Clearly, these factors need

consideration if leaf-litter breakdown studies are to be considered as a cost-effective tool in bioassessment of boreal streams.

Effects of invertebrates and fungi on leaf-litter breakdown (Paper IV)

In this study all biological variables associated with the decaying leaves were significantly correlated to the nutrient gradient (eight sites, 55 days of incubation). Fungal biomass, in both coarse and fine mesh bags, invertebrate density (ind. g⁻¹ DW) and abundance (ind. litter bag⁻¹) increased, whereas taxa richness (number of taxa litter bag⁻¹) decreased with increased nutrients (Table 7). The finding that external nutrients have a positive affect on fungal biomass has been demonstrated earlier (e.g. Grattan & Suberkropp, 2001; Gulis, Ferreira & Graça, 2006). Similarly, negative effects of nutrients on invertebrate richness associated with decomposing leaves but positive effects on invertebrate numbers have also been demonstrated earlier (Pascoal, Cassio & Gomes, 2001; Huryn, *et al.*, 2002; Pascoal, *et al.*, 2003).

Table 7. Linear regression of invertebrates litter bag⁻¹ and g⁻¹ DW (Simuliidae excluded), benthic invertebrate density (ind. m⁻²) and taxa richness against the first (PC1) and second (PC2) PCA axes (eight sites, after 55 days of incubation). p<0.05*, p<0.01**, p<0.001***. R² = Coefficient of determination adjusted for degrees of freedom, RMSE = root mean square error.

		PC1			PC2		
		R ²	Slope	RMSE	R ²	Slope	RMSE
Coarse mesh bags	Taxa leaf-litter bag ⁻¹	0.217**	-	2.178	0.088*	-	2.350
	Individuals leaf-litter bag ⁻¹	0.390***	+	0.248	-0.002	-	0.318
	Individuals g ⁻¹ DW	0.534***	+	0.352	0.156**	-	0.474
	Fungal biomass (mg C g ⁻¹ DW) litter bag ⁻¹	0.295***	+	0.546	0.037	+	0.639
Fine mesh bags	Fungal biomass (mg C g ⁻¹ DW) litter bag ⁻¹	0.330***	+	0.528	0.200**	-	0.577
Benthic kick sample	Taxon richness	0.116*	-	8.699	0.116*	+	8.702
	Individuals m ⁻²	0.141**	-	0.335	-0.019	+	0.365

Our results showed that increased fungal biomass was significantly related to increased leaf mass loss in the fine, but not in the coarse mesh bags (Table 8). However, one site, Husbyån, had >3 times higher breakdown rates than the other seven sites studied (for details see *Paper IV*). Removing this site from the analysis showed that increased breakdown rates in the coarse mesh bags were also related to increased fungal biomass ($R^2 = 0.55$, $p < 0.05$, R^2 adjusted for degrees of freedom used throughout), as well as percentage leaf mass remaining ($R^2 = 0.56$, $p < 0.05$). Breakdown rates (positive) and percentage of leaf mass remaining (negative) were also significantly related to invertebrate density (ind. g^{-1} DW) (Table 8). If Husbyån was removed post hoc, invertebrate abundance (ind. litter bag $^{-1}$) was also significantly related to breakdown rates ($R^2 = 0.49$, $p < 0.05$). We also found that relationships between invertebrate density and leaf-litter decomposition were related to functional feeding groups. For example, the density of detritivores and shredders explained 74.2% and 47.9%, respectively, of the variance in percentage leaf mass remaining (compared to 73.5% for total invertebrates). That invertebrates and aquatic fungi are important mediators of leaf-litter breakdown has also been demonstrated previously (Hieber & Gessner, 2002; Pascoal, *et al.*, 2003).

Table 8. Linear regression of invertebrate taxa and individuals litter bag $^{-1}$ and individuals g^{-1} DW (Simuliidae excluded), *Asellus aquaticus* litter bag $^{-1}$, benthic invertebrate taxa and density (ind m^{-2}), and fungal biomass (mg C g^{-1} DW) against the breakdown rates and percentage leaf mass remaining in coarse and fine mesh bag after 55 days of incubations, $p < 0.05^*$, $p < 0.01^{**}$. R^2 = Coefficient of determination adjusted for degrees of freedom, RMSE = root mean square error.

