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The Ecosystem Approach in Freshwater Ecological Data Analysis

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Contents

List of Tables

List of Figures

Summary

The Ecosystem Approach (EA) is a theoretical concept in ecological assessment and management, which involves the holisitic consideration of multiple ecosystem components. It came into a policy context as the primary framework of the United Nations Convention on Biological Diversity (CBD) in 1992, and since then has been increasingly enforced by environmental legislation worldwide. However, still today, the EA remains mainly a theoretical concept and its practical implementation continues being a great challenge due to the high cost and time intensity of ecosystem assessments, lacks in proper standardized methodology for sampling design and implementation, and knowledge gaps in statistically appropriate data integration. The main goal of this PhD thesis has been the development of new integrative and effective solutions to transform the theoretical grounds of the CBD concept into a praxis method for freshwater ecosystems. Several key-aspects for improving the feasability of the EA are considered, which have so far been insufficiently tested in any type of ecosystem. This involves easily applicable field sampling techniques for destruents and multivariate data integration, as well as adaptations to optimize the time and cost-efficiency. The proposed methods are validated for typical environmental monitoring applications and a universally applicable step-by-step guideline for the implementation of the EA is deduced at the end of the thesis.

Since there still is a lack of appropriate field sampling methods for hyporheic microbial communities, which are essential for a comprehensive understanding of stream ecosystems, a systematic comparison of substratum sampling, interstitial water sampling, and exposing granite, carbonate and glass coupons was conducted in a first study. Multivariate analyses of bacterial community data indicated strong differences in community composition revealed by direct (substratum samples) and indirect (interstitial water samples, coupons) sampling strategies. Substratum samples yielded highest microbial diversity and at the same time proved to be the most cost-effective method as the amounts of time as well as consumables compared to be lowest. Consequently, this technique appears most representative and suitable for extending classical stream ecosystem assessments to microbiota. Moreover, the multivariate analysis of Terminal Restriction Fragment Length Polymorphism (T-RFLP) fingerprinting data applied in this study

was capable of relating bacterial communities to habitat conditions, though only at an arbitrary level of Operational Taxonomic Units (OTUs). Coarser taxonomic levels than species have also often been used in invertebrates to balance accuracy of the results with effort for taxonomic identification (Taxonomic Sufficiency, TS), but their applicability has never been comparatively tested for data analysis in different freshwater groups. The following study analyses the effects of taxonomic resolution, functional groupings, and data transformation on multivariate community patterns and diversity in periphyton, macrophytes, macroinvertebrates, and fishes. The applicability of TS differed strongly among taxonomic groups, depending on the average taxonomic breadth of the species sets, but TS was universally applicable within taxonomic groups for different habitats. For each group, statistical threshold levels of minimum necessary taxonomic resolution were identified. Even though aggregation to family or order was suitable for quantifying biodiversity and environmental gradients, multivariate community analyses required finer resolution in fishes (species) and macrophytes (genus) than in periphyton (order) and macroinvertebrates (family). The key point of the third study focused on statistically sound integration of data from multiple ecosystem components. Since descriptive or univariate analysis of commonly applied compound indices has strong limitations in terms of sensitivity, a standardized procedure for merging species abundance data from different taxonomic groups, which allows multivariate community analyses on ecosystem scale, is proposed herein. A comparison of the indicative power of this new approach with the European Water Framework Directive's Ecological Quality Class revealed that multivariate indication of ecosystem change is much more sensitive than single numeric score indices. Moreover, the simultaneous multivariate analysis of multiple taxonomic groups presented herein is feasible with the same sampling effort, and is independent of the investigation scale or the occurrence of certain indicator taxa.

The following two case studies were developed with a focus on the application and validation of taxonomic sufficiency and the new data integration method for environmental impact assessment and the evaluation of restoration success. Dams and weirs are considered a major threat to aquatic biodiversity. For this reason, a data set on weir effects in stream ecosystems was used for validation in the first study. To quantify the serial discontinuity introduced by weirs, the abiotic stream habitat characteristics and biological community structure of periphyton, aquatic macrophytes, macroinvertebrates and fish were compared between upstream and downstream sides of obstacles at five different study rivers. Multivariate analysis methods that include several taxonomic groups and physicochemical habitat

variables allowed for a precise quantification of effect size in different rivers, yet at the family and order-level. Even with the widespread use of stream restoration due to the strong degredation of riverine habitats, systematic comparisons on the effects of different techniques are still rare. In the last case study of this thesis, further attention is given to the new approach for simultaneous multivariate analysis of multiple taxonomic groups for the evaluation of the success of four substratum restoration techniques in six stream ecosystems. The results are compared with those revealed from indicator-based approaches. Along with the results of the study on weir effects, the integrative multivariate approach proofed to be a highly suitable tool for the quantification and comparison of restoration effect size among techniques and study rivers. Still discriminiating between treatment sites and sampling periods, this method proved to be suitable even when the taxonomic resolution was reduced to family (macroinvertebrates) or order-level (periphyton). However, a more detailed consideration of the effects on target species indicated that these are not necessarily congruent in their results with ecosystem scale effects and an additional consideration of target species related endpoints is advantageous.

In conclusion, the case studies included in this thesis indicate that the implementation of the ecosystem approach in freshwater ecological monitoring and data analysis is achievable based on the available standards for environmental assessments in combination with the new methodological approaches for bacterial field sampling, taxonomic sufficiency and multivariate data integration provided herein. For the future implemenation of the EA, the holistic and integrative nature of such research should already be considered at the stage of project planning. This can ensure an adequately standardized and synchronized sampling design for all ecosystem components and should include a sufficient number of spatial and temporal replicates for statistical verification. By including this most essential prerequesite for the later alignment of data from multiple taxonomic groups, data integration can be simplified by following the step-by-step guideline outlined in the last chapter. The guideline is universally applicable across ecosystem types and boarders, and is highly flexible concerning the type of variables and covariables included. This allows the integration of various biological endpoints, the inclusion of a socioeconomic dimension and the integration of new data types that are currently arising from molecular biology. The broad applicability across ecosystem types, geographic regions, and for a variety of data types makes the data integration and analysis approach presented herein a promising tool for the future development in ecological monitoring.

Zusammenfassung

Der “Ecosystem Approach” (EA) ist ein theoretisches Konzept in Ökologie und Ökosystemmanagement, welches die ganzheitliche Betrachtung mehrerer Ökosystemkomponenten vorsieht. Der “ecosystem approach” kam als Leitkonzept der Biodiversitätskonvention der Vereinten Nationen (CBD) im Jahr 1992 erstmals in einen politischen Zusammenhang und seine Umsetzung wird seither weltweit in Umweltgesetzgebungen gefordert. Dennoch ist der EA heute immer noch ein überwiegend theoretischer Ansatz und für eine breite Umsetzung in die Praxis sind noch große Hürden zu überwinden. Die Hauptgründe dafür sind der große Kostenfaktor derartiger Untersuchungen, das Fehlen einer standardisierten Methodik zur gleichzeitigen Datenerhebung für mehrere Organismengruppen im Feld und Wissenslücken für die statistisch korrekte, multivariate Datenintegration der verschiedenen Teilkomponenten. Als Folge dessen fordern Wissenschaftler, Manager und Politiker heute verstärkt Forschung im Bereich integrativer Lösungen für Fragestellungen der Ökologie und des Biodiversitätsschutzes. Das Hauptziel der vorliegenden Dissertation war die Entwicklung neuer integrativer und effektiver Lösungen zur Verbesserung der praktischen Umsetzbarkeit des EA in Süßwasserökosystemen. Dies beeinhaltet das Schließen methodischer Lücken im Bereich von Felderhebungstechniken und multivariaten Methoden der Datenintegration, sowie die Optimierung der Zeit- und Kosteneffektivität. Abschließend findet eine Validierung der vorgeschlagenen Methodik anhand typischer Fragestellungen im Umweltmonitoring statt. Darüber hinaus wird am Ende der Dissertation eine universell anwendbare Schritt für Schritt Handlungsanweisung für die praktische Umsetzung des EA abgleitet.

Da einfache und effektive Feldbeprobungstechniken für die Mikroorganismen im hyporheischen Interstitial nach wie vor fehlen und Informationen über die Zusammensetzung der Mikrobiozönose von großer Bedeutung für das Verständnis von Fließgewässerökosystemen sind, zielt die erste Studie auf einen systematischen Vergleich der direkten Beprobung des Substrates, der Beprobung des Interstitialwassers und der Beprobung zuvor ausgebrachter, künstlicher Substrate (Glas, Granit und Kalkgestein) ab. Die Ergebnisse der multivariaten Auswertung der Bakteriendaten deuten auf große Unterschiede zwischen direkten (Substrat) und indirekten Beprobungstechniken (Interstitialwasser, künstliche Substrate)

bezüglich der Bakterienzusammensetzung hin. Mit der direkten Beprobung des Substrats wurde die höchste mikrobielle Diversität gemessen. Gleichzeitig ist diese Methode am kostensparendsten, da sie am wenigsten Arbeitszeit und Verbrauchsmaterialien benötigt. Die Substratproben sind daher zur Ausweitung klassischer Ökosystemstudien in Fließgewässern auf die Mikroorganismen am besten geeignet. Weiterhin war das "Terminal Restriction Fragment Length Polymorphism (T-RFLP) fingerprinting" trotz der groben taxonomischen Auflösung von "Operational Taxonomic Units" (OTUs) ausreichend, um Unterschiede in der bakteriellen Zusammensetzung mit den abiotischen Bedingungen in verschiedenen Habitaten in Bezug zu setzen. Gröbere taxonomische Auflösungen als das Artniveau werden auch in Studien über Invertebraten verwendet, damit ein Kompromiss zwischen einer hohen Genauigkeit der Ergebnisse und hohem Aufwand bei den Bestimmungsarbeiten gefunden werden kann. Die tatsächliche Anwendbarkeit solcher Ansätze wurde aber noch nie vergleichend für verschiedene taxonomische Gruppen überprüft. Daher wurden in einer weiteren Studie die Effekte verschiedener taxonomischer Auflösungen, funktioneller Gruppierungen und Datentransformationen auf die Ergebnisse multivariater Auswertungen und Diversitätsindices für Periphyton, Makrophyten, Makrozoobenthos und Fische untersucht. Die Anwendbarkeit des "Taxonomic Sufficiency" (TS) Konzepts unterschied sich deutlich zwischen den einzelnen taxonomischen Gruppen und war abhängig von der taxonomischen Komplexität ("average taxonomic breadth") der jeweiligen Gruppe. Innerhalb einer taxonomischen Gruppe ist das Konzept jedoch für verschiedene Habitattypen universell anwendbar. Für jede der untersuchten taxonomischen Gruppen wurden statistische Schwellenwerte der taxonomischen Auflösung ermittelt, ab denen sich die Ergebnisse ökologischer Analysen signifikant verändern. Ein Zusammenfassen auf Familien- oder Ordnungsebene war im Allgemeinen geeignet, um Biodiversität zu messen und Zusammenhänge mit Umweltgradienten zu untersuchen. Für multivariate Analysen der Artenzusammensetzung von Makrophyten (Gattungsebene) oder Fischen (Artebene) wird jedoch eine höhere taxonomische Auflösung benötigt als für Aufwuchsalgen (Ordnungsebene) oder Makrozoobenthos (Familienebene). Die dritte Studie behandelt die statistisch korrekte Integration von Daten mehrerer Ökosystemkomponenten für spätere multivariate Analysen. Da die momentan im Monitoring übliche deskriptive oder univariate Analyse von Indices starke Limitierungen bezüglich der Sensitivität aufweist, wurde in der vorliegenden Arbeit eine standardisierte Vorgehensweise zur Zusammenführung der Abundanzdaten verschiedener Organismengruppen entwickelt, die eine spätere multivariate Auswertung der ökosystemaren Effekte ermöglicht. In einem Vergleich dieses

neuen Ansatzes mit Index-basierten Auswerteverfahren zeigte sich, dass die simultane multivariate Auswertung mehrerer taxonomischer Gruppen einen wesentlich sensitiveren Indikator für Veränderungen des Ökosystems liefert. Verglichen mit den bisherigen Index-basierten Datenauswertungsmethoden ist der neue Ansatz mit demselben Beprobungsaufwand realisierbar und ist zudem unabhängig vom Untersuchungsmaßstab und dem Vorkommen bestimmter Indikatorarten.

Der Methodenentwicklung und -optimierung der ersten drei Studien folgen zwei weitere Studien, in denen das Konzept der "taxonomic sufficiency" und die neue Methode zur Datenintegration zur Untersuchung anthrophogener Störungen in Fließgewässern und zur Bewertung des Erfolges von Renaturierungsmaßnahmen angewendet werden und bezüglich ihrer Eignung zur Bearbeitung derartiger Fragestellungen validiert werden.

Für ein erfolgreiches Management und die Renaturierung von Fließgewässerökosystemen sind Informationen über die qualitativen und quantitativen Auswirkungen von Querbauwerken von großer Bedeutung. Zur quantitativen Erfassung der seriellen Diskontinuität, die durch Querbauwerke in Fließgewässern entsteht, wurden in der vorliegenden Arbeit die abiotischen Habitateigenschaften oberhalb und unterhalb von Wehren in fünf Fließgewässern systematisch verglichen und deren Auswirkungen auf Aufwuchsalgen, Makrophyten, des Makrozoobenthos und der Fische untersucht. Unter Anwendung multivariater Auswerteverfahren, welche die Effekte auf verschiedene taxonomische Gruppen und auf die abiotischen Habitateigenschaften integrieren, waren die Auswirkungen der Wehre selbst noch auf Familien- und Ordnungsniveau nachweisbar. Aufgrund des schlechten Zustands der Gewässerlebensräume werden heute sehr häufig Maßnahmen zur Fließgewässerrenaturierung durchgeführt. Systematische Vergleiche der tatsächlichen Effekte verschiedener Maßnahmen sind jedoch bislang kaum vorhanden. Im Rahmen der letzten Studie dieser Dissertation wurde die neu entwickelte Methodik zur simultanen multivariaten Analyse verschiedener Organismengruppen bezüglich ihrer Eignung zur Erfolgsbewertung von vier verschiedenen Renaturierungsmaßnahmen in sechs Fließgewässern mit der Reaktion einzelner Indikatorarten verglichen. Der integrative multivariate Ansatz zeigte sich dabei als geeignetes Werkzeug zur Unterscheidung der Effektivität verschiedener Maßnahmen in unterschiedlichen Gewässern. Auch wenn die taxonomische Auflösung der Makroinvertebraten auf Familienebene und des Periphytons auf Ordnungsbene reduziert wurde, konnte mit dieser Methode noch zwischen den verschiedenen Maßnahmen und den Beprobungszeitpunkten unterschieden werden. Eine detaillierte Betrachtung der Auswirkungen auf rheophile Zielarten zeigte allerdings, dass von positiven Effekten auf ökosystemarer Ebene

nicht zwingend auf positive Effekte für einzelne Zielarten geschlossen werden kann. Monitoringprogramme zur Quantifizierung ökosystemarer Veränderungen sollten daher idealer Weise durch die Untersuchung projektspezifischer Zielarten ergänzt werden.

Insgesamt haben die Fallstudien bewiesen, dass die Umsetzung des "ecosystem approach" im Gewässermonitoring auf Basis der verfügbaren Monitoringstandards und unter Erweiterung durch die neuen Methoden und Erkentnisse für die Feldbeprobung von hyporheischen Bakterien, die Anwendung von Taxonomic Sufficiency und die multivariate Datenintegration aus dieser Dissertation möglich ist. Für die zukünftige Umsetzung des EA ist es wichtig, dass die ganzheitliche und integrative Art dieser Forschung schon im Stadium der Projektplanung berücksichtigt wird. So kann ein angemessenenes und standardisiertes Beprobungsdesign erreicht werden, das alle Ökosystemkomponenten synchron erfasst und idealer Weise eine ausreichende Anzahl räumlicher und zeitlicher Replikate für eine statistische Absicherung der Ergebnisse enthält. Wenn diese wichtige Voraussetzung erfüllt ist, kann die spätere Datenintegration einer einfachen Handlungsanweisung folgen, die im letzten Kapitel dieser Dissertation präsentiert wird. Diese Handlungsanweisung ist auch auf andere Ökosysteme oder systemübergreifend anwendbar und ist weiterhin bezüglich der Art der zu integrierenden Variablen und Covariablen sehr flexibel. Dadurch ist beispielsweise die Einbeziehung zusätzlicher biologischer Endpunkte, eine Erweiterung um sozio-ökonomische Aspekte und die Integration neuer Datentypen aus der Molekularbiologie möglich. Die breite Anwendbarkeit für verschiedene Ökosystemtypen, geographische Regionen und Datentypen machen die hier entwickelte Methodik zur Datenintegration und Analyse zu einem vielversprechenden Werkzeug für zukünftige Entwicklungen im Umweltmonitoring.

Preface

Ecosystems can be highly dynamic in space and time, resulting from changes caused by natural factors and human activity. Today, natural ecosystem changes are mostly overlaid by anthropogenic impacts such as transformation of land and sea, alteration of major biogeochemical cycles and the introduction or removal of species (Lubchenco 1998). An understanding of ecosystem change and its consequences is central for the future conservation and restoration of ecosystems to maintain the services they provide (Millennium Ecosystem Assessment 2005). Following the increased recognition of the importance of human-environment relations during the second half of the 20^{th} century, the science of environmental monitoring emerged (Redman 1999). At its beginning, environmental monitoring was mainly focused on chemical or phyisical monitoring of toxic substances (Karr 1987) and further developed to biological monitoring techniques. Due to the high complexity of the biological components and interactions in ecosystems, simplifications that strongly rely on single indicator species are often used in biological monitoring (Cairns & Pratt 1993).

Biological monitoring has become widespread especially in freshwater ecosystems, since these constitute the most essential resource for human existance (Gleick 1993), making them particularly prone to degradation (Ricciardi & Rasmussen 1999, Balian et al. 2008). For a long time, biological studies in freshwater mainly focused on community-based studies of plankton or benthic macroinvertebrates (Cairns & Pratt 1993), but since the beginning of the 21^{st} century there is growing evidence on the very low congruency between different indicator groups in their reaction to environmental changes. This has been reported from different aquatic and terrestrial habitats throughout the world, particularly from European and North American streams and lakes (Allen et al. 1999, Declerck et al. 2005, Heino 2010, Mueller et al. 2011) as well as from tropical rainforests in the Amazon basin (Landeiro et al. 2012). These findings generally question the value of the indicator concept for drawing conclusions on ecosystem scale.

Since environmental monitoring today constitutes a complex evaluation of multiple stressors as well as ecological restoration, there is an increasing need for a more holistic approach including the simultaneous consideration of several indicator groups from different levels of biological organization (Ecosystem

Approach, EA). Current monitoring techniques have strong limitations in terms of sampling design and techniques for multiple taxonomic groups as well as integrative methods for data analysis, which makes it still difficult to get a comprehensive and at the same time clear picture of the ecosystem as a whole. This PhD thesis is intended to provide more integrative and effective solutions for the practical implementation of the EA in freshwater monitoring. It begins with an introductory chapter (Chapter 1), presenting the definition and history of the term EA and discussing its current implementation and limitations in environmental legislation, monitoring and science based on a comprehensive review of legal regulations, monitoring protocols and scientific literature. After a description of the objectives of this thesis at the and of the introductory chapter, the main part comprises five peer-reviewed publications on the development of new sampling techniques and data analysis methods in light of integrating multiple ecosystem components (Chapter 2, publications 1-3), as well as on a validation of the new methods for typical monitoring applications (Chapter 3, publications 4-5). The thesis ends in a general discussion (Chapter 4) of the methods for sampling design and implementation, taxonomic identification and data analysis presented in the previous chapters. The results are discussed in light of the associated scientific literature and synthesized into a universally applicable guideline for multivariate data integration. At the end of Chapter 4, the transferability of this approach to other ecosystem types and geographic regions is discussed.

1 The importance of ecosystem based science and environmental management

1.1 The ecosystem approach

Worldwide, all ecosystem types have been heavily modified due to rapidly growing human population and technological progress (Walker & Salt 2006). This has led to serious consequences for global biodiversity and the ecosystem services provided to human population (Millennium Ecosystem Assessment 2005, United Nations Environment Programme 2007). In response to the strong degradation of the earth's ecosystems the United Nations agreed upon the Convention on Biological Diversity (CBD) in 1992. The main objective of the convention is the conservation of biological diversity. The so called "Ecosystem Approach" (EA) is the primary framework for all actions in environmental monitoring, impact assessment and management under the convention (United Nations 1992). In contrast to former species-focused conservation strategies, the complex and dynamic nature of ecosystems, including all trophic levels and their interactions among each other and with the non-living environment is recognized within the framework of the EA, and humans are considered as an integral part of ecosystems (United Nations Environment Programme 1998). Since biological diversity is inextricably linked to ecosystem processes, functioning, and resilience (United Nations Environment Programme 1998), the EA also requires a holistic view of biodiversity that extends from classical species diversity to functional diversity, diversity of ecosystem processes and habitat diversity.