Biological variables	k-value			% leaf mass remaining		
	R^2	Slope	RMSE	R^2	Slope	RMSE
Taxa leaf-litter bag $^{-1}$	0.044	-	0.017	-0.077	+	23.911
Individuals leaf-litter bag $^{-1}$	0.077	+	0.017	0.371	-	18.276
Individuals g DW $^{-1}$	0.690**	+	0.010	0.735**	-	11.855
Taxon richness	0.359	-	0.014	-0.017	+	23.232
Individuals m^{-2}	0.030	-	0.017	-0.047	+	23.581
<i>Asellus aquaticus</i> litter bag $^{-1}$	0.692**	+	0.010	0.423*	-	17.496
Fungal biomass (mg C/g DW) litter bag $^{-1}$ coarse mesh bags	0.029	+	0.017	0.421	-	17.660
Fungal biomass (mg C/g DW) litter bag $^{-1}$ fine mesh bags	0.419*	+	0.002	0.476*	-	6.869

As mentioned, breakdown rates in coarse mesh bags were significantly related to invertebrate abundance and fungal biomass only when one stream (Husbyån) was excluded from the analyses. Recently it has been demonstrated that certain dominating species can contribute disproportionately to leaf processing rates

(Dangles & Malmqvist, 2004; Carlisle & Clements, 2005). Correspondence analysis (CA) showed that species composition in the litter bags differed among the eight sites (Fig. 5). The CA ordination plot also showed that the species community in litter bags from Husbyån deviated from the other sites. Comparisons of individual abundances of shredder and detritivore taxa against breakdown rates showed that only one species, *Asellus aquaticus*, was positively related to breakdown rates and percentage leaf mass remaining (Table 8). Thus the most likely explanation for the higher breakdown rates in Husbyån was the high abundance of the detritivore *A. aquaticus*. This isopod has many of the species traits described by Dangles and Malmqvist (2004) as characterising a dominant shredder or an organism that could have a strong influence on ecosystem processing. *Asellus aquaticus* is mobile, feeds in a variety of ways (Moog, 1995) and has an aseasonal lifecycle. *Asellus aquaticus* is also relatively tolerant to pollution and more common in regulated than in pristine streams (Giller & Malmqvist, 1998). Hence, the combination of high nutrient concentrations (which induces microbial growth) and detritivore predominance by *A. aquaticus* might explain the disproportionately high breakdown rates noted at Husbyån.

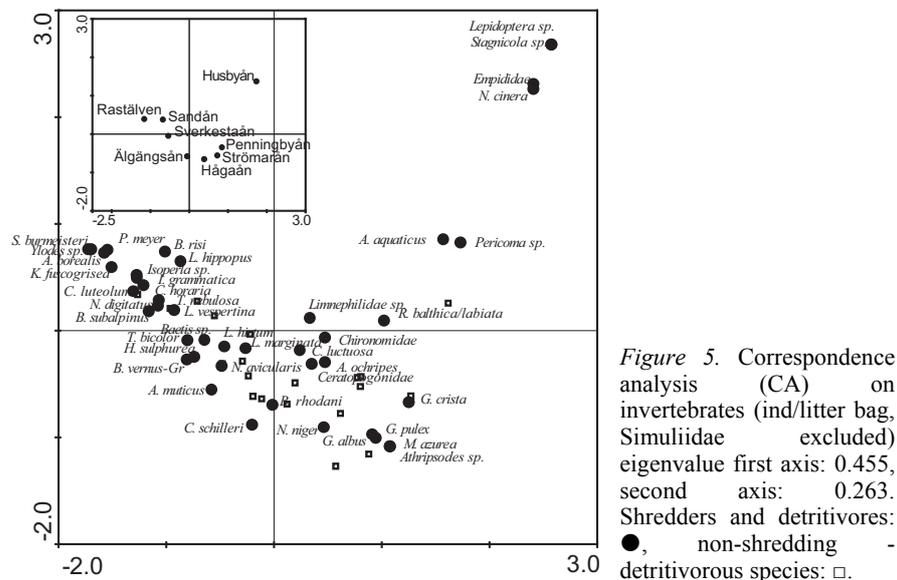


Figure 5. Correspondence analysis (CA) on invertebrates (ind/litter bag, Simuliidae excluded) eigenvalue first axis: 0.455, second axis: 0.263. Shredders and detritivores: ●, non-shredding - detritivorous species: □.