The term ecosystem approach has been referred to in scientific literature long before its first application in a policy context in 1992. It dates back to an article published in *Ecology* by Odum (1957), who suggested the study of ecosystems by sampling and analysis of as many ecosystem components (which can be taxonomic or trophic units) and physicochemical variables as possible in ecological field courses for university students. Odum (1957) proposed this concept as "ecosystem approach in teaching ecology". In the following decades the EA was mentioned in publications on environmental toxicology (e.g. Lakshman 1979, Metcalf 1977) before it was extended to socioeconomic and political dimensions in the 1990s

(e.g. Rowe 1992, Spence & Hughes 1996, Sherman & Duda 1999). Up to now, there are numerous further definitions of what "ecosystem approach" means (Laffoley et al. 2004). So, Rowe (1992), by indicating EA, meant to "shift the focus from parts to wholes, from the interest to the capital, from trees and other plants, animals, stream flow, esthetics and whatever else the earth's surface yields to the three dimensional landscape ecosystems and waterscape ecosystems that produce these valuable things", and establishes an understanding with a strong focus on ecosystem services. Cury et al. (2005) consider the EA in fisheries in a very general way, being an approach that "deals with ecosystems instead of individual stocks", whilst de Jonge et al. (2012) specifically include community structure and function as well as environmental variables into their definition of what EA means: "environmental conditions should be assessed on the basis of the structure and functioning of the biological part of the ecosystem in response to the sum of natural variation and human induced stresses". Beaumont et al. (2007) provide a more management and conservation oriented definition of the EA, being a "strategy for the integrated management of land, water and living resources that promotes conservation and sustainable use in an equitable way". However, all present definitions of the EA are based on an universal understanding of an ecosystem. An ecosystem comprises different components, representing different trophic levels (primary production: e.g. algae, plants; primary consumption: e.g. invertebrates; secondary and higher levels of consumption: e.g. fishes, carnivores; destruents: e.g. bacteria; see Campbell et al. 2009 and Fig. 1.1). The interaction of different ecosystem components with each other and with natural environmental factors as well as human activities, determines the resulting ecosystem services (Fig. 1.1). Ecosystems, and therefore the EA, can be analyzed at many different geographical, biological and temporal scales which are hierarchically structured and can be nested into each other (Fig. 1.1). For instance, the investigation of ecosystem components can be conducted for a single ecosystem from a wide range of ecosystem types (e.g. terrestrial, marine and freshwater biomes), as well as at different geographical (e.g. local, regional, national, continental, global), biological (e.g. genes, individuals, populations, species, communities of each trophic level) and temporal scales (e.g. months, years, decades), but also for multiple ecosystems situated in an entire landscape (Fig. 1.1). The minimum requirement to meet the EA in monitoring is consequently to cover components from all trophic levels of a single ecosystem at the local scale.

In this thesis, the term ecosystem approach refers to the simultaneous consideration and integrative analysis of multiple components from freshwater ecosystems (herein taxonomic groups, including single life stages, individuals, populations,

species and communities) and the natural and anthropogenic factors influencing them at the local and regional scale.

Several decades after defining the EA and integrating it into the CBD objectives, the question is no more wether the EA is required (Cury et al. 2005), but how to implement this complex concept. This still constitutes a key challenge in ecological research and management, even at the minimum level of single ecosystems (de Bello et al. 2010). Consequently, research into more integrative solutions to ecology and biodiversity conservation is now frequently postulated by scientists (Leslie & McLeod 2007, Levin et al. 2009, Geist 2011, Irschick et al. 2013, Pander & Geist 2013), and biodiversity conservation managers, and is increasingly enforced by legislative power.

1.2 International environmental legislation and the ecosystem approach

Legal implementation of the objectives of the CBD has been achieved on various international levels. The main focus of legislative action consists of environmental protection in general as well as laws protecting aquatic resources (Table 1.1). However, only few of these legal regulations (water laws in Europe, Argentina and Namibia) directly refer to the EA in terms of declaring the integration of several biological, physical and chemical ecosystem components into environmental monitoring mandatory (Table 1.1). The European Water Framework Directive (WFD, European Parliament 2000) most specifically regulates the monitoring of aquatic ecosystems by clearly defining the chemical, physical and biological quality elements, the way of presenting the results and a time frame for surveillance monitoring in Annex V (European Parliament 2000). In contrast, details on data collection and analysis methods remain unclear and the development and the implemententation of a monitoring strategy is left to the member states (Scheuer 2006). In general, reference to EA within the framework of most national and international environmental legislation is limited to indirect regulations, like legal achievements such as the protection of "the ecosystem with all its components" without further specification of implementation, monitoring or enforcement regulations. This lack of precise implementation strategies in environmental legislation leads, especially in developing countries where access to scientific knowledge and technology is limited, to weak enforcement and implementation of the EA on the federal level (Alshuwaikhat 2005, Sands & Peel 2012).

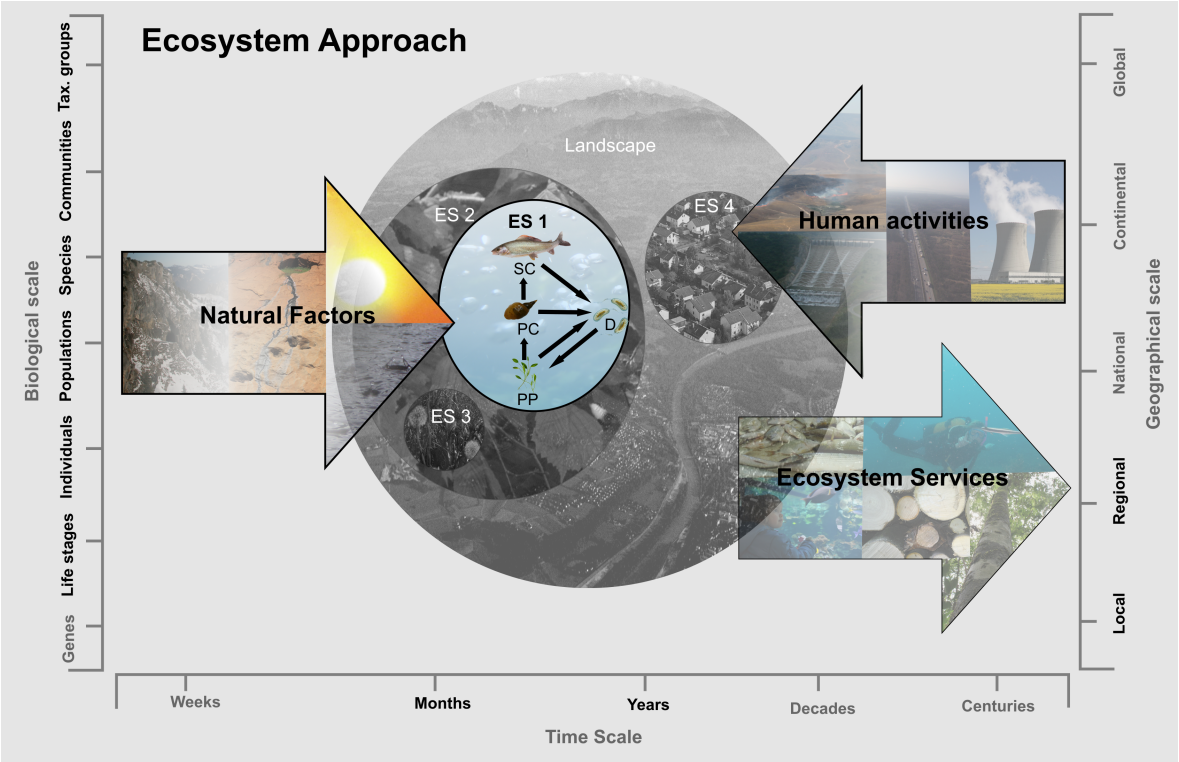

Figure 1.1: Multiple dimensions of the ecosystem approach. Nested circles symbolize different ecosystems that interact with each other (figure part modified from United Nations Environment Programme 1998). ES 1 = ecosystem 1, e.g. freshwater ecosystem; ES 2 = ecosystem 2, e.g. alluvial forest; ES 3 = ecosystem 3, e.g. meadow; ES 4 = ecosystem 4, e.g. urban area. All these different ecosystems are situated within a landscape. PP = primary producers; PC = primary consumers; SC = secondary consumers; D = destruents. Arrows symbolize different factors that affect or emerge from ecosystems: Human activities = all human induced alterations of ecosystems which change their structure, function and composition, e.g. hydropower plants or agricultural land use; Natural factors = physicochemical characteristics resulting from climate (e.g. solar radiation, temperature, rainfall) and geology (e.g. topography, lithology); Ecosystem services = provisioning, regulating, cultural and supporting services provided by ecosystems. The scale bars on the left, right and bottom indicate the different biological, geographical and temporal scales at which the setup in the middle of the figure can be investigated. Words printed in black highlight components and scales of the ecosystem approach that are considered in the following chapters. Note that ecosystem services are only considered in terms of the following aspects: provisioning: fish biomass and abundance, regulating: water quality, cultural: biodiversity and supporting: primary production, while a economic valuation is not provided in this thesis

Table 1.1: Examples of worldwide environmental legislation considering ecological monitoring and impact assessment. PU = political unit; Environ. Law = environmental law; ES Type = ecosystem type. EA indicates reference to the ecosystem approach: + = explicit reference to defined ecosystem components; +/− = mentioning of ecosystem components in general; − = no reference to the EA. EIA = environmental impact assessment. EU = European Union; USA = United States of America; CDN = Canada; AUS = Australia; ZA = South Africa; NAM = Namibia; KEN = Kenya; KOR = Korea; IND = India; CHN = China; RU = Russia; AR = Argentina; CHI = Chile; PAN = Panama. Note that only those environmental policies were considered that are available in English or Spanish language on the web

Continent	PU	Environ. Law	ES Type	EA	Monitoring	EIA
Europe	EU	Habitats Directive (HD, European Parliament 1992)	Multiple	+/−	Surveillance monitoring	Regulated in European Parliament (2011)
Europe	EU	Water Framework Directive (WFD, European Parliament 2000)	Aquatic	+	Physical, chemical, biological components	Adapted monitoring in case of bad condition
North America	USA	Environmental Policy Act (EPA, 91^{st} United States Congress 1970)	Multiple	+/−	Surveillance monitoring	Expert estimation
North America	USA	Clean Water Act (CWA, 92^{nd} United States Congress 1972)	Aquatic	−	Surveillance monitoring	Not specified
North America	CDN	Environmental Protection Act (EPA, Government of Canada 2000)	Multiple	+/−	Environmental quality monitoring	Expert estimation, monitoring
Australia	AUS	Environmental Protection and Biodiversity Conservation Act (EPBCA, Australian Government 1999)	Multiple	+	Voluntary and scientific monitoring, financial support	Expert estimation

Continent	PU	Environ. Law	ES Type	EA	Monitoring	EIA
Australia	AUS	Water Act (WA, Australian Government 2007)	Aquatic	+/−	Quality and quantity of water resources	Expert estimation
Africa	ZA	Environmental Conservation Act (ECA Republic of South Africa 1989)	Multiple	−	Not specified	Expert estimation
Africa	ZA	Water Act (WA, Republic of South Africa 1998*b*)	Aquatic	+/−	Water resources, quantity and quality	Expert estimation
Africa	ZA	Forest Act (FA, Republic of South Africa 1998*a*)	Terrestrial	+/−	multiple components, including biodiversity	Expert estimation
Africa	NAM	Environmental Management Act (EMA, Republic of Namibia 2007)	Multiple	+	Functional integrity and biodiversity	Literature research, field work, monitoring
Africa	NAM	Water Resources Management Act (WRMA, Republic of Namibia 2004)	Aquatic	+	Physical, chemical, biological components	Expert estimation
Africa	KEN	Environmental Management Act (EMCA, Parliament of Kenya 1999)	Multiple	−	Environmental changes	Environmental audit
Asia	KOR	Natural Environmental Conservation Act (NECA, Republic of Korea 1997*b*)	Multiple	+/−	Not specified	Regulated in Republic of Korea (1997*a*)
Asia	KOR	Water Quality and Ecosystem Conservation Act (WQECA, Republic of Korea 1997*c*)	Aquatic	−	Water pollution, water quality, aquatic ecosystems quality	Regulated in Republic of Korea (1997*a*)

Continent	PU	Environ. Law	ES Type	EA	Monitoring	EIA
Asia	IND	Biological Diversity Act (BDA, Parliament of India 2003)	Multiple	+	Areas rich in biological resources	Expert estimation
Asia	CHN	Environmental Protection Law (EPL, Republic of China 1989)	Multiple	−	Establishment of monitoring system	Expert estimation
Asia	CHN	Water Law (WL, Republic of China 1988)	Aquatic	−	Dynamic monitoring of water resources	Comprehensive scientific survey
Eurasia	RU	Law on Environmental Protection (LEP, Russian Federation 2002)	Multiple	+	Environmental condition in areas with man made effects	Not specified
Eurasia	RU	Forest Code (FC, Russian Federation 1997)	Terrestrial	+/−	Obervations, assessments and forecasts	Conservation measures as compensation
Eurasia	RU	Water Code (WC, Russian Federation 2006)	Aquatic	−	Qualitative and quantitative indicators of water body state	Efficiency control for implemented measures
South America	AR	Law on National Environmental Policy (LGA, Republica de Argentina 2002*a*)	Multiple	+/−	Scientific investigations in the field of biodiversity conservation	Expert estimation
South America	AR	Law on the Use of Public Waters (RGA, Republica de Argentina 2002*b*)	Aquatic	+	Physical, chemical, biological components	Expert estimation
South America	CHI	Environmental Law (LMBA, Congreso Nacional de Chile 2010)	Multiple	+/−	Not specified	Expert estimation, monitoring

Continent	PU	Environ. Law	ES Type	EA	Monitoring	EIA
South America	PAN	General Environmental Law (LGA, Republica de Panama 1998)	Multiple	+	Environemtal quality, support of scientific studies	Regulated in Republica de Panama (2009)

However, the increasing number of international legal regulations creates an urgent need for ecosystem-based environmental monitoring throughout the world. To improve precision and enforcement of environmental legislation also in developing countries, research in the field of standardized monitoring systems that are adaptable to these regions is essential.

1.3 Current implementation of the ecosystem approach

The monitoring of anthropogenic effects on the environment is a prerequisite for environmental management and sustainable decision making (Spellerberg 2005) and the integration of the EA into monitoring systems is essential for the implementation of ecosystem-based environmental management. For the development of monitoring systems suitable for assessments of entire ecosystems, scientific knowledge on ecosystem functioning, processes, and interactions as well as on standardized methods for adequate data collection, analysis and integration is needed as a foundation (Biermann 2002). An overview of studies related to the EA available in the scientific literature up to the year 2012 presented in Fig. 1.2 (for details on review methods see figure legend) indicates a strong temporal development in this field, but also a clear differentiation between studies concerning ecosystem type, continent, purpose of study and the applied methods of data integration. Especially in the field of aquatic ecology there is a long historical acceptance of the importance of studying systems from a holistic perspective and not only partially (de Jonge et al. 2012). The first published ideas on the need for integrative ecosystem research date back to Patrick (1949), who investigated zooplankton, macroinvertebrates and fishes in streams and concluded that the best type of biological measure is one that is based on all groups of plants and animals living in a stream. Following the establishment of a more holistic view of ecosystems in environmental legislation, the number of scientific studies considering multiple biological groups is continuously increasing in all ecosystem types (Fig. 1.2) and the EA has also been integrated in some protocols for management monitoring (e.g. in European, US and South African water quality monitoring, Table 1.2). However, in consideration of the large body of scientific literature available on environmental monitoring and impact assessment in general (e.g. reviewed by Lindenmayer & Likens 2010, 5,500 studies between 1985 and 2009), 82 case studies on multiple assemblages since 1949 that resulted from the literature review presented herein (Fig. 1.2, Appendix 7.2) are still a low number compared to the numerous studies on single species or taxonomic groups.

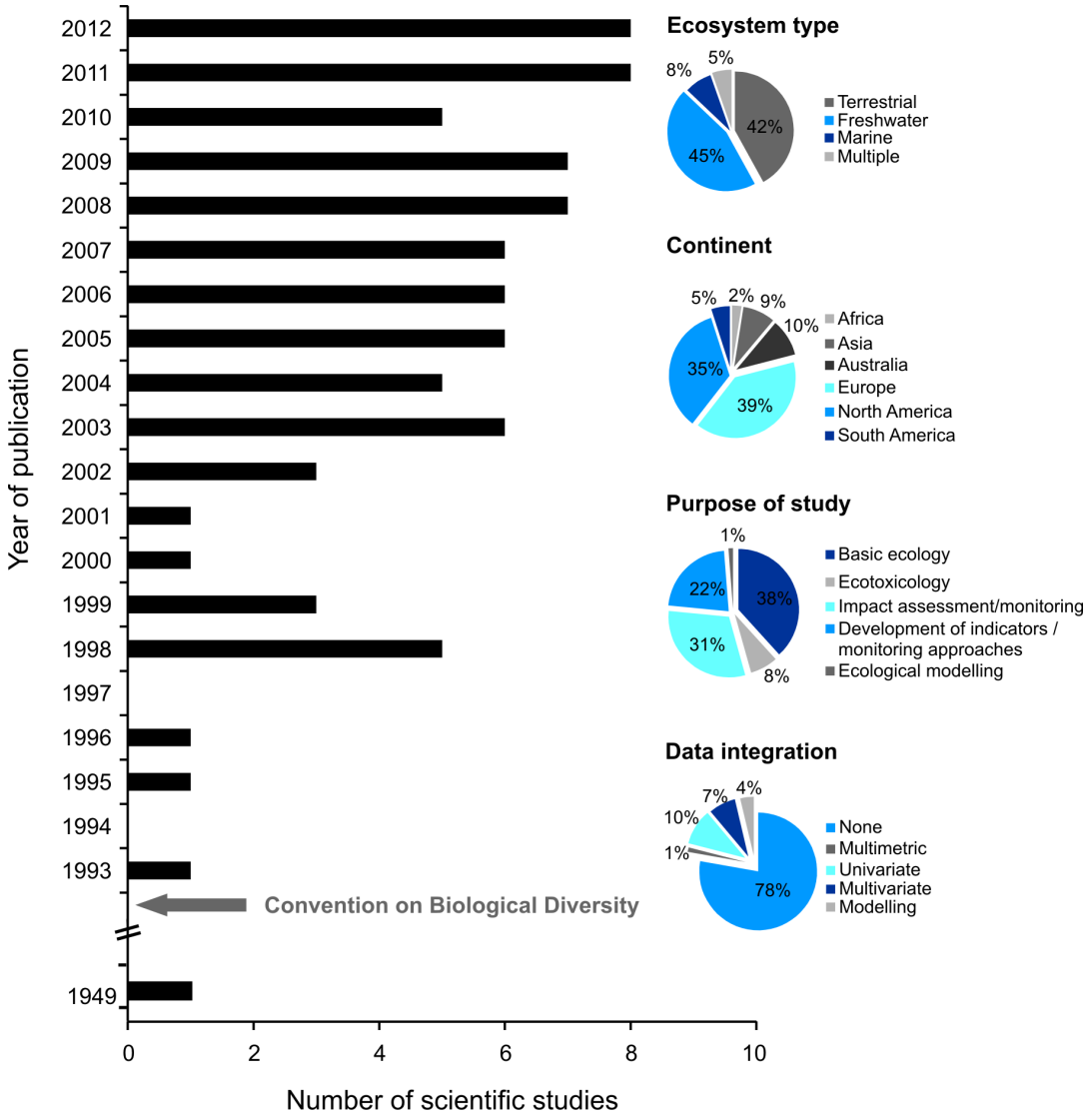

Figure 1.2: Literature review of ecological studies including multiple taxonomic groups. The review was carried out in the search engines "Google Scholar" and "ISI Web of Knowledge" in May 2013, using the following search terms: "monitoring different taxonomic groups", "monitoring multiple taxonomic groups", "monitoring several taxonomic groups"; "effects different taxonomic groups", "effects multiple taxonomic groups", "effects several taxonomic groups". The first 200 results for each search term per search engine were analyzed. Review papers that analyzed already published data were excluded to avoid dublication. If several publications from the same authors on the same data sets were found, only the first publication was considered. Studies that were not peer-reviewed or not published in international journals were not included. The search resulted in a total number of 82 studies that were considered for the analyses. A complete list of these studies is supplied in Appendix 7.2

Existing monitoring and scientific investigation of multiple taxonomic groups is mainly carried out in freshwater and terrestrial ecosystems in Europe and North America, while there are only few implementations in less developed parts of the world (Fig. 1.2, Table 1.2). Mostly up to four taxonomic groups from two or three trophic levels are considered, mainly comprising primary production, primary consumption and higher trophic levels. In contrast, micro- and meiobiota (e.g. bacteria, archaea, fungi, protozoa) are widely ignored in ecosystem based studies and monitoring protocols (Thompson et al. 2012, see also Fig. 1.2 and Table 1.2), though they play a central role in ecosystem functioning, being the interlinkage between trophic levels (Townsend et al. 2009). While standardized data collection methods are available for most disciplines (e.g. electrofishing: freshwater fish, Braun-Blanquet (1932) plant coverage estimation method: terrestrial vegetation, pitfall traps: terrestrial vertebrates), there are no standards used for the study design (i.e. selection of included ecosystem components) and the further proceeding of complex ecosystem level data sets (Dabrowski et al. 2011). Especially, the integration of data from different ecosystem components, which would provide an important overall picture of an ecosystem's condition for management, is so far not supported by effective and universally applicable standards. Consequently, data integration is rarely practiced as well in scientific studies as in applied monitoring throughout freshwater, marine and terrestrial habitats (Fig. 1.2, Table 1.2). Instead, data is analyzed and presented separately for each taxonomic group using a range of different methods, including descriptive methods, univariate statistics, modeling approaches and multivariate statistics. In the few cases where an overall consideration of the results is presented this is mostly realized by the calculation of single score indices, which are analyzed applying univariate statistics (e.g. Crosswhite et al. 1999, Maes & Dyck 2005, Lougheed et al. 2007, Schouten et al. 2009). Data integration approaches that conserve the multiple dimensions of ecosystem level data are applied only in very few studies and the methodologies used strongly differ in the degree of data simplification and the statistics used. For instance, Kruk et al. (2009) calculated the species richness of all the assemblages studied and correlated them with multivariate ReDundancy Analysis (RDA) plots of environmental variables. Thompson & Townsend (2000) applied Non-metric Multidimensional Scaling (NMDS) on multimetric indices derived from different taxonomic groups and Guerra-García et al. (2006) as well as Martínez-Crego et al. (2010) used ordination methods (Canonical Correspondence Analysis, CCA; NMDS) for full resolution abundance data combining algae and marine macroinvertebrates that were sampled with the same method.