Effects of nutrient enrichment on stable isotope and stoichiometric ratios (Paper V)

Our results showed that $\delta^{15}\text{N}$ was a sensitive indicator of nutrient enrichment in medium-sized boreal streams; with increasing nutrients the $\delta^{15}\text{N}$ signatures at various trophic levels (i.e. CPOM, periphyton, invertebrates and fish) increased (Fig. 6a). Regression of mean $\delta^{15}\text{N}$ against the nutrient gradient (i.e. loadings on the first PC axis) confirmed this conjecture (R^2 : 0.77 – 0.91 for all biological variables, $p < 0.05$). These findings lend support to a number of recent studies that have shown increases in $\delta^{15}\text{N}$ along nutrient and agricultural gradients (Harrington, *et al.*, 1998; Vander Zanden, *et al.*, 2005; Udy, *et al.*, 2006). In fact,

Vander Zanden *et al.* (2005) and Udy *et al.* (2006) concluded that land use and nutrient loading variables were the best predictors of $\delta^{15}\text{N}$ in aquatic biota.

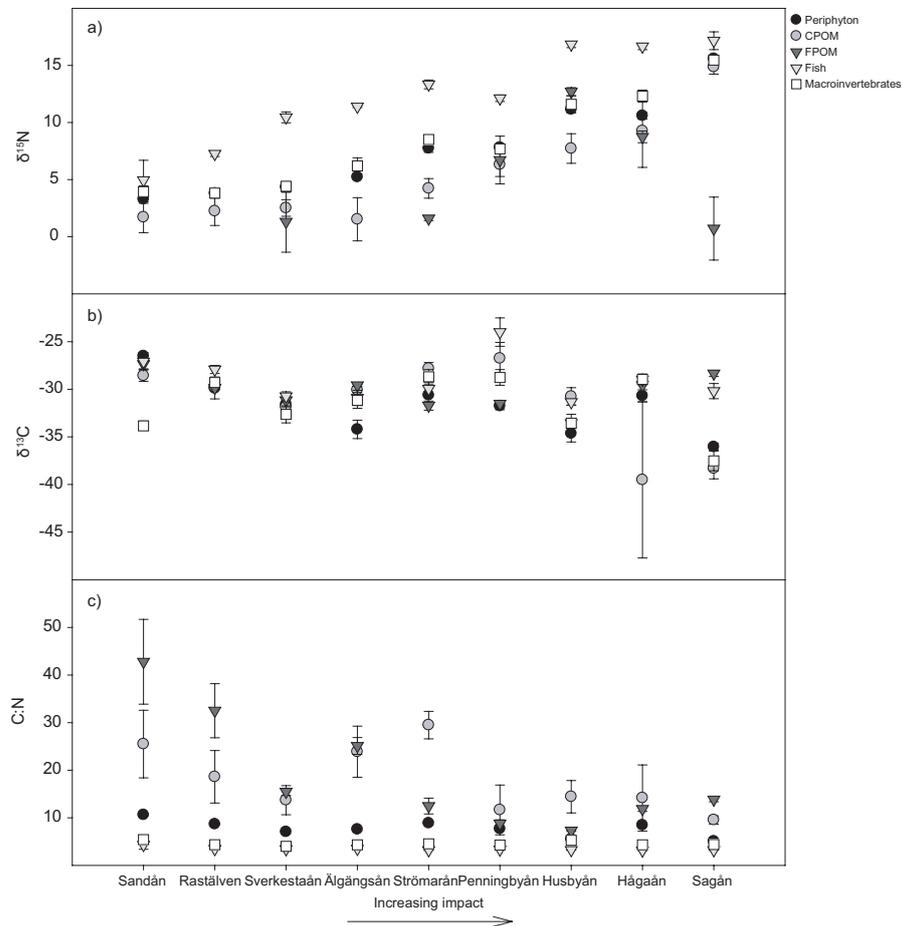


Figure 6. Mean $\delta^{15}\text{N}$ (a), $\delta^{13}\text{C}$ (b) and C:N (c) ratios for four basal resources (periphyton $n = 3$ except Strömarån where $n = 2$, CPOM $n = 3$ and FPOM $n = 2$), invertebrates and fish. Error bars denote standard error (SE). Increasing impact from nutrient enrichment is extracted by Principal Components Analysis (see text for details).