Table 1.2: Examples of environmental monitoring systems. REL = respective environmental law (abbreviations from Table 1.1). ES Type = Ecosystem type: T = terrestrial; FW = freshwater; M = marine. Monitoring comp. = Monitoring components: SE = socio-economic factors; PP = primary producers; PC = primary consumers; SC = secondary consumers and higher trophic levels; D = destruents; EV = environmental variables. TG = Number of taxonomic groups. AT = Analysis Type: UV = univariate statistics; MV = multivariate statistics; SA = statistics in general; MM = multimetric; d = descriptive. DI = method of data integration: SSI = single score index; SRP = sunray plots. RF = type of reference: RC = reference condition; BACI = before after control impact design; nsp. = not specified

Continent	PU	Monitoring Protocol	REL	ES Type			Monitoring comp.						TG	AT	DI	RF
				T	FW	M	SE	PP	PC	SC	D	EV				
Europe	GER	Natura 2000 Monitoring (Sachtleben 2010)	HD	x	x	x		x	x	x		x	6	d	–	nsp.
Europe	GER	PHYLIB (Schaumburg et al. 2007), PERLODES (Meier et al. 2006), FiBS (Diekmann et al. 2005)	WFD		x			x	x	x		x	4	MM	SSI	RC
North America	USA	Rapid Bioassessment Protocols (Barbour et al. 1999)	CWA		x			x	x	x		x	4	MM	SRP	RC
North America	CDN	Marine and Estuarine Biodiversity Monitoring Protocols (Environment Canada 1997)	EPA			x		x	x	x			5	UV, MV	–	BACI
North America	CDN	Field Manual Wadable Streams (Environment Canada 2012)	EPA		x				x	x		x	1	nsp.	–	RC
North America	CDN	Framework for Monitoring Biodiversity Change (Roberts-Pichette 1995)	EPA	x				x	x	x	x	x	7	MM, MV	–	nsp.

Continent	PU	Monitoring Protocol	REL	ES Type			Monitoring comp.						TG	AT	DI	RF
				T	F	M	SE	PP	PC	SC	D	EV				
Australia	AUS	Methods for Ecological Monitoring of Coral Reefs (Hills & Wilkinson 2004)	AUS-EPBCA, WA			x		x	x	x		x	3	SA	–	BACI
Australia	AUS	AUSRIVAS (Nichols et al. 2000)	EPBCA, AUS-WA		x				x	x		x	1	MV	–	RC
Africa	ZA	River Health Programme (Dallas et al. 2008)	ZA-WA		x			x	x	x		x	4	MM, MV	SCI	RC
Africa	NAM, ZA	BIOTA Africa (Jürgens et al. 2012)	ECA, EMA	x			x	x	x	x	x	x	7	SA	–	nsp.
Asia	KOR	Aquatic Ecological Monitoring Program (Lee et al. 2011)	WQECA		x			x	x	x		x	3	MM	–	RC
Asia	IND	CLEAN India (http://www.cleanindia.org)	BDA	x	x		x	x	x	x		x	2	d	–	nsp.
South America	AR	Monitoreo Ambiental Rural (Zaccagnini et al. 2007)	LGA, RGA	x	x		x	x	x	x		x	10	nsp.	nsp.	nsp.

The development of standards for data integration so far plays a minor role, since the main focus of a large percentage of the scientific literature on ecosystem scale assessments is still to answer basic ecological questions, such as congruency and interactions between taxonomic groups (e.g. Padial et al. 2012, Larsen et al. 2012, Ficetola et al. 2007, Mykrä et al. 2008, Allen et al. 1999), biodiversity of certain systems (e.g. Bailey et al. 2007, Fabricius et al. 2003, Niemelä & Baur 1998) and community response to environmental gradients (e.g. Johnson & Hering 2009, Stendera & Johnson 2006). Some others are still aiming at the selection of single indicators for monitoring approaches (e.g. Roth & Weber 2008, Blasi et al. 2010, Maes & Dyck 2005) and consequently also do not consider data integration. According to the low consideration of data integration in ecological sciences, the simplification of the complex data to a single score multimetric index is currently the only method practiced in applied monitoring (e.g. European WFD Monitoring, South African River Health Programme, Table 1.2). Most monitoring protocols leave data analysis up to the user, resulting in scarce statistical approval of the results (less than half of the monitoring protocols reviewed herein, Table 1.2) and low comparability between studies. Monitoring approaches in general are highly specific to certain geographic regions, since they strongly rely on reference conditions and the occurence of certain indicator taxa for index calculation. This also limits the transferability of monitoring approaches between ecosystem types and geographic regions and the comparability of their results. However, an examination across ecosystems and geographic regions would be essential for the sustainable management of biodiversity at the global level.

1.4 Limitations of current monitoring techniques for the implementation of the ecosystem approach

The majority of the studies reviewed herein that judge the value of single indicators (88%) clearly support a “shopping basket approach” to conservation, which proposes the use of a suite of taxa instead of single indicators (Pullin 2002). However, the systematic integration of organisms from all trophic levels is rarely considered in current monitoring systems due to the high workload involved, though it seems to be most promising for strategic assessment of the status of biodiversity and successful ecosystem management. Despite several attempts to incorporate the EA into environmental legislation and monitoring systems and after several decades of ecosystem research having passed since the implementation of the CBD there are still problems of scientific knowlegde gaps.

These are e.g. lacks in standardized methodology and insufficient information on the biodiversity of many ecosystems (Novacek & Cleland 2001, Pereira & Cooper 2006). Therefore, measuring changes in ecosystems and their communities (e.g. following disturbance) remains a great challenge in ecological sciences and up to now science and management in this context still heavily relies on single indicator values or computation of simple diversity indices. These limitations probably arise from the complex nature of ecosystems, being interconnected, highly dynamic in space and time and constantly changing. Comprehensive ecosystem studies so far have been highly time and cost consuming even at the lowest possible scale (e.g. a specific river without considering the floodplain or socioeconomic factors) and generally have a reduced feasability due to enhanced requirements of skills, increased stakeholder involvement and a high probability of restrictions (Pander & Geist 2013). Moreover, the dynamic nature of ecosystems brings along the challenges of applying the appropriate scale for the purpose of the investigation and defining reference conditions. The output of comprehensive ecosystem research following the current discipline-specific approaches is often a complex pattern of results, especially if the reaction of different ecosystem components to the investigated stressor is very inhomogenous. As a consequence, it is currently not possible to make ecosystem based management decisions without being hampered by oversimplification of the results through the combination of opposite trends in single score indices in monitoring programs (Caroni et al. 2013) or by a complex presentation of multiple components in science which is hardly accessible for non-scientists, and by lacking estimations of uncertainty. The recent multimetric and compound index based approaches to data integration strongly reduce information content of the data. For this reason they are prone to loose important information on functional ecosystem processes, e.g. on changes in productivity, and run the risk of cancelling out underlying trends when the component indicators change in different directions (Millennium Ecosystem Assessment 2005). Since the importance of including adequate numbers of spatial and temporal replicates in environmental monitoring is rarely considered (Pander & Geist 2013), a statistical plausability backup of the results is mostly impossible. Dahm et al. (2013) recently concluded from an analysis of the effects of different stressors using Water Framework Directive monitoring data that the multimetric system can only afford a rough characterization of the ecological status at a geographically broad scale. An integrative and sensitive analysis of local or regional stressors is not possible with the WFD assessment system. To transform the results from monitoring and ecosystem science into effective management, a more integrative, holistic and statistically appropriate way to analyse data from

multiple assemblages is necessary. In this context, there is a strong need to find the optimal balance between effort, level of detail of the results and accessibility for environmental managers.

The major remaining challenges for a successful implementation of the EA seem to be the development of more time and cost effective but still accurate methods for data collection of all ecosystem components, including the trophic level of destruents (especially bacteria, archae and fungi) and a conceptual standard for the selection of ecosystem components and the analysis of the resulting data that is applicable across ecosystems. In this context, the establishment of a methodological framework for coupling and integratively analysing data from diverse fields that eliminates the shortcomings of the recent compound index approach is crucial (Lindenmayer & Likens 2010, de Jonge et al. 2012).

In conclusion, practical implementation of the EA still requires extensive research on possible aspects and methods of standardization to ensure a high transferability of ecosystem monitoring systems from data collection (e.g. investigation scale) to analysis and presentation (i.e. data integration). For this research it is most reasonable to start with single ecosystems and develop standardized methods that can be extended to other systems and scales in a further step.

Freshwater ecosystems represent one of the most important reservoris of biodiversity (Balian et al. 2008), constitute the most essential resource for human existance (Gleick 1993) and at the same time have up to five times higher extinction rates than any terrestrial or marine biome (Pander & Geist 2013), making biodiversity research an especially important topic in these systems (Geist 2011). Despite increasing scientific evidence on the variable responses of different taxonomic groups to environmental stressors, biodiversity monitoring in freshwaters to date still heavily relies on single indicators.

1.5 Objectives

With the results of this PhD thesis methodological gaps for the implementation of the EA in freshwaters have been closed, while aiming at a more holistic and integrative approach to freshwater ecological data analysis, i.e. the simultaneous multivariate community response analysis of multiple taxonomic groups.

First, methodological gaps concerning the standardized field sampling of benthic bacterial communities in streams were addressed, establishing the basis for the future integration of bacteria and other microbiota into ecosystem assessments. The suitability of different sampling options (direct substratum analyses, inter-

stitial water samples, and three different coupon types) for easy and rapid field assessment of bacterial communities was tested in a standardized experimental setup. A special focus was given to the capability to resolve effects of differences in stream substratum texture and the following physicochemical habitat characteristics on microbial community structure (Chapter 2.1). The two hyptheses being tested were as follows:

- Substratum texture affects important environmental variables, as well as bacterial community composition and diversity
- Direct and indirect techniques for microbial community sampling differ in their capability to recover important microbiome distinctions

Second, taxonomic sufficiency approaches that are frequently used to reduce monitoring costs in marine systems were extended to four freshwater groups (periphytic algae, macrophytes, macroinvertebrates and fishes) by identifying statistical threshold levels of minimum necessary taxonomic resolution for multivariate community analyses. Furthermore, the importance of numerical data resolution and the use of functional groupings as surrogates for species-level data were evaluated using statistical methods (Chapter 2.2). Specifically, the following hypotheses were tested:

- Taxonomic and numerical resolution of biological community data affects the outcome of multivariate analyses and diversity indices in periphyton, macrophytes, macroinvertebrates and fishes
- Classification into functional groups resolves a different outcome of ecological analyses than grouping individuals according to Linnean taxonomy and can improve the capability to detect environmental gradients

Third, technical solutions for the integration of abundance data from multiple taxonomic groups with variable data structure were developed. In this context, the main focus was on integration methods that result in a data matrix, which allows in contrast to commonly applied compound index approaches, the multivariate analyses of overall community data (Chapter 2.3). The following three hypotheses were subject to research:

- The generation of a combined species abundance data matrix from multiple taxonomic groups based on different sampling techniques and investigation scales is possible, but needs some standardization/normalization to account for differences in species numbers and numerical scale

- The combination of multiple taxonomic groups does not reduce the capability of detecting environmental gradients and differences between treatments compared to single taxonomic groups if multivariate statistics are applied
- Combining information from multiple taxonomic groups using multivariate analysis is a more sensitive tool for the monitoring of environmental changes than the calculation of single score indices integrating multiple groups

In the following chapter, these methods were validated for applied research questions in disturbance ecology and restoration ecology. In a first validation step, taxonomic sufficiency and the data integration method were used to quantify the serial discontinuity introduced into streams by weirs, which is one of the most crucial disturbance factors in fluvial ecosystems (Chapter 3.1). The hypotheses tested were as follows:

- The serial discontinuity of streams introduced by weirs and dams is detectable on all levels of biological organization
- Different taxonomic groups differ in their response to weirs concerning effects on biodiversity and community composition
- Multivariate methods that include abiotic and biotic effects are more suitable for the quantification of weir effects compared with the univariate consideration of single taxonomic groups

Since the mitigation of anthropogenic disturbance is of increasing importance in ecological sciences, the second validation step comprised the application of the new data analysis approach for a holistic evaluation of four different stream substratum restoration measures (Chapter 3.2). The following hypotheses were tested:

- The investigated substratum restoration treatments differ in their effects on target species as well as in their ecosystem effects
- There is low congruency between restoration success for target species and ecosystem scale effects

Based on the results of the method validations, finally the most essential prerequisites for the application of the ecosystem approach considering the sampling design, field sampling techniques, taxonomic identification and data analysis were discussed and synthesized into a step-by-step flowchart guiding data integration. In a last step, aspects of transferability of the guiding concept to other ecosystem types were outlined and further research perspectives were discussed (Chapter 4).

2 New methods to realize the ecosystem approach

2.1 The effects of stream substratum texture on interstitial conditions and bacterial biofilms: methodological strategies

A similar version of this section is published:
Mueller, M., Pander, J., Wild, R., Lueders, T. & Geist, J. (2013) The effects of stream substratum texture on interstitial conditions and bacterial biofilms: Methodological strategies. *Limnologica-Ecology and Management of Inland Waters* **43**(2), 106-113.

Abstract

Hyporheic substrates play a key role in aquatic ecosystems, and increasing loads of fine sediment are considered one of the major threats to stream ecosystems. Knowledge concerning the interaction of stream substratum properties with habitat quality and microbial community structure is essential for a comprehensive understanding of the functionality of the hyporheic zone. To date, there is a lack of optimal field sampling methods for hyporheic microbial communities in streams. We systematically tested the effects of defined substratum textures on the physicochemical properties of interstitial water and on bacterial communities utilizing T-RFLP fingerprinting. We also tested the representativeness of different methodological approaches of investigating bacterial diversity comparing sampling of substratum, interstitial water, and exposed coupons made of granite, carbonate and glass. The temporal development of physicochemical habitat characteristics in the interstitial zone, especially of fish-toxic nitrogen compounds and oxygen supply, significantly depended on substratum texture and was strongly correlated with bacterial community composition. Multivariate analyses of bacterial community data indicated strong differences in community composition between direct (substratum samples) and indirect (interstitial water samples, coupons) sampling strategies. Substratum samples yielded highest richness of Operational Taxonomic Units (OTUs) and the most pronounced temporal dynamics of bacterial community composition. Consequently, this technique appears most representative for assessing bacterial community structure and diversity in hyporheic habitats. The observed couplings between substratum texture,

physicochemical habitat conditions and bacterial community structure expand current knowledge of previously described negative effects of fine sediments on taxa from higher levels of biological organisation.

Introduction

River bed substratum provides a key habitat for many species in stream ecosystems, including e.g. rheophilic fishes, macroinvertebrates and benthic algae (Boulton et al. 1998, Bretschko 1995, Denic & Geist 2010, Müllner & Schagerl 2003, Österling et al. 2010). Besides these organisms, which have been in the focus of limnologists for decades (e.g. Bretschko 1981, Grossman et al. 1987, Malcolm et al. 2003, Schwoerbel 1964), microbes are important components for the functionality of hyporheic habitats, but links between micro- and macrobiology are only rarely established. Microbial communities in streams play a key role for the turnover of organic matter and pollutants as well as for aquatic food webs (Fischer et al. 1996, Findlay 2010), mostly in the form of attached biofilms. Biofilm structure and metabolic activity are known to control the matter fluxes and hydrochemical conditions within the hyporheic zone (Hancock 2002). These can in turn affect organisms of higher levels of biological organization, such as eukaryotic biofilms, macrophytes, macroinvertebrates and fishes.

Due to changes in landuse and flow regulation measures, stream substratum quality has been altered dramatically over the last centuries (Hancock 2002, Geist & Auerswald 2007). This includes changes in substratum composition (texture and porosity) and consequently exchange rates between free-flowing water and the hyporheic zone. The resulting changes in physicochemical habitat characteristics and community composition of organisms on higher trophic levels have already been studied intensively. In particular, siltation and increased loads of fine sediments were shown to negatively affect the reproduction of many stream-dwelling species, e.g. including salmonid fishes (Soulsby et al. 2001), freshwater mussles (Geist & Auerswald 2007, Österling et al. 2010), and insect larvae (Richards & Bacon 1994).

In contrast to the direct consequences of siltation for these species, effects of stream bed alterations on hyporheic microbial communities are still rarely studied in stream ecology (Febria et al. 2010). Today, molecular ecology methods such as Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis are routinely applied for characterising microbial community composition in environmental systems (reviewed in Schütte et al. 2008). Strategies for representative sampling of microbial communities in stream substratum are less clearly defined.

Hence, methods for standardized and optimal field sampling of hyporheic microbial communities are key requirements for holistic ecosystem analysis. Due to the high temporal and spatial variability in stream ecosystems (Pringle et al. 1988, Ward & Tockner 2001, Winemiller et al. 2010), an ideal sampling strategy needs to consider the option to carry out multiple samplings (i.e. different levels of biological organization and abiotic habitat factors) at the same time and in the same spot.

In this study, we systematically compared alternative direct and indirect sampling strategies for interstitial conditions and bacterial community composition on different substratum textures (coarse vs. fine). We hypothesize that substratum texture affects important environmental variables (dissolved oxygen, redox potential, nitrate, nitrite, ammonium, pH, electric conductance), and bacterial community composition and diversity. Thus, several direct and indirect techniques for microbial community sampling are compared in their capability to recover important microbiome distinctions.

Material and Methods

Sampling design

To evaluate the suitability of different bacterial biofilm sampling techniques and to detect effects of substratum texture on bacterial community and interstitial conditions, a standardized experiment was set up in an artificial flow channel (25 cm water depth, 0 - 0.06 m s^{-1} flow velocity (measured 2 cm above substratum surface with a Flow Measuring Instrument HFA, Höntzsch, Waiblingen, Germany), 2.7 - 15.2°C, water supplied by the river Moosach) at the Aquatic Systems Biology Unit (Technische Universität München, Germany). In order to simulate authentic geochemical conditions of natural rivers, defined fractions of washed and dried substratum from the river Günz (a calcareous river in Germany, for location details see Pander & Geist 2010*b*) was used (fine: < 0.85 mm, coarse: 6.3 - 20 mm). Six replicates of fine substratum (6.4 ± 0.1 kg dry weight each) as well as six replicates of coarse substratum (7.9 ± 0.2 kg dry weight each) were filled into open plastic boxes (30 cm × 17 cm × 10 cm, ROTHO clear boxes, ROTHO Kunstoff AG, Würenlingen, Switzerland) and exposed to surface water by placing the boxes on the bottom of the artificial flow channel. Three methods to sample bacterial communities in the hyporheic zone of the substratum-filled boxes were compared: substratum samples, interstitial water samples and exposed artificial substratum surfaces (coupons). For the latter, carbonate slides (lithographic limestone), granite

slides (75 mm × 25 mm × 5 mm) and smooth standard glass slides of equal size (Menzel Gläser, Braunschweig, Germany) were exposed vertically in each of the 12 plastic boxes. All artificial substrates were placed into the plastic boxes with the top edge of each slide being 2.5 cm below the substratum surface.

Physicochemical variables

First measurements of physicochemical variables were taken at the start of exposure and they continued in a weekly cycle throughout the duration of the experiment (9 weeks). Redox potential was measured in each box in 5 cm depth as well as in the free flowing water according to Geist & Auerswald (2007). Interstitial water samples for further physicochemical measurements and for DNA extraction were collected from the same depth as the coupon exposure following the method described in Geist & Auerswald (2007). pH, temperature, electric conductance and dissolved oxygen were measured with WTW handheld pH315i and Multi340i (Wissenschaftlich-Technische Werkstätten (WTW), Weilheim, Germany). Nitrate, nitrite and ammonium were analyzed using Spectroquant® Test-Kits (Merck KGaA, Darmstadt, Germany) and concentrations were determined photometrically with Photolab S12 (WTW, Weilheim, Germany) immediately after sampling.

Bacterial community analysis

Bacterial community analysis was carried out by DNA-based T-RFLP fingerprinting two, five and nine weeks after start of exposure. Total DNA was isolated in biological triplicates from the substratum, from interstitial water samples and from exposed artificial substratum of each replicate box. For each sampling method, one biological replicate was split into three technical replicates which were analyzed separately. Since these tests revealed exactly the same composition of operational taxonomic units and extremely low variation in terms of relative abundances, subsequently only biological replicates were considered. Samples of each substratum type as well as each of the coupon types were analyzed prior to contact with water as a zero control. Bacteria from free-flowing water were also analyzed to compare bacterial diversity to that of the hyporheic zone. To obtain material from the substratum for direct DNA extraction, 50–100 g of substratum was collected from the same depth as the coupon exposure in each box using a 50 mL falcon tube. Samples were then instantly frozen at -20°C. For water sampling, 50 mL of interstitial water as well as reference samples from the

free-flowing water were filtered through 0.22 μm CME membrane filters (Carl Roth GmbH & Co. KG, Karlsruhe, Germany). For sampling of biomass from the artificial coupons, the front and backside of each slide was brushed twice with a toothbrush while continuously flushing with autoclaved water. A new sterilized toothbrush was used for each slide to avoid contamination. The resulting suspension was then filtered through 0.22 μm CME membrane filters. All filters were transferred to sterile Petri dishes and frozen immediately. The first samples were taken after two weeks of exposure from three replicates of each substratum type. Five weeks after exposure, additional substratum and interstitial water samples were taken. After nine weeks the artificial substrata, interstitial water samples and substratum samples in the three remaining replicates of each substratum type were collected. The isolation of bacterial DNA differed for filters and substratum for practical handling reasons. Filters with adherent biomass from interstitial water and artificial substratum were cut into small pieces (0.5 cm^2) and transferred into 2 ml bead-beating cups. Biomass from the fine grain size ($\leq$ 0.85 mm) was directly added to the lysis buffer (0.7 g of substratum) in bead beating cups. Bead beating is a cell lysis step, in which cells are disrupted and DNA is dissolved in the lysis buffer. Further DNA extraction followed the protocol described in Lueders et al. (2004). For the coarse grain size, this protocol had to be upscaled. Approximately 15 g of substratum (up to $\varnothing$ 2.5 cm) was transferred into aseptic stainless steel cups (Retsch, Haan, Germany) containing 900 µL $NaPO_4$ buffer (112.87 mM Na_2HPO_4, 7.12 mM NaH_2PO_4; pH 8), and approximately 0.35 g of 0.1 mm zirconia/silica beads (Roth, Karlsruhe, Germany). Samples were shaken in a mixer mill (MM 200, Retsch, Haan, Germany) for 2 min at 20 Hz. Afterwards, the resulting cell lysis suspension was transferred to 50 mL Falcon tubes. Steel cup walls were washed with additional 100 μL $NaPO_4$ buffer, which were then added to the Falcon tubes. Samples were centrifuged at 4,000 $\times$ g for 5 min and the collected lysate was then transferred to 2 mL Eppendorf tubes for subsequent organic extraction and DNA precipitation as described in Lueders et al. (2004). After extraction and precipitation, DNA pellets were resuspended in 15 – 50 μL EB buffer (10 mM Tris-HCl pH 8.5, Qiagen). DNA concentration was quantified by photometric measurement (ND-1,000 Nanodrop Spectrophotometer, Peqlab, Erlangen, Germany) and samples were frozen at -20°C until further analysis. T-RFLP analysis of bacterial 16S rRNA gene amplicons was performed as described in detail in Pilloni et al. (2011). Briefly, FAM-labeled amplicons were amplified using the primers Ba27f (5'FAM-aga gtt tga tcm tgg ctc ag-3') and 907r (5'-ccg tca att cct ttg agt tt-3') in a Mastercycler ep gradient (Eppendorf, Hamburg, Germany) with the following thermal profile: 5 min of initial denaturation at

94°C; 28 cycles of 30 s denaturation (94°C), 30 s annealing (52°C), 60 s elongation (70°C); and a final extension of 5 min at 70°C. The 50 µL PCR reactions contained 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 1.25 U Taq polymerase (Fermentas, St. Leon-Rot, Germany), 0.2 $\mu g\,\mu L^{-1}$ BSA (Roche, Penzberg, Germany), 0.5 µM of each primer (Biomers, Ulm, Germany), and 1 μL of template DNA. Amplicons were restricted using MspI and resolved by capillary electrophoresis on a 3730 DNA analyzer (Applied Biosystems, Carlsbad, CA, USA) according to Lueders et al. (2006). Electropherograms were analyzed using GeneMapper 4.0 software (Applied Biosystems, Carlsbad, CA, USA) as reported in Winderl et al. (2008).

Data analysis

Fingerprinting data was analyzed with the T-REX online T-RF analysis software (Culman et al. 2009). Background noise filtering (Abdo et al. 2006) was on default factor 1.2 and the clustering threshold for aligning peaks across the samples was set to 1.5 using the default alignment method of Smith et al. (2005). Relative T-RF abundance was inferred from peak heights. For reduction of data complexity, T-RFs that occurred in less than 10% of the samples were excluded from further analysis. From comprehensive aligned peak data, operational taxonomic unit (OTU) richness, Shannon Index and Evenness were computed for each sample using PRIMER v.6 (Plymouth Marine Laboratory, Plymouth, United Kingdom).

Detrended correspondence analysis (DCA) was performed using the R-package vegan (Oksanen et al. 2009) to visualize differences between samples in microbial community composition. For comparison of all investigated sampling techniques, DCA was performed for all samples in a first step. In order to account for differences between fine and coarse substratum and sampling times, a separate DCA was performed for each sampling technique. To establish a link between physicochemical variables and microbial community composition, the mean values of all measured variables at the respective sampling time points were considered for the environmental fitting on the DCA ordination plots. Environmental fitting was performed with 1,000 permutations. Only environmental variables with significant correlation with the DCA were considered as ordination plot vectors. Analysis of Similarity (ANOSIM) in PRIMER v6 was used to determine significant differences in the microbial community of the different texture types, sampling techniques and sampling time points.

Differences between coarse and fine substratum in physicochemical variables as well as differences in diversity and richness between treatments were analyzed using standard univariate statistics in R (http://www.r-project.org). Normality of

data was tested with the Shapiro-Wilk-Test and the homogeneity of variances was tested with the Levene-Test. Since all data were not normally distributed, non-parametric tests were applied. The Mann-Whitney *U*-Test (Bonferroni corrected) was used for pairwise comparisons between treatments, sampling time-points and sampling methods. Significance was accepted at $P \leq 0.05$, given as Bonferroni corrected values in the Results section.

Results

Effects of substratum texture on physicochemical habitat development

Physicochemical properties of dissolved oxygen, electric conductance, pH, redox potential and nitrite strongly differed between coarse and fine substratum throughout exposure time (Fig. 2.1). For dissolved oxygen, electric conductance and ammonium the differentiation between coarse and fine substratum increased with time (Fig. 2.1). Redox potential and nitrate concentrations were similar between coarse and fine substratum at the beginning and at the end of the experiment, but strongly differed between both substrate types from week two to week eight (redox potential) and from week one to week four (nitrate). In the coarse substratum, oxygen concentrations and redox potential was two-fold higher ($P < 0.001$), whereas ammonium and nitrite concentrations were significantly lower than in the fine substratum ($P < 0.001$; averaged across the duration of the experiment). Electric conductance was significantly different between coarse and fine substratum exposures ($P < 0.001$). Values were lower in coarse substratum and remained constant over exposure time ($780 \pm 24\,\mu\mathrm{Scm}^{-1}$). In fine substratum electric conductance averaged $1556\,\mu\mathrm{Scm}^{-1}$ and the standard deviation was more than 10-fold higher ($\mathrm{SD} = 293\,\mu\mathrm{Scm}^{-1}$) than in coarse substratum. No differences in water temperature between fine and coarse substratum were detectable ($9.7 \pm 4.1^{\circ}\mathrm{C}$ in coarse substratum, $9.7 \pm 4.2^{\circ}\mathrm{C}$ in fine substratum, $P > 0.05$). The rising temperature along the experiment was related to seasonal variation between February and April. Oxygen concentration and redox potential strongly decreased over time, with effects being most pronounced in fine substratum. Consequently, nitrate and ammonium both exhibited strong temporal variations over the experiment. Nitrate concentrations generally decreased for both grain sizes during the experiment. Ammonia concentrations were low and showed only moderate fluctuations in coarse substratum ($0.46 \pm 0.42\,\mathrm{mg\,L}^{-1}$), while ammonia in fine substratum increased by a factor of 8 from 0.5 to $4.0\,\mathrm{mg\,L}^{-1}$ after week 5.

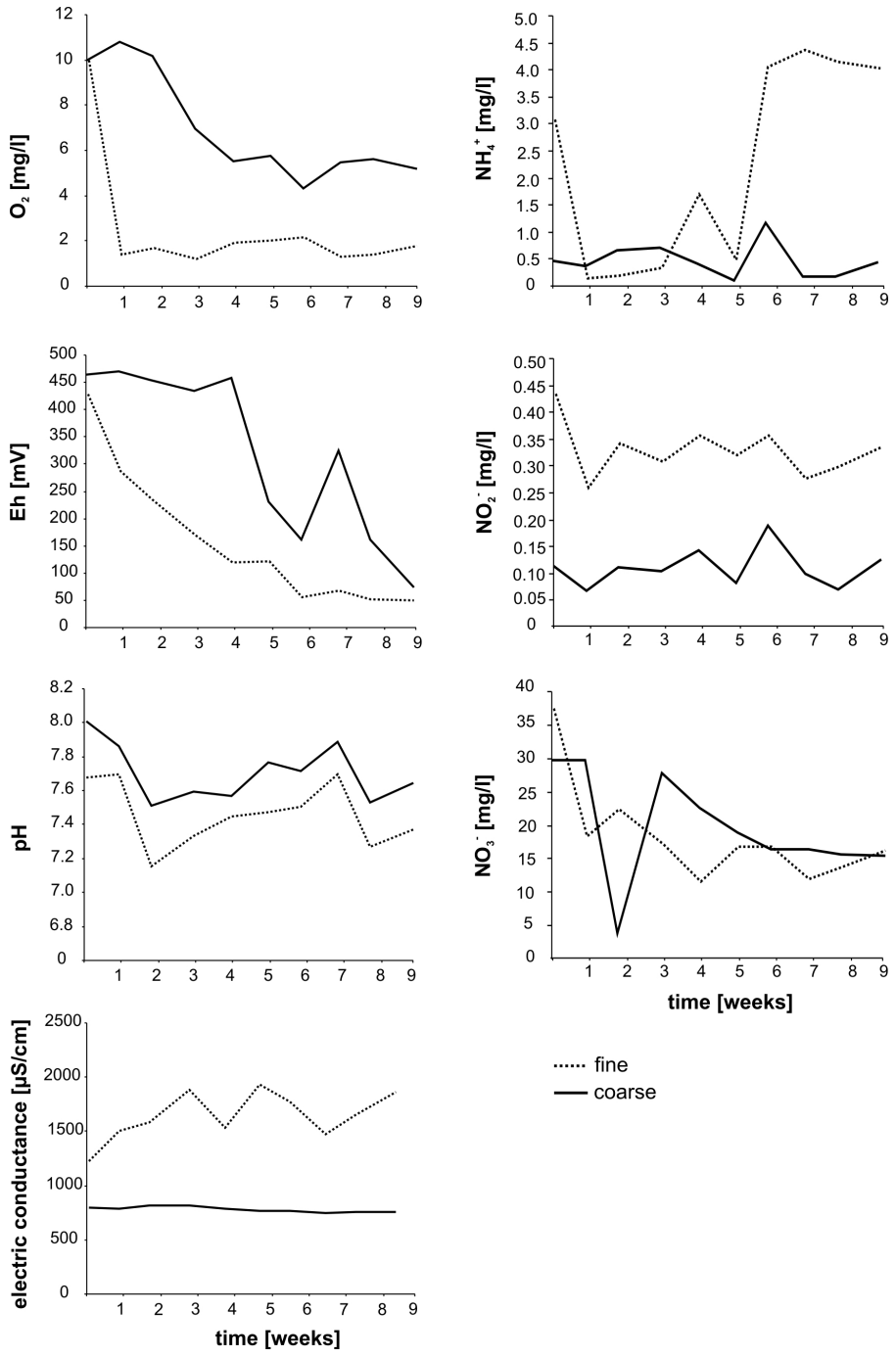

Figure 2.1: Time course of interstitial concentrations of NO_3^- = nitrate; NO_2^- = nitrite; NH_4^+ = ammonium; O_2 = dissolved oxygen; and Eh = redox potential; in coarse (continuous line) and fine (dashed line) substratum during exposure. Displayed values are arithmetric means of six replicates per substratum type and time point. All concentrations are in mg L^{-1}

Effects of substratum texture on bacterial communities

Analogous to the physicochemical habitat differentiation between coarse and fine substratum, pronounced differences in bacterial community structures in the different grain sizes ($P < 0.001$) and at different time points ($P < 0.05$) were evident (Fig. 2.2). The correlation of the DCA ordination plots with the investigated physicochemical variables reflects the pronounced differences in interstitial concentrations of dissolved oxygen and nitrite between coarse and fine substratum. Redox potential, electric conductance, nitrate and pH were correlated with substratum type and the temporal effects. However, ammonium and temperature were only correlated with temporal differences in OTU composition. In terms of bacterial diversity, no significant differences in taxonomic richness and Shannon Index were found between coarse and fine substratum (Tables 2.1 and 2.2). However, Evenness was significantly higher in the coarse substratum than in the fine (coarse, 0.9 ± 0.1,; fine, 0.8 ± 0.1, $P < 0.01$). Averaged over all treatments, OTU richness, diversity and Evenness increased from the 2^{nd} week of incubation (O, 33.8 ± 13.4; H, 2.8 ± 0.5; J, 0.8 ± 0.1) to the 5^{th} week (O, 58.0 ± 4.6, $P < 0.001$; H, 3.3 ± 0.3, $P < 0.01$; J, 0.8 ± 0.1, $P > 0.05$), and decreased again towards week 9 at the end of the experiment (O, 41.4 ± 8.8, $P < 0.001$; H, 3.2 ± 0.3, $P > 0.05$). Only Evenness continuously increased throughout the experiment (0.8 ± 0.1, $P > 0.05$).

Representativeness of different microbial sampling techniques

Comparing direct and indirect extractions, all three tested methods were suitable to yield sufficient amounts of DNA for T-RFLP analysis. However, the amount of DNA extracted from fine substratum was by far the highest (81–233 $\mu g\, g^{-1}$). DNA yield from filtered interstitial water (0.15–9.65 $\mu g\, g^{-1}$) and coarse substratum (0.24–3.04 $\mu g\, g^{-1}$) was very variable and considerably lower than from finer substrata. The amount of DNA extractable from coupon biofilms was generally very low (0.0003–0.06 $\mu g\, cm^{-2}$), especially for the shortest exposure time, as well as for samples derived from filtering free-flowing water. The highest richness of operational taxonomic units (OTUs) could be gained by direct extraction from substratum samples (48.5 ± 11.8; Tables 2.1 and 2.2). Substratum OTUs were significantly richer than those from carbonate slides (28.9 ± 12.6) ($P < 0.001$) and granite slides (37.7 ± 9.6) ($P < 0.01$). The number of OTUs found in interstitial samples (42.9 ± 13.8) was the second highest, closely followed by the glass slides (40.7 ± 11.1) and also differed from richness of carbonate slides ($P < 0.01$). Diversity and Evenness were highest for substratum samples (H, 3.3 ± 0.3; J, 0.9 ± 0.1)

Table 2.1: Diversity of the bacterial community from fine substratum of all treatments. G = glass slide; Gr = granite slide; C = carbonate slide; S = substratum samples; I = interstitial water samples. O = OTU-richness; H = Shannon Index; J = Evenness. Time = time of exposure in weeks. Mean values are given with ± standard deviation

Treatment	Time	O	H	J
G	2	46 ± 11	3.0 ± 0.3	0.78 ± 0.04
	8	44 ± 13	3.4 ± 0.3	0.91 ± 0.01
Gr	2	38 ± 11	2.9 ± 0.3	0.79 ± 0.05
	8	42 ± 9	3.2 ± 0.4	0.87 ± 0.08
C	2	14 ± 5	1.8 ± 0.1	0.73 ± 0.09
	8	45 ± 4	3.3 ± 0.1	0.86 ± 0.02
S	2	50 ± 4	3.1 ± 0.1	0.80 ± 0.01
	5	62 ± 6	3.5 ± 0.5	0.81 ± 0.11
	8	48 ± 5	3.6 ± 0.1	0.93 ± 0.01
I	2	33 ± 2	2.3 ± 0.0	0.67 ± 0.02
	5	58 ± 5	3.0 ± 0.1	0.73 ± 0.01
	8	33 ± 13	2.5 ± 0.2	0.73 ± 0.03

Table 2.2: Diversity of the bacterial community from coarse substratum of all treatments. Descriptors are the same as in Table 2.1

Treatment	Time	O	H	J
G	2	30 ± 4	2.9 ± 0.1	0.86 ± 0.02
	8	43 ± 12	3.2 ± 0.3	0.86 ± 0.02
Gr	2	26 ± 4	2.7 ± 0.1	0.82 ± 0.01
	8	45 ± 4	3.1 ± 0.1	0.81 ± 0.04
C	2	24 ± 4	2.3 ± 0.2	0.71 ± 0.05
	8	28 ± 4	2.9 ± 0.2	0.87 ± 0.01
S	2	34 ± 20	3.2 ± 0.6	0.95 ± 0.03
	5	57 ± 4	3.3 ± 0.2	0.83 ± 0.06
	8	40 ± 6	3.3 ± 0.0	0.89 ± 0.02
I	2	33 ± 17	3.1 ± 0.6	0.94 ± 0.02
	5	55 ± 2	3.6 ± 0.0	0.91 ± 0.00
	8	45 ± 5	3.4 ± 0.1	0.90 ± 0.00

and lowest for carbonate slides (H, 2.6 ± 0.6; J, 0.8 ± 0.1). Multivariate analyses of the bacterial community data indicated strong differences in community composition between direct (substratum samples) and indirect (interstitial water samples, artificial substrata) extraction methods ($P < 0.01$; Fig. 2.2). Differences between substratum samples and interstitial samples were more pronounced in fine than in coarse substratum. These differences were mainly caused by operational taxonomic units (OTUs) that were exclusively detected in substratum samples (Fig. 2.2), implying that the biofilm communities on the slides were a subset of the substratum communities. The bacterial community of the exposed artificial substrata strongly differed from the community composition in substratum and interstitial water samples ($P < 0.01$). Carbonate slides revealed a bacterial community that was significantly different from that on glass slides for both substratum types, while the bacterial community of granite slides was similar to that on carbonate and glass slides ($P > 0.05$) (Fig. 2.2). Substratum samples exhibited more pronounced temporal differences in bacterial community composition than interstitial water samples. In coarse substratum, bacterial community composition only differed between the start of the exposure (week 2) and its end (9 weeks). In fine substratum, the differentiation of bacterial communities over experimentation time was more pronounced, with a clear separation evident between two, five and nine weeks exposure time (Fig. 2.2). This matches results of the interstitial water sampling, where temporal effects were also most pronounced in the fine substratum (Fig. 2.2). Temporal effects were also detectable in each type of coupon, with the bacterial community becoming more similar to the community of the substratum samples after nine weeks (Fig. 2.2 and Table 2.3).