Weak, albeit significant, decreases in $\delta^{13}\text{C}$ were detected for all basal resources (i.e. periphyton, CPOM and FPOM, $R^2: 0.41 - 0.09$, $p < 0.05$) along the nutrient enrichment gradient (Fig. 6b). However, no significant changes in either fish or invertebrate $\delta^{13}\text{C}$ values were noted with increased nutrient concentrations. If the dissolved inorganic carbon (DIC) pool in streams is derived to a large extent from respiration then $\delta^{13}\text{C}$ values of algae are expected to be highly depleted (i.e. more negative $\delta^{13}\text{C}$) (Peterson & Fry, 1987). The periphyton in our study streams was more depleted in ^{13}C along the nutrient gradient, indicating that DIC increasingly originated from decomposed organic matter as impact increased. A decrease in CPOM $\delta^{13}\text{C}$ could be expected if CPOM originated from surrounding vegetation

with low $\delta^{13}\text{C}$ values. Unfortunately, I can not test this assumption since no samples of the surrounding vegetation were collected. In contrast to periphyton and CPOM, $\delta^{13}\text{C}$ values of the benthic consumers did not change along the nutrient gradient as expected from the C isotopic signature of their food (i.e. CPOM and periphyton) (Rounick & Winterbourn, 1986; Peterson, Fry & Deegan, 1993).

Decreasing C:N ratios in all basal resources (R^2 : 0.29 – 0.67, $p < 0.05$) were also noted along the nutrient gradient studied here (Fig. 6c). Reduced C:N ratios in algae with increased nutrient enrichment has also been shown in previous studies (Bowman, Chambers & Schindler, 2005; Liess & Hillebrand, 2006), and as a result the nutritional value of basal resources increases with increasing nutrient enrichment. However, invertebrate C:N ratios did not change significantly with increased nutrients. The theory of homeostasis predicts that consumers do not change their C:N ratio even if the food resources do (Sterner & Elser, 2002) and our findings on invertebrate C:N ratios lend support to this theory. By contrast, a decrease was noted for fish C:N ratios along the nutrient enrichment gradient ($R^2 = 0.55$). Homeostasis theory also predicts that C:N ratios of consumers should be lower than the food resource they assimilate (e.g. Cross, *et al.*, 2003; Evans-White, Stelzer & Lamberti, 2005; Liess & Hillebrand, 2006). Our findings, like those of many others, lend support to this conjecture; invertebrate and fish C:N ratios were lower than the basal resources (Fig. 6c).

Species function has been shown to vary across different environmental gradients (Wellnitz & Poff, 2001; Abreu *et al.*, 2006; Northington & Hershey, 2006) and N limitation could induce consumers to preferentially feed on more nutrient-rich food sources (Fagan *et al.*, 2002). To investigate potential differences in food preferences between high and low impact sites we used one-way ANOVA followed by Tukey's HSD ($\alpha = 0.05$) on PCA loadings to separate the nine study sites into two groups (low impact sites: Sandån, Rastälven and Sverkestaån, high impact sites: Sagån, Hågaån and Husbyån).

As stable C isotope ratios of consumers are similar to their food resource, but vary among basal resources, $\delta^{13}\text{C}$ has frequently been used to determine the origin of different food sources in stream ecosystems (Vander Zanden & Rasmussen, 1999; Finlay, 2001). However, C isotope ratios often overlap between terrestrial plant detritus and algae, confounding their use in stream studies (France, 1995a; France, 1996). In our study, we found no difference between the $\delta^{13}\text{C}$ signatures of periphyton and CPOM at either the high- or the low-impact sites (Fig. 7 a, b), making stable C isotopes ratios of limited use for determining food preferences. Moreover, our finding that many benthic consumers were more depleted than basal resources indicate that we did not sample all important food sources.

Given the limitations of using C isotope ratios in aquatic ecosystems, recent focus has shifted to the use of $\delta^{15}\text{N}$ to determine the relative importance of allochthonous and autochthonous inputs to stream function. In particular, terrestrial autotrophs generally have lower $\delta^{15}\text{N}$ signatures than freshwater autotrophs (France, 1995b). Our findings support this conjecture, i.e. periphyton

was more enriched in ^{15}N than CPOM (Two-way ANOVA: $F_{df1} = 14.6$, $p = 0.0005$). However, determining whether the invertebrates fed primarily on periphyton or CPOM was still difficult since invertebrates that feed on two or more food sources have $\delta^{15}\text{N}$ intermediate of their diet (Fry, 2006). Also at the high impact sites, $\delta^{15}\text{N}$ ranges of periphyton and CPOM were not distinguishable from each other (Fig. 7 c). However, our results suggests that periphyton was of greater importance at both high and low impact sites as invertebrate species in trophic levels two and three were more enriched or similar to periphyton. Moreover, periphyton had significantly lower C:N ratios than CPOM and FPOM (Two-way ANOVA: $F_{df2} = 8.38$, $p = 0.0008$), making it the most nutritious resource.