Discussion

The importance of the relationship between physicochemical habitat variables, microbiota, and species at higher levels of biological organization for the functionality of stream substratum habitats are widely recognized among stream ecologists, but are rarely accounted for in scientific studies (e.g. Geist 2011, Findlay 2010). Bacterial biofilms interact with habitat conditions and determine particle surface properties, porosity and available microhabitat volume (i.e. interstitial spaces), which all influence the exchange rates of the hyporheic zone with the free-flowing water (Pusch et al. 1998). In turn, these exchange rates are considered the most important factor for the ecological integrity of stream ecosystems (Geist & Auerswald 2007). Previous studies considered the effects of physicochemical properties in the hyporheic zone on microbial assemblages applying single sam-

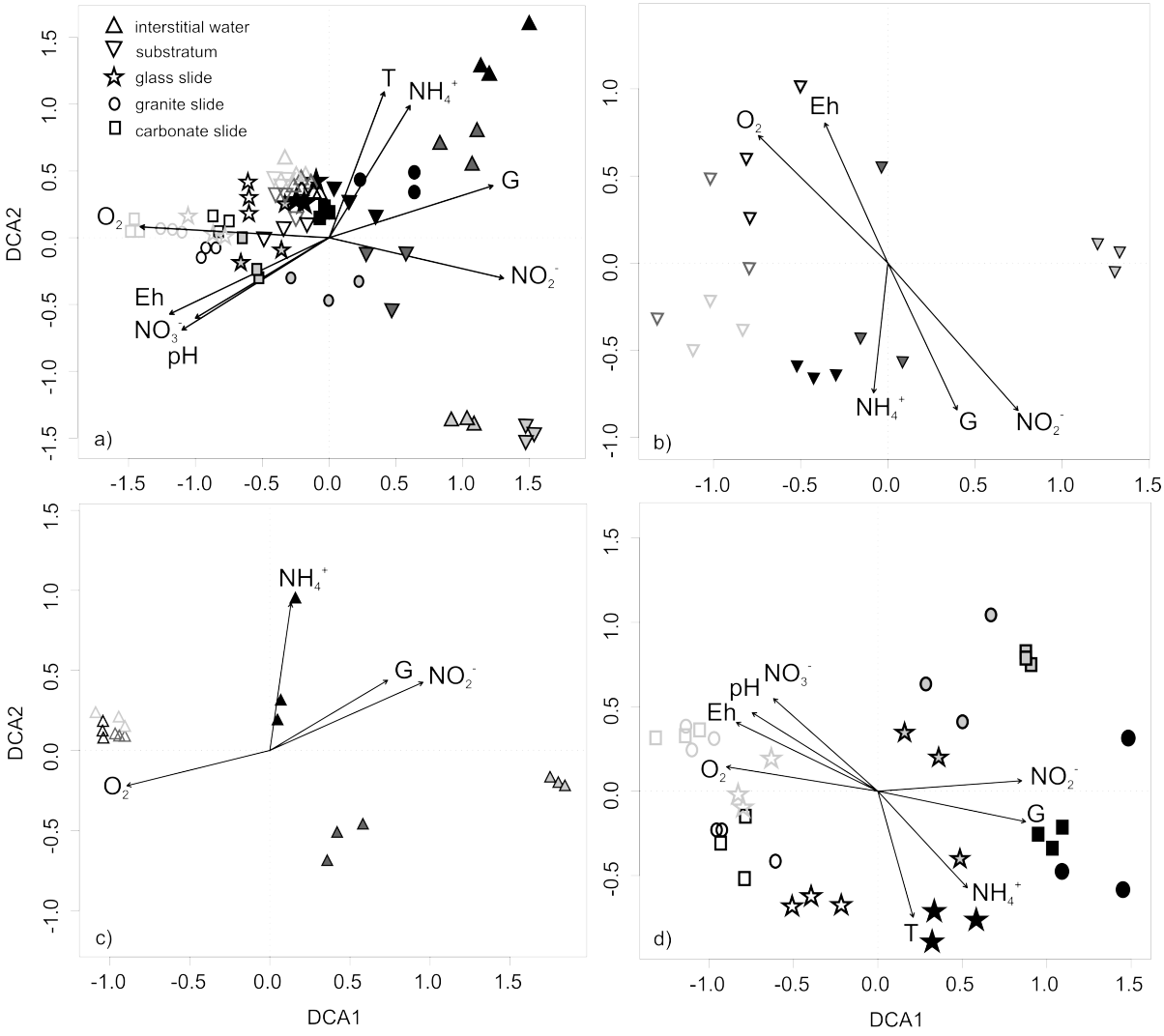

Figure 2.2: Detrended correspondence analysis (DCA) performed for T-RFLP data. Open symbols refer to samples from coarse substratum, filled symbols are from fine substratum. The three sampling time points are displayed in different shades of grey: light grey = 2 weeks; dark grey = 5 weeks; black = 9 weeks. Discriminant functions of environmental variables ($P < 0.05$ based on 1,000 permutations) are displayed as arrows. T = temperature; G = electric conductance; Eh = redox potential; NO_3^- = nitrate; NO_2^- = nitrite; and NH_4^+ = ammonium. a) T-RFLP data from all samples, percent variance accounted by the axes: DCA1 = 36%; DCA2 = 31%; b) T-RFLP data from substratum samples only, percent variance accounted by the axes: DCA1 = 55%; DCA2 = 22%; c) T-RFLP data from interstitial water samples only, percent variance accounted by the axes: DCA1 = 67%; DCA2 = 18%; d) T-RFLP data from artificial substratum only, percent variance accounted by the axes: DCA1 = 45%; DCA2 = 26%

Table 2.3: Environmental correlations on the DCA ordination plots in Fig. 2.2. Eh = redox potential; T = temperature; O_2 = dissolved oxygen; pH = pH-value; G = electric conductance; NO_3^- = nitrate; NO_2^- = nitrite; and NH_4^+ = ammonium. DCA1 and DCA2 = vector properties; r^2 = spearman rank correlation coefficients; *P*-value = level of significance. Numbers printed in bold indicate significant *P*-values

		Eh	T	O_2	pH	G	NO_3^-	NO_2^-	NH_4^+
All	DCA1	-0.90	0.35	-0.99	-0.85	0.95	-0.86	0.97	0.52
	DCA2	-0.43	0.94	0.06	-0.53	0.30	-0.52	-0.23	0.85
	r^2	0.38	0.30	0.44	0.37	0.36	0.30	0.39	0.30
	P-value	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Sediment	DCA1	-0.41	-0.99	-0.71	-0.37	0.43	-0.63	0.66	-0.11
	DCA2	0.91	0.01	0.70	0.93	-0.91	0.77	-0.75	-0.99
	r^2	0.46	0.20	0.64	0.09	0.52	0.14	0.75	0.33
	P-value	**0.00**	0.10	**0.00**	0.40	**0.00**	0.24	**0.00**	**0.01**
Interstitial	DCA1	-0.62	-0.86	-0.97	-0.99	0.86	-0.97	0.91	0.14
	DCA2	-0.78	0.52	-0.24	0.13	0.51	-0.24	0.41	0.99
	r^2	0.26	0.09	0.40	0.15	0.35	0.07	0.52	0.41
	P-value	0.09	0.44	**0.02**	0.27	**0.04**	0.51	**0.00**	**0.02**
Coupons	DCA1	-0.90	0.27	-0.99	-0.85	0.98	-0.75	0.99	0.68
	DCA2	0.44	-0.96	0.16	0.53	-0.20	0.66	0.07	-0.73
	r^2	0.88	0.61	0.83	0.78	0.81	0.69	0.74	0.61
	P-value	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

pling strategies under field conditions (e.g. effects of grain size applying direct substratum sampling: Santmire & Leff 2007, effects of flow velocity applying artificial substrata: Sliva & Williams 2005; seasonal variation using previously collected and sterilized native substratum: Febria et al. 2010). However, only few authors analyzed the relationships between bacterial biofilms and taxa from higher levels of biological organization (e.g. Kim et al. 2008). Due to the high complexity of field assessments on ecosystem level, standardized optimal sampling techniques, which are easy to apply in the field, are required to link microbial constituents of the system with the community composition of organisms from higher taxa and food webs in streams. It was the main objective of our study to provide such standardized workflow. This study demonstrated the differences in the temporal development of bacterial communities and interstitial conditions in two simultaneously exposed substratum types and compared different methodological approaches for the analysis of microbial communities in stream substratum under standardized conditions in an artificial flow channel. In addition, it expands current knowledge on the previously described negative effects of stream bed siltation in vertebrates, molluscs and insect larvae to the effects on abiotic habitat properties and microbial communities.

The effects of substratum texture on abiotic and biotic habitat characteristics

Physicochemical variables showed a strong discrimination between simultaneously exposed coarse and fine substratum at identical ambient water conditions (Fig. 2.1), indicating a powerful influence of substratum texture on interstitial habitat quality. After a period of six weeks, fine substratum displayed almost ten-fold higher concentrations of ammonium than coarse substratum. Moreover, substrate-related differences in the nitrogen compounds ammonium and nitrite (higher in fine-textured substratum), are likely to have a negative impact on survival and development of critical life stages of fishes (Williams & Eddy 1989) and freshwater molluscs (Newton et al. 2003). As with interstitial habitat conditions, multivariate analyses of bacterial community data also revealed strong differences between coarse and fine substratum. Environmental fitting indicates that dissolved oxygen, electric conductance, pH and nitrite were the major factors correlating with the composition of the bacterial community. Only in coarse and well-sorted substratum, intense exchange between the free flowing water and the hyporheic zone is possible (Geist & Auerswald 2007). This causes a generally high supply with dissolved oxygen as well as high export rates of metabolic products (e.g. nitrite or ammonium), resulting in no or small differences of pH and

electric conductance between interstitial zone and free-flowing water. Under oxic conditions, ammonium can be oxidized by nitrifying bacteria (Strauss & Lamberti 2000), explaining the low levels of ammonia in the coarse substratum throughout the experiment. In contrast to the coarse substratum, exchange with free-flowing water is heavily restricted in fine substrata, resulting in a decreased exchange of water between the interstitial and the free-flowing water (Brunke & Gonser 1997). This was confirmed by the low and decreasing concentrations of dissolved oxygen and the low redox potential measured in the fine substratum. The observed temporal shift in water chemistry was associated with community composition change. As nitrate was the dominant form of nitrogen input, it is likely that nitrate-reducing bacteria established under oxygen limiting conditions in the fine substratum, possibly not only catalyzing full denitrification, but dissimilatory reduction of nitrate to ammonium, as expected for nitrate-limited environments (Dong et al. 2009). This could explain the observed enrichment of ammonium in this treatment, and also the differences in bacterial community composition observed between coarse and fine substratum. Changing interstitial conditions during the exposure time, especially in fine substratum, were also strongly correlated with the development of bacterial communities. These processes were also observed in the coarse substratum, but more slowly and at lower intensity. Here, suspended fine sediment particles from the free-flowing water of the river Moosach may have filled interstitial spaces with time and caused an increasing decoupling of free-flowing water and hyporheic zone towards the end of the experiment. In fact, fine sediment input in natural rivers, which is considered a major threat to aquatic biodiversity (Bo et al. 2007, Geist 2011, Larsen et al. 2011, Wood & Armitage 1997), could have similar effects on interstitial conditions and the microbial community composition in hyporheic substrata.

The applicability of different microbial sampling techniques

Bacterial community analysis via T-RFLP fingerprinting is a highthroughput method. This allows the time-effective analysis of high sample numbers, which is required due to the naturally high patchiness of stream ecosystems (Pringle et al. 1988, Ward & Tockner 2001, Winemiller et al. 2010). Each of the three evaluated field sampling techniques (interstitial water, stream substratum, slides) provided adequate material for T-RFLP analysis and yielded comparatively high numbers of OTUs (e.g. Besemer et al. 2009, Feris et al. 2003, Islam-ud-din et al. 2010). Only the reference samples from the free-flowing water did not provide sufficient DNA for reliable conclusions about the bacterial community, reflecting the low

abundance of planktonic in comparison to benthic bacteria in stream ecosystems (Findlay 2010). For studies into the microbial community of the free-flowing water, a higher sample volume of several litres is thus required. Due to better defined information on spatial allocation, we recommend sampling substratum microbial communities, especially if conditions and processes in the hyporheic zone are of interest. However, relations of the strong discrimination in physicochemical variables between coarse and fine substratum and the temporal development of the bacterial community in the substratum could be detected with each of the tested sampling techniques. These results indicate that the bacterial community data gained with the evaluated sampling procedures can be successfully linked with simultaneously assessed physicochemical variables. Hence, all of the tested sampling approaches can be considered capable of detecting a response of hyporheic microbial communities to changing environmental conditions.

Direct vs. indirect microbial sampling techniques

The sampling techniques differed significantly in their capability for qualitative and structural community recovery, cost and time effectiveness, and consequently in their adequacy for certain scientific questions. Substratum sampling is very cost-effective as it needs fewest consumables and at the same time proved to be most time-effective. As the direct extraction from substratum samples revealed the highest number of T-RFs and the highest diversity of all samples, this technique appears most adequate for recovering natural microbial community diversity in river bed substratum. Filtering interstitial water may still be a good alternative, especially in cases where substratum structure precludes substrate sampling or handling in the laboratory. Due to the low amount of DNA obtained from free-flowing water in our study, the sampling of a higher volume seems more appropriate, ideally several litres as common, e.g. also in groundwater sampling (Brielmann et al. 2009). As DNA yield, taxonomic richness and diversity were significantly lower on coupon samples compared to substratum samples, we cannot recommend the use of these slides for a comprehensive and representative sampling of microbial community composition in situ. On the other hand, artificial substrata can be beneficial if the colonization of defined surfaces is in focus and if the effect of substratum differences needs to be excluded, e.g. in studies which require standardization for comparisons between rivers, and in enclosures. After two weeks of exposure, community composition of each of the slide types differed significantly from the substratum samples, while after 9 weeks no significant differences could be detected. Therefore, longer exposure times may help to

alleviate sampling associated bias for artificial surfaces in experiments.

Conclusions

This study provides important evidence of the effects of substratum texture on interstitial habitat characteristics and intrinsic bacterial community structures. Possible adverse effects of microbial nitrate reduction on habitat quality for macrobiota were clearly coupled to substrate texture and porosity. The utilized T-RFLP fingerprinting of substratum samples was most capable of relating bacterial communities to habitat conditions, albeit only at an arbitrary level of OTUs. For more detailed taxonomic insights into bacterial communities, more elaborate approaches will be necessary such as sequencing of dominant bacterial taxa, e.g. via clone libraries, or high-throughput pyrotag sequencing of marker genes. This may be valuable for a better understanding of the ecology of hyporheic habitat functionality, not only in a microbial perspective, but also for critical links to higher trophic levels such as macroinvertebrates or fishes.

2.2 Taxonomic sufficiency in freshwater ecosystems: Effects of taxonomic resolution, functional traits and data transformation

A similar version of this section is published:
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Abstract

Taxonomic sufficiency (TS) was proposed for assessing community composition and environmental impacts, balancing the need to indicate the biology of the organisms present with time and effort for species identification. While TS has mostly been applied to marine and freshwater macroinvertebrates, there is a lack of studies that test its usefulness in other freshwater groups. Herein, we analysed the effects of taxonomic resolution, functional groupings and data transformation on multivariate community patterns in periphyton, macrophytes, macroinvertebrates and fishes, and on the quantification of biodiversity and environmental gradients. The applicability of TS strongly differed between taxonomic groups, depending on the average taxonomic breadth of the species sets. Numerical data resolution had more pronounced effects on community patterns than taxonomic resolution. Richness was strongly affected by data aggregation, but the calculation of diversity indices was statistically reliable up to order-level. Taxonomic aggregation had no significant influence on the capability to detect environmental gradients. Functional surrogates based on biological traits such as feeding type, reproductive strategy and trophic state revealed high correlations ($\rho = 0.64$-0.85) with taxonomic community composition. However, environmental correlations with data aggregated to functional traits were generally lower than for species-level data. Consequently, TS is universally applicable within taxonomic groups for different habitats. Aggregation to family or even order-level was suitable for the quantification of biodiversity and environmental gradients, whereas multivariate community analyses require higher resolution in fishes and macrophytes compared to periphyton and macroinvertebrates. Generally, sampling effort in environmental impact studies and monitoring programs should rather be invested into quantitative data and number of spatial and temporal replicates than in taxonomic detail.

Introduction

Loss of biodiversity is proceeding faster in freshwater than in any other major biome (Dudgeon et al. 2006, Strayer & Dudgeon 2010, Geist 2011). Time- and cost-effective methods for the quantification of changes in ecosystem community structure are needed in the context of biodiversity conservation and for assessing and monitoring of human impacts (Bevilacqua et al. 2012). On the other hand, a comprehensive scientific picture of their current status is essential to provide for the conservation of freshwater ecosystems (Millennium Ecosystem Assessment 2005, Bellier et al. 2012). Several authors and monitoring protocols regard species-level identification (Maurer 2000, Giangrande 2003, Drew 2011), identification of subspecies (Schaumburg et al. 2007), or DNA-based identification methods (e.g. Sweeney et al. 2011) as most appropriate in this context. However, identification of many freshwater species, e.g. algae or macroinvertebrates, and excessive quantitative sampling can be very difficult and time consuming (Johnson et al. 2006). Consequently, several attempts have been made to increase the time and cost efficiency of monitoring efforts by minimizing sampling or laboratory effort by reducing taxonomic or numerical resolution. Ellis (1985) proposed taxonomic sufficiency (TS) as a concept for assessment of marine pollution that balances the need to interpret the biology of the organisms present against the time and effort needed for species identification. TS involves identifying taxa to the coarsest taxonomic level possible without losing significant ecological detail (e.g. differences in multivariate community patterns or diversity). In the last two decades, an increasing number of studies addressing the applicability of identification levels coarser than species has been published. These studies mainly covered marine ecosystems (42%) and especially marine macroinvertebrates (36% of all studies; reviewed by Bevilacqua et al. 2012). To our knowledge, in freshwater ecosystems, TS has been applied systematically only to macroinvertebrates (reviewed by Jones 2008), and its suitability for other groups is only known from single case studies (plankton: Hansson et al. 2004, diatoms: Heino & Soininen 2007). Especially for vertebrates, plants, and algae, few studies have been done to test the applicability of TS for all ecosystem types (Bevilacqua et al. 2012). Multivariate community patterns of marine and freshwater macroinvertebrates seem to be consistent from species at least up to family-level (Bevilacqua et al. 2012), but biodiversity measures, such as richness, Evenness, Shannon Index, or Simpson's Index have the potential to be strongly underestimated by the use of coarser taxonomic-levels (Maurer 2000). Multivariate community patterns and biodiversity measures are used often in freshwater science to assess effects of

anthropogenic disturbance and to determine areas of conservation priority (e.g. Balmford et al. 1996), so such information would be of high value for freshwater ecologists. The degree to which the results of ecological analyses change if a coarser level of taxonomic resolution is used is largely influenced by the number of species that are being condensed (Bevilacqua et al. 2012). Furthermore, the degree of taxonomic relatedness among the investigated species and the distribution of species to coarser taxonomic levels (even or uneven distribution) also may influence the applicability of TS. These characteristics can differ strongly among taxonomic groups and ecosystem types. Several indices are available for measuring taxonomic diversity (e.g. average taxonomic breadth Clarke et al. 2001), but their usefulness for predicting the appropriate taxonomic resolution before undertaking time- and cost-intensive identification work has never been tested systematically in freshwater ecosystems. Moreover, ecological similarity of species is not necessarily correlated with taxonomic relatedness (Losos et al. 2003, Poff et al. 2006) Alternative groupings, such as feeding type, reproductive strategy, locomotion type, and habitat preference, can provide valuable information about ecosystem functions and processes (Usseglio-Polatera et al. 2000, Mouillot et al. 2005, Siefert 2012). However, the effects of aggregation to functional guilds on multivariate community patterns have not yet been investigated comprehensively for all relevant taxonomic groups in freshwater systems. Quantification of the impact of environmental factors on biological communities plays an important role in assessments of human disturbance and natural variability that goes beyond simple description of changes in multivariate community patterns or diversity. To date, only few studies of marine macroinvertebrates have considered the capability of detecting environmental gradients using coarser taxonomic levels than species or other classification types like functional groupings (e.g. Olsgard et al. 1997, 1998). The outcome of ecological analyses also can be influenced by the numerical resolution of the data (Clarke et al. 2001). Differences in numerical resolution can be founded on the degree of quantitative detail in the sampling strategy (quantitative, % abundance, or presence–absence data) or on post-hoc data transformation. Especially in applied freshwater science, presence–absence data or % abundance data often are used subsequent to nonquantitative sampling methods (e.g. Schaumburg et al. 2007, Barbour et al. 1999). Various degrees of transformation are used commonly in multivariate analyses of ecological data. These techniques include $\sqrt{x}$-transformation to allow the intermediate abundant species to play a part, $log(x)$- or $4\sqrt{x}$-transformation to increase consideration of rarer species, or use of presence–absence data to down-weight the effects of common and abundant species. However, choice of the numerical resolution of

the data is more a biological than a statistical question. This choice can affect the conclusions of an analysis more than the choices of similiarity measure or ordination method (Clarke et al. 2001) and may affect the applicability of TS. Knowledge on the applicability of different taxonomic and numerical resolutions and functional surrogates currently is limited to single-case studies (e.g. algae: Hansson et al. 2004) or remains untested (e.g. macrophytes, fishes: none of 678 publications reviewed by Bevilacqua et al. 2012) for most freshwater groups. Furthermore, most studies are based on only one data set, do not consider taxonomic levels coarser than family-level (see Bevilacqua et al. 2012), compare different taxonomic groups, include effects of numerical resolution and alternative groupings, or test the detected threshold levels statistically. These constraints limit direct comparisons between data sets and taxonomic groups within one ecosystem type. Application of TS and other taxonomic surrogates in freshwater ecosystems require identification of uses for which TS or functional surrogates could be advantageous and situations in which the lack of taxonomic information might severely limit the quality of assessments for all major taxonomic groups. This knowledge will help to find an optimal balance between detail of the results and effort. To our knowledge, our study is the first to analyze comprehensively the applicability of TS (up to phylum-level) and functional surrogates for multivariate analyses of community patterns, univariate analyses of diversity indices, and detection of environmental gradients with 3 freshwater data sets, each including abundance data for several taxonomic groups (periphyton, macrophytes, macroinvertebrates, and fishes). The following hypotheses were tested: (I) Taxonomic resolution of periphyton, macrophyte, macroinvertebrate, and fish community data affects the outcome of ecological analyses (multivariate community pattern, diversity measures, capability to detect environmental gradients). The extent of loss of information is expected to depend on the average taxonomic breadth of the investigated taxonomic group and set of species. (II) Classification into functional groups resolves a different outcome of ecological analyses (multivariate pattern, diversity) than grouping individuals according to Linnean taxonomy and can improve the capability to detect environmental gradients. (III) Numerical resolution of the data (e.g relative abundance or presence–absence data instead of quantitative data) has a stronger influence on the results of community analyses than taxonomic resolution (e.g. genus- or family- instead of species-level).

Material and Methods

Data sets

The hypotheses were tested using 3 large, full-resolution data sets (quantitative species abundance data) from lentic and lotic freshwater habitats. Data set 1 was focused on a pairwise comparison of sites upstream and downstream of weirs in 5 different rivers (10 sampling locations; Mueller et al. 2011). Data set 2 was aimed at a comparison of biodiversity and abiotic habitat variables in different floodplain habitats (river stretches, oxbow sections, small ponds). Data were collected in the River Danube floodplain in Bavaria, Germany (42 sampling locations; Stammel et al. 2012). Data set 3 was collected to compare abiotic habitat characteristics and community composition in 3 calciferous and 3 silicious rivers distributed throughout Bavaria, Germany (30 sampling locations; Mueller et al. 2014). Each data set included the taxonomic groups periphyton, macroinvertebrates, macrophytes, and fishes and a set of environmental variables (water temperature, dissolved O2, specific conductance, pH, water depth, current speed), but the data sets differed in data structure (sampling methods, number of sampled river stretches, and treatments; Table 2.4). In our study, periphyton refers to all groups of periphytic algae, including diatoms. The level of taxonomic identification was species for macrophytes and fishes. Periphyton and macroinvertebrates were identified to species-level as far as possible. Taxonomically difficult groups (chironomids, oligochaeta, mites, chlorophyceae $< 5\,\mu$m) and small juveniles were identified to genus or lowest possible level.

Data aggregation

Fine-resolution data sets are needed to study the applicability of different taxonomic or numerical data resolutions and functional groupings for ecological analyses. The resolution of these data sets can then be modified by summarizing the data to coarser levels. This procedure is referred to as data aggregation (taxonomic resolution) in the following text. Understanding the effects of data aggregation is essential to ensure that the most suitable classification system and data resolution can be chosen before undertaking the effort involved in species identification in future studies.

Table 2.4: Characterisation of the three data sets used for the analyses. Str. = number of investigated river stretches; Riv. = number of investigated rivers; Tr. = number of different treatments within one river; S = number of species in the respective group and data set; N = number of individuals in the respective group and data set (representing the number of recorded presences for macrophytes). P = Periphyton; MP = Macrophytes; MIV = Macroinvertebrates; F = Fishes

Data set	Tax. group	Sampling effort	Sampling method	Habitat type	Source of variation	Str.	Riv.	Tr.	S	N
1	P	Semi-quantitative	Scraping from stones (Mueller et al. 2011) and following sedimentation method (DIN EN 15204 2006)	Headwater streams	Upstream and down-stream sides of weirs	10	5	2	108	42,384
	MP	Semi-quantitative	Point-abundance sampling with garden rake (Deppe & Lathrop 1993)						18	49
	MIV	Quantitative	Surber-Sampling (Surber 1930)						103	9,405
	F	Quantitative	Electrofishing (Mueller et al. 2011)						28	2,508
2	P	Quantitative	Scraping from stones (Mueller et al. 2011) and following sedimentation method (DIN EN 15204 2006)	Lotic and lentic floodplain habitats	Natural variability between floodplain habitats	42	1	3	138	61,856

Data set	Tax. group	Sampling effort	Sampling method	Habitat type	Source of variation	Str.	Riv.	Tr.	S	N
	MP	Semi-quantitative	Point-abundance sampling with garden rake (Deppe & Lathrop 1993)						53	395
	MIV	Quantitative	Kick-Sampling (Hauer & Lamberti 2011)						140	23,047
	F	Quantitative	Electrofishing (Pander & Geist 2010*b*)						26	1,779
3	P	Quantitative	Scraping from stones (Mueller et al. 2011) and following sedimentation method (DIN EN 15204 2006)	Headwater streams	Natural variability between rivers	30	6	1	144	92,793
	MP	Quantitative	Visual plot assessment (Hauer & Lamberti 2011)						14	72
	MIV	Quantitative	Surber-Sampling (Surber 1930)						149	15,580
	F	Quantitative	Electrofishing (Pander & Geist 2010*b*)						23	2,899

Taxonomic resolution (hypothesis I) was modified by aggregating the full-resolution species abundance data to coarser levels of taxonomic resolution. In our study, species-level is the finest level of taxonomic resolution, whereas phylum-level is the coarsest level of taxonomic resolution. Species abundance data for periphyton, macroinvertebrates, and macrophytes from each data set were aggregated to genus, family, order, class, and phylum-level by calculating the sum of all individuals from the respective level per sample with the aggregation tool in PRIMER v6 (Clarke & Gorley 2006). All freshwater fishes were from the class Osteichthyes and the phylum Chordata, so species abundance data were aggregated only to order for this group. The effects of functional groupings on the multivariate community patterns of periphyton, macrophytes, macroinvertebrates, and fishes (hypothesis II) were tested by aggregating data to commonly used functional traits (representing a mixture of biological traits and ecological requirements; Table 2.5). Fishes and macroinvertebrates were assigned to groups commonly used in assessments in context of the European Water Framework Directive (see Table 2.5). The use of functional traits to assess macrophytes and periphyton is less established in standard evaluation. Therefore, commonly applied functional classifications were selected from different literature sources for these groups. For each taxonomic group, all traits were summarized in a matrix (All Traits) containing the number of specimens from each trait state per sample (e.g. 61 fishes with reproduction type rheophilic, 12 fishes with reproduction type indifferent, 10 with trophic status omnivore; cf. functional trait niche, Poff et al. 2006). Details about the selected functional traits and the respective literature sources are provided in Table 2.5. The numerical resolution (hypothesis III) of each data set, taxonomic group, and level of taxonomic resolution was modified by data transformation using the pre-treatment transformation (overall) procedure in PRIMER v6 (Clarke & Gorley 2006). Numerical resolution in our study reached from untransformed quantitative data (finest level) over $\sqrt{x}$-transformed and % abundance data to presence–absence data (coarsest level).

Table 2.5: Classification and literature source for the functional traits used as taxonomic surrogates for the taxonomic groups (TG) periphyton (P), macrophytes (MP), macroinvertebrates (MIV) and fishes (F). The classification scheme and autecological data were adopted from the respective reference

TG	Functional trait	Classification	Literature
P	Life form	Unbranched filaments, branched filaments, single cells, colonial, colonial sheet-like	John et al. (2002)
	Habitat preferences	Planctonic, attached, benthic, benthic or planctonic	Blum (1956), Bellinger & Sigee (2011)
	Motility	Flagellated, non-motile, monoraphid, biraphid, araphid	Wehr (2002)
	Saprobic state	Oligosaprobic, mesosaprobic, eusaprobic, polysaprobic	Rott et al. (1997)
	Trophic state	Oligotrophic, mesotrophic, eutrophic, polytrophic, hypertrophic	Rott et al. (1999)
MP	Substrate type	Mud-silt, mud-sand, mud-gravel, mud, silt, silt-sand, sand, sand-gravel	Henry et al. (1996), Ettl et al. (1978-1999), Rothmaler et al. (2002)
	Trophic state	Oligotrophic, mesotrophic, eutrophic, polytrophic, hypertrophic	Schneider & Melzer (2003), Ettl et al. (1978-1999), Rothmaler et al. (2002)
	Flow preference	Neglegible, neglegible-slow, neglegible-moderate, neglegible-fast, slow, slow-fast, moderate, moderate-fast	Henry et al. (1996), Ettl et al. (1978-1999), Rothmaler et al. (2002)
	Assimilation type	CO_2 only, HCO_3^-- and CO_2	Madsen & Sand-Jensen (1991), Maberly & Madsen (1998), Keeley & Sandquist (1992)

TG	Functional trait	Classification	Literature
	Life form	Emergent, floating-leafed, sumberged, free-floating	Brix & Schierup (1989), Hauer & Lamberti (2011)
MIV	Feeding types	Active filterers, passive filterers, detrivores, predators, parasites, grazers, shredders, and combinations	Moog (1995)
	Zonation	Litoral, potamal, rhithral, indifferent	Moog (1995)
	Saprobic state	Oligosaprobic, alphasaprobic, betasaprobic, xenobiontic	Moog (1995)
F	Feeding types	Herbivores, invertivores, piscivores, omnivores, inverti-piscivores	(Dußling & Blank 2005)
	Reproductive strategy	Marine, psammophilic, phytophilic, lithophilic, ostracophilic, phytolithophilic, lithopelagophilic	(Dußling & Blank 2005)
	Habitat preferences	Rheophilic, stagnophilic, indifferent	(Dußling & Blank 2005)
	Migration	Long distance, short distance, middle distance	(Dußling & Blank 2005)

Multivariate analyses

Comparison of resemblance matrices After data aggregation, resemblance matrices (Bray–Curtis Similarity) were calculated for all taxonomic groups, respective levels of taxonomic resolution, levels of numerical resolution (note that Bray–Curtis Similarity is equal to Sørensen Index for presence–absence data), and functional traits (taxonomic and numerical resolution: 3 data sets × 4 taxonomic groups × 6 taxonomic levels × 4 numerical resolutions = 288 Bray–Curtis matrices; functional groupings: 3 data sets × 18 traits = 54 Bray–Curtis matrices). The 2^{nd}-stage approach in the PRIMER package was used to analyze differences among multivariate community patterns derived from different data-aggregation modes (taxonomic resolution, numerical resolution, functional groupings). This procedure uses multivariate Spearman rank correlation (ρ) to compare resemblance matrices based on Bray–Curtis Similarity and is commonly applied in assessments of TS (Somerfield & Clarke 1995). Nonmetric multidimensional scaling (NMDS) was run on the resulting 2^{nd}-stage matrices (ρ as resemblance measure) to visualize similarities and dissimilarities among Bray–Curtis matrices. To test hypotheses I and III, Bray–Curtis matrices derived from different taxonomic and numerical resolutions were compared in a 2^{nd}-stage analysis, resulting in twelve 2^{nd}-stage matrices and NMDS plots (3 data sets × 4 taxonomic groups). To test hypothesis II, Bray–Curtis matrices derived from functional groupings were compared with those from the taxonomic groupings (species, genus, family, order, class, and phylum) in a separate 2^{nd}-stage analysis, resulting in twelve 2^{nd}-stage-matrices and NMDS plots (3 data sets × 4 taxonomic groups). Permutational multivariate analysis of variance (PERMANOVA; Anderson et al. 2008) was run in PRIMER v6 to compare the effects of numerical resolution on multivariate community patterns with those of taxonomic resolution (hypothesis III). PERMANOVA is a routine for testing the multivariate response to one or more factors on the basis of any resemblance measure. The values in the matrix are not treated as independent of one another (Anderson et al. 2008), which enables comparison of matrices derived from the same data (e.g. different taxonomic or numerical levels). For each taxonomic group and data set, two PERMANOVA analyses were run on the respective 2^{nd}-stage matrices. Two separate 1-way PERMANOVA designs were applied. Taxonomic resolution (6 factor levels: species, genus, family, order, class, and phylum) was used as a fixed factor in the first design and numerical resolution (4 factor levels: untransformed quantitative, $\sqrt{x}$-transformed, % abundance, and presence–absence data) was used as the fixed factor in the 2^{nd} design. Pseudo-F values and permutational P-values were used to compare the effect

strength of taxonomic vs numerical resolution.

Taxonomic resolution and environmental gradients The ability to recover ecological patterns of periphyton, macrophytes, macroinvertebrates, and fishes at different taxonomic resolutions (hypothesis I)/functional groupings (hypothesis II) was tested using Biota-Environmental-Stepwise matching (BEST) analyses in PRIMER (Clarke et al. 2001). Taxa data sets were used as response variables (all 342 Bray–Curtis matrices generated in the previous analyses) and environmental variables (water temperature, dissolved O_2, specific conductance, pH, water depth, current speed) were used as predictors. The BEST procedure uses a stepwise search and Spearman rank correlation to find a minimum combination of environmental variables that maximizes correlation with the biotic data. The taxonomic level and transformation type resulting in the maximum Spearman correlation coefficient between environmental variables and biotic data (r^2) was used to identify the best description of community patterns (following Olsgard et al. 1997).

Univariate analyses of Spearman rank correlation coefficients and diversity measures

To identify the threshold of significant loss of information for each taxonomic group, we used nonparametric univariate statistical analysis with ρ-values, r^2-values, and diversity indices as the response variables and taxonomic resolution as the factor (with factor levels: species, genus, family, order, class, and phylum). This approach is applied similarly in microarray analyses (Listgarten & Emili 2005) and machine learning (Demšar 2006) to test the validity of classification algorithms, and Demšar (2006) proposed the use of nonparametric tests for comparisons across data sets. A significant loss of information resulting from coarsening taxonomic resolution (hypothesis I) in our study is defined as a statistically significant drop of ρ-values (indicating a change in multivariate community pattern; 2^{nd}-stage analysis), r^2-values (indicating a change in the capability to detect environmental gradients; BEST analysis), or diversity indices (indicating a change in richness, Evenness, Shannon Index, or Simpson's Index) from a finer level of taxonomic resolution to the next coarsest level (e.g. from species to genus). Richness, Evenness (Pielou 1975), Shannon Index (Shannon & Weaver 1949), and Simpson's Index (Simpson 1949) were calculated for each taxonomic level using the DIVERSE procedure in PRIMER v6. In addition, we calculated the functional diversity (measured as richness, Evenness, Shannon Index, Simpson's Index) for

each functional trait (hypothesis II). Diversity values were pooled over all data sets, whereas ρ-values and r^2-values were pooled over all data sets and levels of numerical resolution. All data were tested for normality using the Shapiro-Wilk-Test and for homogeneity of variances using the Levene-Test. Because correlation coefficients and diversity indices were not normally distributed, the Kruskal–Wallis-Test and post-hoc pairwise Mann-Whitney *U*-Test were used to test for differences between aggregation levels. Bonferroni correction was applied to correct for multiple comparisons. All univariate statistics were carried out in the software program R (http://www.r-project.org). To test if the extent of the loss of information depends on taxonomic diversity (higher-taxa/species ratio [Φ] and the distribution of species to higher taxa) of the investigated set of species (hypothesis I), the average taxonomic breadth (Δ^+) for each aggregation level was correlated with the ρ-values between the respective resemblance matrices using linear regression and Spearman rank correlation. According to Clarke et al. (2001), the average taxonomic breadth is defined as

$$\Delta^+ = (\sum\sum_{i<j} \omega)/(S[S-1]/2)$$

where S is the observed number of species in the sample and the double summation ranges over all pairs i and j of these species, ω represents the taxonomic distances through the classification tree between every pair of individuals. It was calculated for each data set, taxonomic group, and aggregation level using the function DIVERSE in PRIMER v6. Linear regressions and correlation analyses were carried out in the software program R.