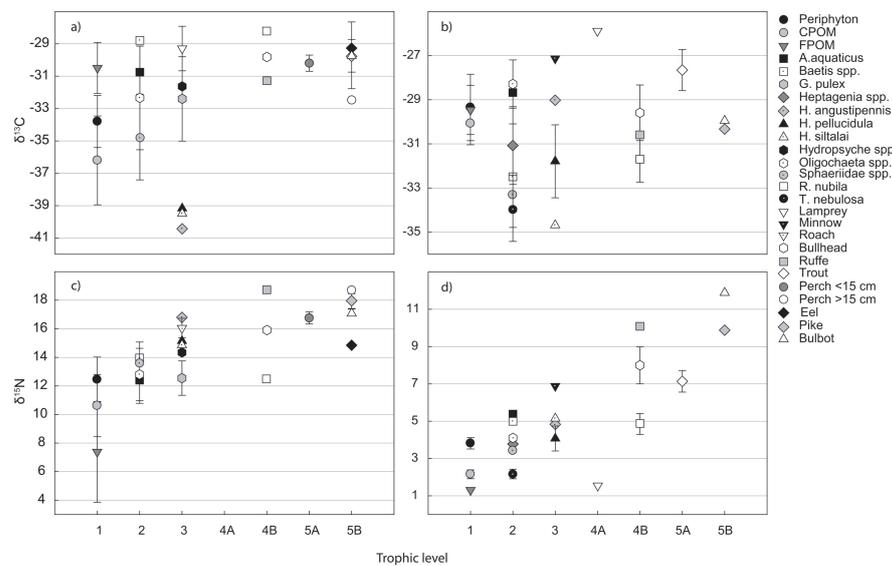


Figure 7. Mean $\delta^{13}\text{C}$ of basal resources, invertebrates and fish at high impact sites (a) and low impact sites (b) and mean $\delta^{15}\text{N}$ isotope ratio at high impact sites (c) and low impact sites (d). Legends on x-axis is explained in Table 3 (increasing value = higher trophic level). Error bars denotes standard error (SE). Note different scales on each y-axis.

Due to inherent differences in community composition with nutrient stress we were not able to collect the same species at the all sites. Consequently, it is difficult to unequivocally show how food preferences changed with increased nutrient enrichment. However, for some of the species found at both high- and low-impact sites we noted differences in food preference that could be related to stress. For example, *A. aquaticus* at high-impact sites was less enriched in ^{15}N than the other primary consumers and more similar to CPOM $\delta^{15}\text{N}$. The opposite was found at the low-impact sites; *A. aquaticus* was enriched in ^{15}N and more similar to periphyton. This finding suggests that *A. aquaticus* was more inclined to feed on CPOM at high- than at low-impact sites.

Finally, our findings of the food preference of the caddisfly *Rhyacophila nubila* did not agree with its previously described “exclusively” predacious behaviour

(Moog, 1995). Our results showed that this species was one of the most ^{15}N depleted species found, especially at the high impact sites, indicating a food preference for periphyton/detritus. However, this conclusion may be biased and an artefact of our study design, since we were not able to sample an adequate biomass of the small “prey” invertebrates (e.g. simuliids and chironomid midges) for elemental analysis. We also noted that some fish species changed food preference along the nutrient gradient. For example, both eel and bulbot had relatively low $\delta^{15}\text{N}$ values at high impact sites, indicating an invertebrate rather than a fish diet, whilst, conversely, at low impact sites bulbot was highly enriched, indicating piscivorous feeding. The finding that some species change their food preferences as a result of anthropogenic stress can have serious implications for the functional integrity of ecosystems (Wellnitz & Poff, 2001).

Conclusions and future perspectives

In this thesis I show that leaf-litter breakdown rates are limited in their ability to assess effects of nutrient enrichment from non-point sources (e.g. run-off from agricultural land use) on stream integrity. The findings also imply that biotic indices were better predictors of nutrient enrichment on stream conditions than functional measures. Somewhat disconcerting was the finding that the relation between decomposition in coarse and fine mesh bags across the nutrient gradient, and hence interpretation of the degree of impairment, changed with time.