Results

Effects of taxonomic resolution on multivariate community patterns

Effects of taxonomic resolution on multivariate community patterns strongly differed among the taxonomic groups periphyton, macrophytes, macroinvertebrates, and fishes (Figs 2.3 & 2.4 A–D). The lowest effects of coarsening taxonomic resolution were detected by 2^{nd}-stage analysis in periphyton, followed by macroinvertebrates. A significant loss of information for macrophytes and fishes occurred at the genus- (Mann-Whitney *U*-Test, macrophytes, $P < 0.001$; Figs 2.3 & 2.4 B) and family-levels (Mann-Whitney *U*-Test, fishes, $P < 0.01$; Figs 2.3 & 2.4 D), respectively. In contrast, macroinvertebrate and periphyton community structure changed significantly from species- to order- (Mann-Whitney *U*-Test, macroinvertebrates, $P < 0.01$; Figs 2.3 & 2.4 C) and from species- to class-level (Mann-Whitney

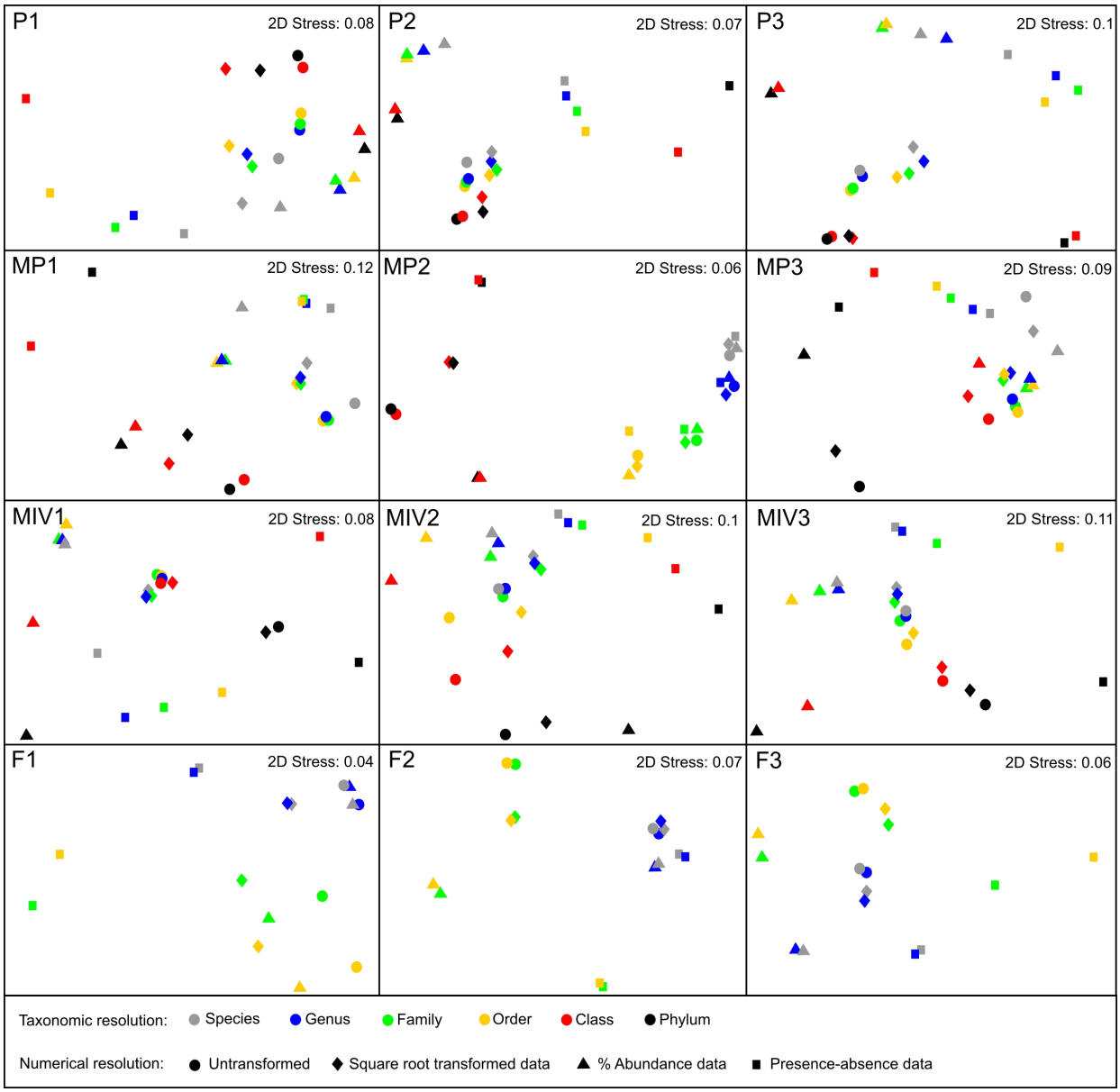


Figure 2.3: 2^{nd}-stage nonmetric multidimensional scaling (NMDS) plots of resemblance matrices from different taxonomic and numerical resolutions. The taxonomic groups periphyton (P), macrophytes (MP), macroinvertebrates (MIV), and fishes (F) are arranged in rows with data sets (1, 2, 3) in columns

U-Test, periphyton, $P < 0.01$; Figs 2.3 & 2.4 A). A strong aggregation up to phylum- or class-level revealed a significantly different community composition for each taxonomic group (Figs 2.3 & 2.4 A–D). This structure was constant across data sets, but as a comparison of ρ-values from the 3 data sets indicates, the correlations among taxonomic levels were higher for anthropogenic disturbance (effects of weirs, data set 1, mean $\rho = 0.79$) than for natural variability both for large-scale (different rivers, data set 3, mean $\rho = 0.67$) and small-scale data (different habitats within one river system, data set 2, mean $\rho = 0.66$). Regression and correlation analysis of Δ^+ and ρ-values between resemblance matrices of different taxonomic levels revealed a strong relationship between both parameters for all taxonomic groups with ρ ranging between 0.70 for fishes and 0.87 for periphyton (Fig. 2.5). In contrast, a comparison of Φ and the decline in ρ for periphyton and macrophytes indicates a less pronounced relationship in these groups. Φ between species and genus-level was lower for periphyton ($\Phi = 0.56$) with ρ staying constant, whereas for macrophytes, Φ was higher ($\Phi = 0.68$), but ρ decreased significantly from species to genus-level. At the same time, the Δ^+ was higher for periphyton (Δ^+ species–genus = 0.98) than for macrophytes (Δ^+ species–genus = 0.89).

Effects of taxonomic resolution on the quantification of biodiversity

The univariate comparisons of richness, Evenness, Shannon Index, and Simpson's Index from different taxonomic levels suggest that diversity measures are generally affected by taxonomic resolution in a very similar way as the multivariate community patterns. As expected, increasing aggregation resulted in a decrease of richness and diversity indices, but with differences in the extent of decrease among different taxonomic groups. In the groups of macroinvertebrates and macrophytes, the quantification of biodiversity applying TS did not strongly differ from using species-level data as a baseline. The detected decrease with coarser taxonomic level was strongest for richness (Table 2.6), moderate and identical for Shannon Index and Simpson's Index (Table 2.6), and less pronounced for Evenness. Significant differences in Evenness occurred only by shifting from species to class and phylum-level for periphyton (Mann-Whitney *U*-Test, $P < 0.01$), macrophytes (Mann-Whitney *U*-Test, $P < 0.001$), and macroinvertebrates (Mann-Whitney *U*-Test, $P < 0.001$), and to order-level for fishes (Mann-Whitney *U*-Test, $P < 0.05$). Shannon Index and Simpson's Index did not change significantly up to the same or even at coarser taxonomic level as observed for the respective community pattern in each taxonomic group. Evenness was significantly lower only on class and phylum-level for all taxonomic groups except for fishes, where it decreased

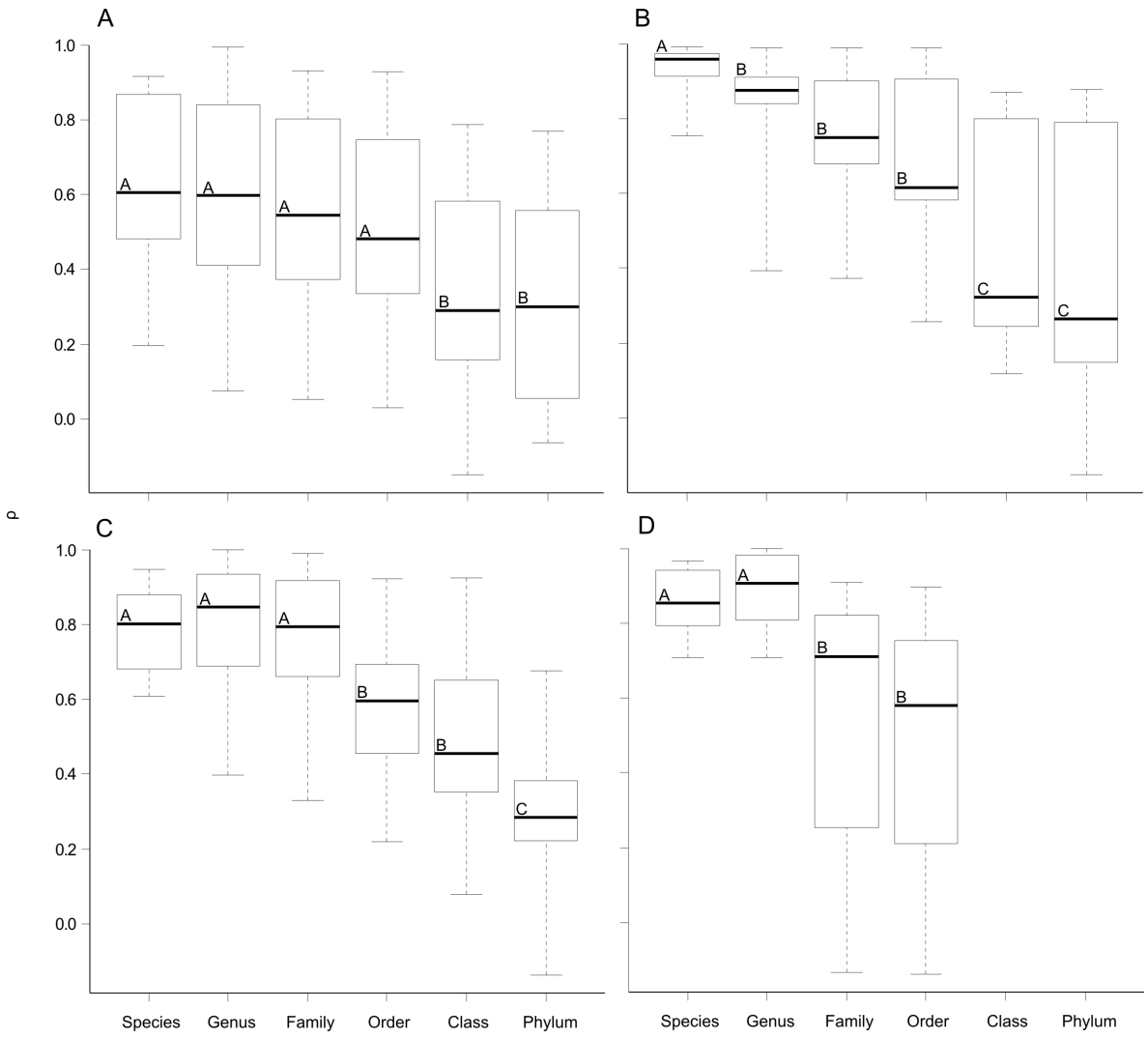


Figure 2.4: Box-and-whisker plots showing efficiency of taxonomic sufficiency in periphyton (A), macrophytes (B), macroinvertebrates (C), and fishes (D) for pooled data from all studies. Class and phylum-level were not considered for fishes because all species were from the same class and phylum. Efficiency is measured by similarity of Spearman rank correlation coefficients (ρ) of the coarser taxonomic levels to those of species-level. Significant differences in median Spearman rank correlation coefficients between taxonomic levels are indicated by different capital letters (A, B, C) above the median line. Lines in boxes show medians, box ends show quartiles, and whiskers show ranges.

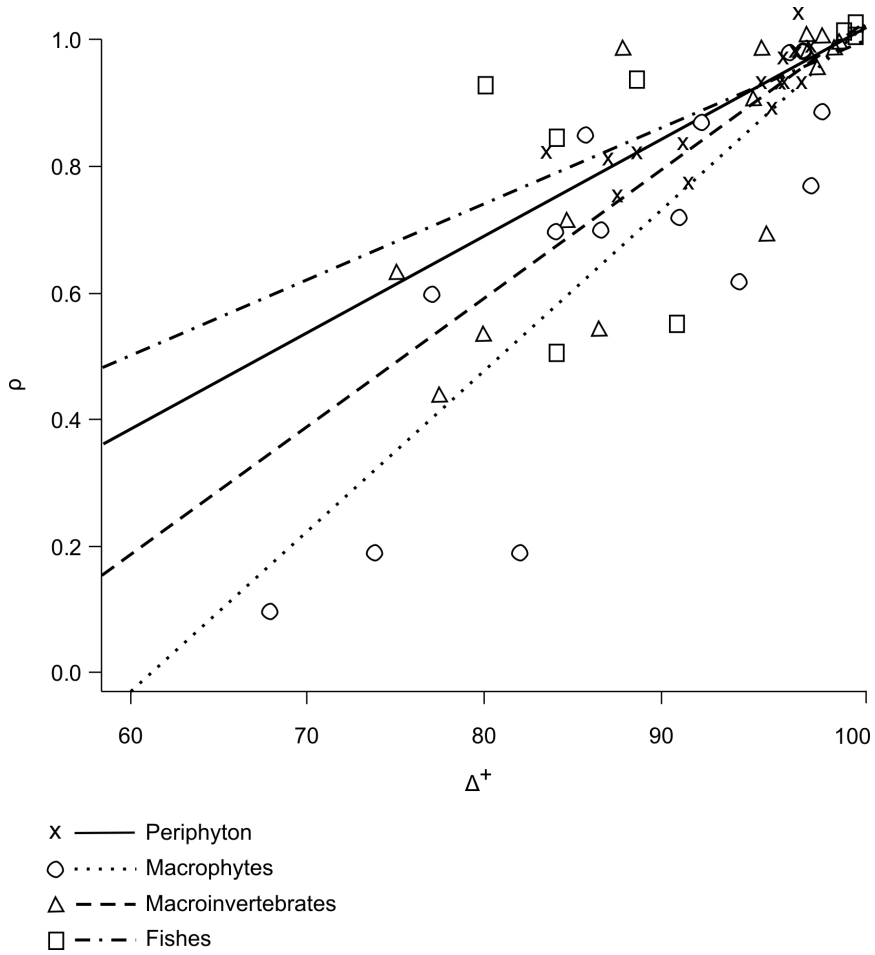


Figure 2.5: Spearman rank correlation coefficient (r^2) of ρ from correlation of species and higher taxa matrices against the corresponding average taxonomic breadth (Δ^+) for periphyton ($r^2 = 0.87$, adjusted $r^2 = 0.70$, $P < 0.001$); macrophytes ($r^2 = 0.82$, adjusted $r^2 = 0.70$, $P < 0.001$); macroinvertebrates ($r^2 = 0.79$, adjusted $r^2 = 0.68$, $P < 0.001$); and fishes ($r^2 = 0.70$, adjusted $r^2 = 0.12$, $P < 0.05$)

significantly on order-level.

Taxonomic resolution and environmental gradients

Because there were almost no differences in the results of BEST analyses between taxonomic levels (Table 2.7), the applied taxonomic resolution generally had low effects on the capability to detect environmental gradients. Significant differences between taxonomic levels occurred only for macroinvertebrates, with the correlation of environmental variables being significantly lower on family, order, class, and phylum-level than on species-level (Table 2.7). For macrophytes, the BEST analysis revealed slightly higher correlation on species-level, but there were no significant differences and the standard deviation of BEST correlation coefficients was very high (Table 2.7). For periphyton, a slight decrease in values from family- to phylum-level was detected, whereas for fishes only the standard deviation increased from family-level to higher taxonomic classifications (Table 2.7). Numerical resolution had no significant effects on BEST results for all investigated taxonomic groups.

Effects of numerical resolution

PERMANOVA Pseudo-F values were constantly higher and P-values lower for the factor numerical resolution than for taxonomic resolution, so different types of data transformation (none, $\sqrt{x}$, % abundance data, presence–absence data) obviously had stronger effects on community patterns than taxonomic levels (PERMANOVA taxonomic resolution: Pseudo-$F = 1.01$–68.87, $P = 0.001$–0.5; numerical resolution: Pseudo-$F = 1.01$–252.32, $P = 0.001$–0.49). Matrices from $\sqrt{x}$-transformed data and untransformed data strongly clustered in most cases (except for the periphyton data from data set 1, see Fig. 2.3). Percent abundance data and presence–absence data had more pronounced effects on the multivariate community pattern across data sets and taxonomic groups. The effects of coarsening taxonomic resolution on multivariate community patterns increased with coarsening numerical resolution, which also differed between taxonomic groups (Fig. 2.3). Evaluating the arrangement of matrices in the 2^{nd}-stage NMDS, the strongest change of community patterns resulting from numerical data resolution was detected for periphyton (PERMANOVA data set 1: Pseudo-$F = 252.32$, $P < 0.001$; data set 2: Pseudo-$F = 198.94$, $P < 0.001$; data set 3: Pseudo-$F = 1.01$, $P = 0.35$). A moderate impact was found in macrophytes (PERMANOVA data set 1: Pseudo-$F = 133.14$, $P < 0.001$; data set 2: Pseudo-$F = 15.56$, $P < 0.01$; data set 3: Pseudo-

Table 2.6: Mean (± SD) richness, Evenness, Shannon Index, and Simpson's Index for periphyton (P), macrophytes (MP), macroinvertebrates (MIV), and fishes (F) at different levels of taxonomic resolution. Class and phylum-level were not considered for fishes because all species were from the same class and phylum. Values with the same uppercase letters are not significantly different ($P > 0.05$) within taxonomic groups.

Tax. Group	Level	Richness	Evenness	Shannon Index	Simpson's Index
P	Species	36 ± 10^{A}	0.60 ± 0.18^{A}	2.11 ± 0.67^{A}	0.76 ± 0.17^{A}
	Genus	24 ± 5^{B}	0.59 ± 0.16^{A}	1.86 ± 0.52^{AB}	0.72 ± 0.16^{AB}
	Family	21 ± 4^{C}	0.55 ± 0.18^{A}	1.68 ± 0.56^{B}	0.67 ± 0.20^{B}
	Order	7 ± 2^{E}	0.56 ± 0.18^{AC}	1.63 ± 0.54^{B}	0.66 ± 0.20^{B}
	Class	18 ± 3^{D}	0.45 ± 0.16^{B}	0.89 ± 0.35^{C}	0.46 ± 0.18^{C}
	Phylum	6 ± 1^{F}	0.49 ± 0.18^{BC}	0.82 ± 0.29^{C}	0.45 ± 0.17^{C}
MP	Species	5 ± 3^{A}	0.87 ± 0.17^{A}	1.25 ± 0.63^{A}	0.64 ± 0.25^{A}
	Genus	5 ± 3^{A}	0.85 ± 0.20^{A}	1.18 ± 0.71^{A}	0.58 ± 0.28^{A}
	Family	4 ± 2^{A}	0.84 ± 0.20^{A}	1.10 ± 0.64^{A}	0.57 ± 0.28^{A}
	Order	4 ± 2^{A}	0.81 ± 0.20^{A}	0.99 ± 0.61^{A}	0.52 ± 0.29^{A}
	Class	2 ± 1^{B}	0.64 ± 0.24^{B}	0.41 ± 0.36^{A}	0.25 ± 0.22^{B}
	Phylum	2 ± 1^{B}	0.63 ± 0.23^{B}	0.32 ± 0.34^{A}	0.19 ± 0.21^{B}
MIV	Species	26 ± 9^{A}	0.62 ± 0.15^{A}	1.97 ± 0.55^{A}	0.75 ± 0.17^{A}
	Genus	24 ± 8^{A}	0.60 ± 0.15^{A}	1.87 ± 0.53^{A}	0.73 ± 0.18^{A}
	Family	19 ± 5^{B}	0.61 ± 0.15^{A}	1.77 ± 0.47^{A}	0.72 ± 0.17^{A}
	Order	10 ± 2^{C}	0.58 ± 0.15^{A}	1.34 ± 0.36^{B}	0.63 ± 0.17^{B}
	Class	5 ± 1^{D}	0.42 ± 0.21^{B}	0.62 ± 0.30^{C}	0.34 ± 0.18^{C}
	Phylum	3 ± 1^{E}	0.29 ± 0.21^{C}	0.30 ± 0.22^{D}	0.16 ± 0.15^{D}
F	Species	6 ± 4^{A}	0.72 ± 0.18^{A}	1.11 ± 0.52^{A}	0.62 ± 0.22^{A}
	Genus	6 ± 4^{A}	0.72 ± 0.18^{A}	1.11 ± 0.52^{A}	0.62 ± 0.22^{A}
	Family	3 ± 2^{B}	0.59 ± 0.29^{AB}	0.58 ± 0.36^{B}	0.37 ± 0.25^{B}
	Order	3 ± 1^{B}	0.56 ± 0.31^{B}	0.52 ± 0.36^{B}	0.34 ± 0.26^{B}
	Class	-	-	-	-
	Phylum	-	-	-	-

Table 2.7: Mean (± SD) and range of Spearman rank correlation coefficients between biotic and environmental data received from Biota Environmental Stepwise matching analysis (BEST Clarke & Gorley 2006) including the environmental variables: water temperature; dissolved O_2; specific conductance; pH; water depth, and current speed. Class- and phylum-level were not considered for fishes because all species were from the same class and phylum. Values with the same uppercase letters are not significantly different ($P > 0.05$) within taxonomic groups

	Periphyton	Macrophytes	Macroinvertebrates	Fishes
Species	0.46 ± 0.17	0.64 ± 0.15	0.60 ± 0.09^{AB}	0.57 ± 0.08
	[0.24-0.76]	[0.47-0.85]	[0.49-0.79]	[0.49-0.70]
Genus	0.43 ± 0.15	0.61 ± 0.16	0.61 ± 0.08^{AB}	0.59 ± 0.08
	[0.23-0.66]	[0.42-0.83]	[0.51-0.79]	[0.49-0.70]
Family	0.42 ± 0.13	0.58 ± 0.16	0.58 ± 0.09^{BC}	0.53 ± 0.18
	[0.24-0.66]	[0.41-0.82]	[0.45-0.77]	[0.26-0.76]
Order	0.40 ± 0.13	0.58 ± 0.16	0.54 ± 0.12^{BC}	0.54 ± 0.20
	[0.23-0.66]	[0.44-0.82]	[0.38-0.73]	[0.25-0.83]
Class	0.41 ± 0.19	0.51 ± 0.15	0.51 ± 0.12^{BC}	-
	[0.20-0.79]	[0.31-0.73]	[0.33-0.73]	
Phylum	0.39 ± 0.20	0.45 ± 0.22	0.46 ± 0.18^{C}	-
	[0.20-0.83]	[0.17-0.80]	[0.27-0.74]	

$F = 1.01$, $P = 0.49$), and fishes (PERMANOVA data set 1: Pseudo-$F = 65.42$, $P < 0.01$; data set 2: Pseudo-$F = 12.69$, $P < 0.001$; data set 3: Pseudo-$F = 41.63$, $P < 0.001$). The least pronounced effects were found for macroinvertebrates (PERMANOVA data set 1: Pseudo-F = 12.73, $P < 0.001$; data set 2: Pseudo-$F = 17.76$, $P = 0.001$; data set 3: Pseudo-$F = 17.35$, $P < 0.001$; Fig. 2.3).