Although a number of studies have used percentage of leaf mass remaining after a pre-determined time period as a measure of stream integrity, few have validated the power of this approach to quantify impairment. Studies using this approach have calculated the exponential breakdown rates (k-values) based on a single measure of leaf mass loss (Hury, *et al.*, 2002; Woodcock & Hury, 2005). In this thesis I propose the use of percentage leaf mass remaining at a defined date as an alternative to the breakdown rate. In fact, since this measure was better correlated to the nutrient gradient than the k-values and as correlation increased with time, it might be of interest to only visit the sites after a defined period of time with more bags left in the field to ensure adequate recovery, this is also economically most interesting. Moreover, due to natural factors such as high flows (both autumn-winter and spring) and heavy snow-fall, the success of the field studies was severely compromised. Thus, the use of litter-bag studies as a biomonitoring tool was more cost-intensive than expected. These factors, together with the information gained from using leaf-litter decomposition as a measure of ecosystem integrity, should be considered in the design and implementation of a robust monitoring program.

This thesis also shows that invertebrates and fungi are important mediators of leaf-litter decomposition and that both are affected by nutrient enrichment. Invertebrate community composition was also found to significantly influence breakdown rates. High breakdown rates at one site were associated with the high abundance of *A. aquaticus*. As some species can have a disproportionate effect on

ecosystem processes, uneven distributions of species abundances can result in a single species having a strong influence on a particulate process (Cardinale, Nelson & Palmer, 2000). The preservation of biodiversity is an important issue as it has been argued that the loss of sensitive taxa might have dramatic implications for ecosystem function, in particular, if the species is a dominant contributor to the ecosystem process. However, if ecosystem processes are supported by dominant species and only rare species become locally extinct, ecosystem processes would largely be unaltered (Giller, *et al.*, 2004). Moreover, the finding that certain species can have a stronger influence on ecosystem function than others has implications for the interpretation of ecosystem function studies, as the roles of different species needs to be considered when assessing impairment in streams (Carlisle & Clements, 2005).

In this thesis I also show that $\delta^{15}\text{N}$ of organic matter has potential as a good indicator of nutrient enrichment in boreal streams. Stable isotope analysis could be a simple low-cost method as it would not need taxonomic expertise nor elaborate sampling procedures and further studies should focus on the applications of stable isotope studies in bioassessment.

It was difficult to unequivocally show food preferences of aquatic organisms due to overlapping isotopic signatures of both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the basal resources. Since this study was conducted in autumn during leaf abscission, the streams were undergoing a marked change regarding the available food resources. Studies have shown changes in invertebrate stable isotope ratios with seasonal variation in both streams (Zah *et al.*, 2001) and lakes (Bohman, 2005). Thus species that have their main growth period after abscission could show marked changes in stable isotope ratios. Therefore, further studies on changes in stable isotopes due to seasonal variations should be of interest to elucidate trophic interactions in temperate stream ecosystem.

Svensk sammanfattning

Människan har alltid varit beroende av rinnande vatten, som åar och floder, som därmed har utnyttjats kraftigt som dricksvatten, för bevattning, kraftproduktion och transport av avfall. Tyvärr har människans aktiviteter inneburit att dessa ekosystem blivit starkt påverkade, vilket har resulterat i att bland annat den biologiska mångfalden minskat. Det finns också en oro för att olika ekosystemfunktioner också kan vara påverkade. För att komma tillrätta med dessa problem har flera lagar och direktiv införts som reglerar hur vi får använda och påverka akvatiska miljöer, EUs Ramdirektiv för vatten är ett exempel på ett sådant direktiv.

De övervakningsmetoder för sötvatten som utvecklats är oftast baserade på kemiska förhållanden och organismers förekomst och samhällsstruktur. Exempel på organismer som används är fisk, bottenfauna och påväxtalger. Då de index som beräknas oftast enbart tar hänsyn till strukturen hos ett ekosystem missar man ofta

hur olika ekosystemprocesser påverkas. Ett exempel på en ekosystemprocess i rinnande vatten är lövnedbrytning. Lövnedbrytningshastigheten är ofta korrelerat till olika typer av påverkan som t.ex. näringsberikning och försurning och därmed så har det föreslagits att man kan använda lövnedbrytning i miljöövervaknings syfte. Stabila isotoper i organiskt material är ett annat mått som föreslagits kunna ge en indikation på påverkan i vattendrag är. Till exempel kan naturligt förekommande stabila isotoper av t.ex. kväve ($\delta^{15}\text{N}$) ge ett integrerat mått på kväveprocesser i akvatiska system. $\delta^{15}\text{N}$ ökar vanligtvis med ökad näringstillförsel från till exempel jordbruksmark. I denna avhandling studerar jag hur en störning, näringsberikning, påverkar ekosystemstruktur och funktion. Jag studerar också möjligheten att använda ekosystemfunktioner för att utvärdera tillståndet hos rinnande vatten.

I den första uppsatsen studeras artrikedomen hos fisk, bottenfauna, makrofyter och påväxtalger i 28 vattendrag i södra Sverige längs en näringsgradient. Syftet med studien var att undersöka om någon organismgrupp kan fungera som en potentiell ersättare för andra organismgrupper för att indikera påverkan. Detta har betydelse då många av de organismer som finns i sötvattenmiljöer är dåligt studerade och även ibland är dyra att övervaka. I denna studie fann vi enbart ett svagt samband mellan fiskartrikedom och bottenfauna- och makrofyter – artrikedom, vilket innebär att fiskartrikedom skulle kunna användas som indikator för generell artrikedom i rinnande vatten. Detta bör dock studeras ytterligare då sambanden var svaga.

I den andra uppsatsen studerades lövnedbrytning i nio vattendrag i mellersta Sverige under hösten - vintern 2003. Jag fann att lövnedbrytningshastigheten inte var lika känslig som olika strukturella biologiska index (ASPT, DSFI och EPT) för att indikera näringsberikning. Jag fann också att resultaten förändrades beroende på försöksupställningen. Till exempel var nedbrytningshastigheten i grovmaskiga lövpåsar starkare korrelerat till påverkan ju längre inkubationstid som användes. Dessutom var ett alternativt mått på lövförlust, den procentuella massa löv som fanns kvar efter en förutbestämd tid, en bättre indikator på påverkan än den exponentiella lövnedbrytningshastigheten.

I den tredje studien återupprepade jag det försök som ställts upp i den första artikeln under tre ytterligare säsonger, nämligen våren 2005, hösten - vintern 2005 och våren 2006. Jag fann att endast under hösten vintern 2005, i finmaskiga lövpåsar, fanns en signifikant skillnad i lövförlust mellan påverkade och opåverkade vattendrag. Det fanns också signifikanta säsongskillnader i nedbrytning, vilket kunde förklaras med temperaturskillnader mellan de fyra säsongerna. Då många lövpåsar försvann på grund av is och fluktuerande vattennivåer under mina studier så kom detta att påverka tolkningen av resultaten. Dessutom var jag tvungen att återbesöka lokalerna flera gånger för att garantera att tillräckligt många lövpåsar kunde hämtas i slutet av studierna. Trots detta så förlorades ett stort antal lövpåsar. Detta innebär att lövnedbrytning inte var det billiga och enkla övervakningsverktyg som hade förväntats.

I den fjärde artikeln studerades bottenfaunasamhället samt svampbiomassan som fanns i lövpåsarna under den första studien, hösten – vintern 2003. Jag fann att näringsberikning ökade svampbiomassan som i sin tur ökade nedbrytningshastigheten. Jag fann även att bottenfaunan i lövpåsarna ökade i densitet och abundans med ökad påverkan och att detta ökade lövnedbrytningshastigheten i de påverkade systemen. Även artsammansättningen i lövpåsarna påverkade nedbrytningshastigheten. Hög nedbrytning på en lokal, Husbyån, berodde på dominans av vattengråsugga (*Asellus aquaticus*). Att lövnedbrytning kan påverkas av artsammansättningen kan ha betydelse för tolkningen av liknande studier. Detta är också något som måste beaktas om lövnedbrytning ska ingå i övervakningsprogram.

I den femte artikeln studerades olika isotop signaturer ($\delta^{15}\text{N}$ och $\delta^{13}\text{C}$) samt C:N kvoten i olika fraktioner av organiskt material; CPOM och FPOM, samt i olika sötvattensorganismer; fisk, bottenfauna och påväxt, från de nio lokalerna. Jag fann att med ökad påverkan ökade $\delta^{15}\text{N}$ i alla prover utom FPOM. Dessa resultat tyder på att $\delta^{15}\text{N}$ skulle kunna användas som indikator på näringsberikning i boreala vattendrag.

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