Functional traits as alternative grouping

A multivariate comparison of Bray–Curtis matrices from alternative groupings according to functional characteristics of species with those from taxonomic groupings indicates strong differences between taxonomic groups and the applied functional traits (Fig. 2.6). Some functional groupings, e.g. feeding types of fishes and macroinvertebrates and the trophic state of macrophytes, revealed community patterns that were very similar to those on species or other taxonomic levels in 2^{nd}-stage analysis, but other traits, such as reproductive strategies and habitat preferences of fishes and the zonation of macroinvertebrates, resulted in a clustering that differed from taxonomic groupings (Fig. 2.6). Similarity between functional groupings and species-level was highest for periphyton ($\rho = 0.71$–0.82) and lowest for fishes ($\rho = 0.25$–0.65; Fig. 2.7). Correlations with environmental variables were lower or in the same range for functional groupings as for species-level data and declined with decreasing similarity to species-level (Fig. 2.7). Functional diversity, measured by Shannon Index and Simpson's Index, summarized for all traits per taxonomic group was always higher than species diversity, no matter if richness was increased or decreased in comparison to species richness by summarizing all traits. Richness was generally strongly reduced by functional grouping (Table 2.8) because it was limited by the maximum number of trait states. Nevertheless, functional diversity (especially when measured by Evenness) of single traits often reached similar values as species diversity (e.g. habitat preferences of periphyton, functional feeding groups of macroinvertebrates, substrate preferences, and trophic state of macrophytes and reproductive strategies of fishes; Table 2.8, Fig. 2.6).

Discussion

Our study provides new baseline data about the effectiveness of taxonomic surrogates in freshwater ecosystems, including taxonomic resolution coarser than species, functional groups, diversity measures, and effects of numerical data resolution in periphyton, macrophytes, macroinvertebrates, and fishes. This

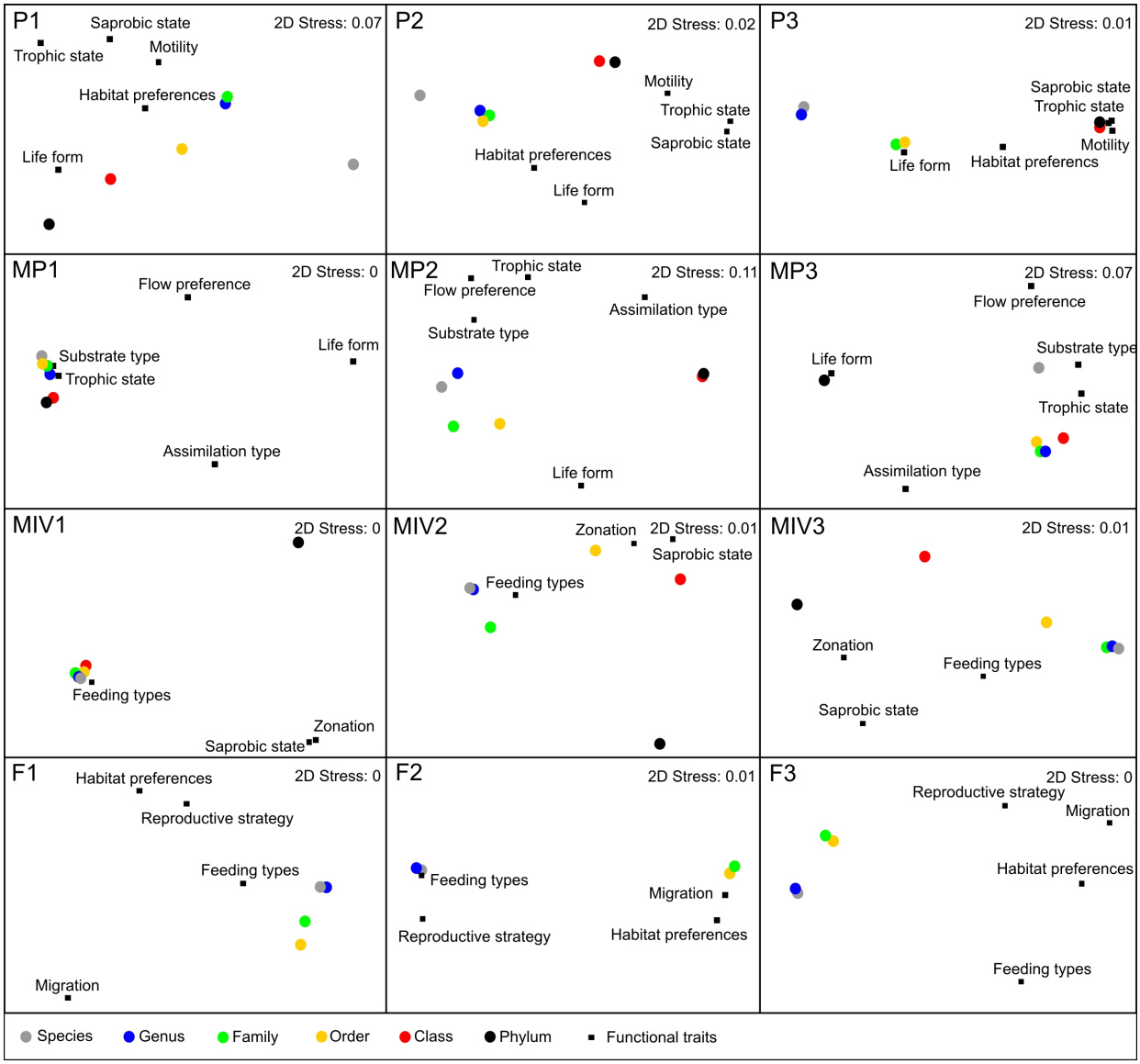


Figure 2.6: 2^{nd}-stage nonmetric multidimensional scaling (NMDS) plots of resemblance matrices from different taxonomic levels and functional groupings. The taxonomic groups periphyton (P), macrophytes (MP), macroinvertebrates (MIV), and fishes (F) are arranged in rows with data sets (1, 2, 3) in columns. A detailed classification of the functional traits can be drawn from Table 2.5

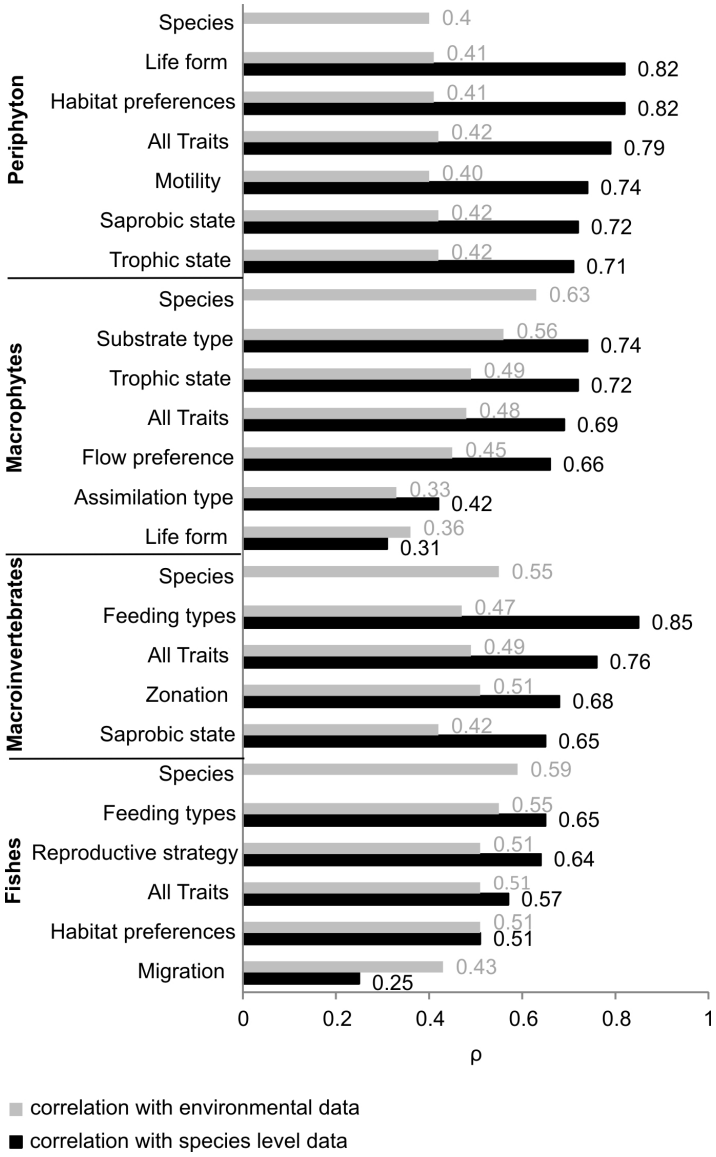


Figure 2.7: Spearman rank correlation coefficients (ρ) for abundance of periphyton, macrophytes, macroinvertebrates, and fishes aggregated to functional traits, species-level and environmental variables (Biota Environmental Stepwise matching analyses, BEST). Numbers adjacent to the bars represent the mean values of ρ for untransformed data of the 3 data sets. Functional traits are arranged in decreasing order according to their correlation with the respective species-level. Species is listed first for each taxonomic group as a reference for environmental correlations. All Traits represents a summary of all single traits per taxonomic group

Table 2.8: Mean (± SD) functional diversity for functional traits of periphyton (P), macrophytes (MP), macroinvertebrates (MIV), and fishes (F) in comparison to species diversity (Species). Functional traits are arranged according to the order in Fig. 2.7. A detailed description of each trait is given in Table 2.5. All Traits represents a summary of all single traits per taxonomic group. Values with the same uppercase letters are not significantly different ($P > 0.05$) within taxonomic groups

	Grouping	Richness	Evenness	Shannon Index	Simpson's Index
P	Species	36 ± 10^{A}	0.60 ± 0.18^{A}	2.11 ± 0.67^{A}	0.76 ± 0.17^{A}
	Life form	5 ± 1^{B}	0.68 ± 0.18^{BC}	1.12 ± 0.31^{B}	0.58 ± 0.16^{B}
	Habitat preferences	6 ± 1^{C}	0.62 ± 0.18^{BA}	1.08 ± 0.31^{B}	0.55 ± 0.17^{B}
	All Traits	25 ± 2^{D}	0.75 ± 0.09^{C}	2.41 ± 0.27^{C}	0.88 ± 0.03^{C}
	Motility	5 ± 0^{B}	0.50 ± 0.24^{AD}	0.81 ± 0.38^{D}	0.42 ± 0.22^{D}
	Saprobic state	4 ± 3^{E}	0.34 ± 0.21^{E}	0.50 ± 0.37^{E}	0.25 ± 0.19^{E}
	Trophic state	5 ± 1^{B}	0.31 ± 0.20^{E}	0.51 ± 0.34^{E}	0.25 ± 0.19^{E}
MP	Species	6 ± 4^{A}	0.97 ± 0.03^{A}	1.59 ± 0.61^{A}	1.06 ± 0.07^{AB}
	Substrate type	4 ± 2^{AB}	0.85 ± 0.16^{A}	1.04 ± 0.49^{AB}	0.72 ± 0.30^{BC}
	Trophic state	3 ± 1^{BC}	0.83 ± 0.20^{A}	0.86 ± 0.46^{AB}	0.62 ± 0.33^{C}
	All Traits	14 ± 5^{E}	0.91 ± 0.06^{A}	2.32 ± 0.33^{C}	0.91 ± 0.05^{A}
	Flow preference	3 ± 2^{B}	0.84 ± 0.16^{A}	0.92 ± 0.49^{B}	0.63 ± 0.33^{C}
	Assimilation type	2 ± 1^{D}	0.69 ± 0.20^{B}	0.42 ± 0.34^{D}	0.32 ± 0.27^{D}
	Life form	2 ± 1^{E}	0.48 ± 0.24^{B}	0.17 ± 0.25^{E}	0.10 ± 0.16^{E}
MIV	Species	21 ± 6^{A}	0.56 ± 0.14^{A}	1.69 ± 0.46^{A}	0.68 ± 0.17^{A}
	Feeding type	9 ± 2^{B}	0.65 ± 0.17^{B}	1.41 ± 0.39^{B}	0.66 ± 0.17^{B}
	All Traits	18 ± 3^{C}	0.74 ± 0.10^{C}	2.15 ± 0.32^{C}	0.85 ± 0.06^{C}
	Zonation	4 ± 1^{D}	0.54 ± 0.21^{A}	0.79 ± 0.30^{D}	0.45 ± 0.18^{D}
	Saprobic state	5 ± 1^{E}	0.59 ± 0.21^{AC}	0.96 ± 0.38^{E}	0.52 ± 0.21^{D}
F	Species	5 ± 4^{A}	0.74 ± 0.21^{A}	1.08 ± 0.61^{A}	0.63 ± 0.29^{A}
	Feeding type	3 ± 1^{B}	0.66 ± 0.30^{ABC}	0.58 ± 0.36^{B}	0.41 ± 0.28^{B}
	Reproductive strategy	3 ± 1^{C}	0.69 ± 0.23^{AC}	0.72 ± 0.35^{B}	0.47 ± 0.23^{B}
	All Traits	9 ± 3^{D}	0.85 ± 0.10^{D}	1.82 ± 0.24^{C}	0.84 ± 0.05^{C}
	Habitat preferences	2 ± 1^{E}	0.56 ± 0.28^{BC}	0.29 ± 0.30^{D}	0.20 ± 0.21^{D}
	Migration	2 ± 1^{E}	0.46 ± 0.29^{B}	0.17 ± 0.23^{D}	0.11 ± 0.17^{D}

information is crucial for assessing the applicability of the concept of TS in freshwater ecosystems, e.g. for understanding advantages and limits of using coarser taxonomic resolution than species (Bevilacqua et al. 2012) or functional surrogates instead of classical species data.

The applicability of taxonomic sufficiency for ecological analyses

The post-hoc univariate comparison of 2^{nd}-stage correlation coefficients clearly demonstrates that the threshold of losing statistically significant information when applying TS strongly differs between taxonomic groups. In addition, the applicability of TS is influenced by the scale of the investigated effects. The influence of effect scale is evident by the higher 2^{nd}-stage correlation between finer and coarser taxonomic levels throughout all investigated taxonomic groups for data sets considering very pronounced differences between treatments (e.g. upstream and downstream sides of weirs in data set 1 or different rivers in data set 2) than for data sets considering small-scale natural variability (e.g. natural variation between habitat types within one river in data set 3). These findings indicate that the required taxonomic resolution rather depends on the investigated taxonomic group and the extent of the studied effects than on the ecosystem type. The differences in the applicability of TS between taxonomic groups are probably founded in the complexity of the systematic classification of the respective groups in a certain geographic region (Heino & Soininen 2007), which in turn results in differences in taxonomic diversity. In groups with a relatively low taxonomic diversity, the application of coarser taxonomic levels for community analyses can cause significant loss of information because of the aggregation of species with differing ecological requirements. For instance, in the group of freshwater fishes, species with contrasting specialization are aggregated on family-level. In the data sets investigated in our study, species having very different ecological requirements but belonging to the same family (cyprinids) co-occurred (e.g. high current preference: *Chondrostoma nasus* L. or *Barbus barbus* L. and preference for lentic habitats: *Scardinius erythrophthalmus* L. or *Rhodeus amarus* A.). This grouping of ecologically different characteristics may have limited the habitat-type separation in the multivariate community pattern analysis, resulting in a low correlation between species- and family-level. The high discrepancy between genus- and family-level limits the practical use of TS for fishes, e.g. in European freshwater ecosystems because many genera are species-poor, and genus identification requires the same expert knowledge as species identification, so it is not more effective than species identification (Mandelik

et al. 2007). However, the practical use of TS for fishes may be different in regions where fish species diversity is very high compared to our data sets (e.g. Amazon basin, Congo basin, Southern Asia Rosenzweig & Sandlin 1997). In contrast to fishes, a comparison of the results for macroinvertebrates from our study with previous studies from other types of habitats (freshwater: Jones 2008, Buss & Vitorino 2010; marine: Olsgard et al. 1997, Chainho et al. 2007, Sajan et al. 2010; terrestrial: Blanche et al. 2001, Cagnolo et al. 2002, Landeiro et al. 2012) generally suggests a high robustness for multivariate community analyses up to family-level. A similar result was obtained for periphyton. Because of the high numbers of families and orders in macroinvertebrates and periphyton, the probability that ecological differences are conserved on coarser taxonomic levels is increased for these groups compared to fishes. This explanation is supported by Bevilacqua et al. (2012), who found a significant relationship between Φ and correlations between species and coarser taxonomic level community patterns. Bevilacqua et al. (2012) also could detect this relationship for randomly aggregated data. For this reason, it is likely that the phenomenon of higher Φ increasing the probability of similarity between species and coarser taxonomic levels is a simply stochastic relationship. However, the use of Φ as a predictive measure for the effectiveness of TS in a certain set of species does not include the possible effects of an uneven distribution of species to higher taxa. This influence is evident from comparing Φ and Δ^+ of periphyton and macrophytes in relation to the change in multivariate community patterns between genus- and species-level. The lower predictive power of Φ is probably caused by the more even hierarchical taxonomic distribution of periphyton species on genera than the distribution of macrophyte species on genera. Taxonomic diversity measured by Δ^+ (Clarke et al. 2001) includes these effects and was suitable to predict the applicability of different taxonomic levels as evident from the high correlation coefficients. Thus, the average taxonomic breadth can be used as surrogate for a pre-estimation of the minimum necessary taxonomic level. This measure can easily be calculated from species lists obtained from pre-assessments in all geographic regions and ecosystem types. 2^{nd}-stage NMDS is a universally applicable and powerful method for the selection of a combination of functional traits with minimum phylogenetic- and autocorrelation that maximize information content.

The capability to detect environmental gradients using coarser levels than species

Previous studies on macrobenthic communities in marine and freshwater environments suggested that coarser taxonomic levels may be more appropriate for the quantification of environmental changes than the species-level (Ferraro & Cole 1990, Warwick 1993, Bailey et al. 2001). This finding was explained by the high noise that is caused by the individual reaction to natural environmental gradients of each species, which can disguise the effects of anthropogenic disturbance (Warwick 1993). Based on our results, this hypothesis has to be rejected for freshwater systems. Aggregation level had no significant influence on the capability to detect environmental gradients in our data sets, and correlation values decreased at levels coarser than species. This finding is also supported by results from marine meiofauna (Olsgard et al. 1997). Consequently, the application of moderate taxonomic levels in taxa identification may be justifiable if financial resources are limited. However, as indicated by the decreasing trend of correlation values and rising standard deviations, the reliability of the results suffers from aggregation, at least for class and phylum-level. Subtle environmental changes, which may have an effect on rare or threatened species, can be overlooked if TS is applied.

The applicability of higher-taxon diversity as surrogate for species diversity

Besides the effects of TS on multivariate community patterns, there were also effects on the quantification of biodiversity, depending on the diversity measure applied. Although Heino & Soininen (2007) found a strong correlation between species richness and higher taxon richness for stream macroinvertebrates and diatoms, the strong decrease of richness already occurring by aggregation to genus- or family-level in our study indicates that biodiversity can be strongly underestimated if richness is used as the only diversity measure. In contrast, Evenness, Shannon Index, and Simpson's Index are even less affected by taxonomic data aggregation than multivariate community patterns. However, the most pronounced loss of information was detected from order- to class-level for all data sets, taxonomic groups, and diversity indices. Consequently, the order-level seems to be a critical threshold of taxonomic resolution for aquatic ecology, below which the explanatory power of biodiversity measures strongly decreases. If the concept of TS is being applied to new systems or habitats, an initial combination of several measures of diversity (Heino 2008*a*) and multivariate community analyses, calculated from genus-, family-, or order-level (adapted to the specific

taxonomic group and the available resources) can serve as a reliable surrogate for species diversity for periphyton and macroinvertebrates. In contrast, the strong changes in the outcome of the analysis from species to family-level observed for macrophytes and fishes suggest that genus- or species-level identification is necessary for these groups.

The applicability of functional surrogates for multivariate analyses

Alternative groupings according to functional traits can potentially reveal additional information concerning ecosystem properties beyond taxonomic composition (Usseglio-Polatera et al. 2000, Poff et al. 2006). Thereby, low statistical and phylogenetic correlations among traits, e.g., as detected in our study for migration types of fishes and life form of macrophytes (ρ species-level ≤ 0.31), are desirable to ensure statistical independence and to maximize information content (Townsend & Hildrew 1994, Cadotte et al. 2011, Poff et al. 2006). For the faunal groups in our study, functional traits related to feeding types of fishes and macroinvertebrates were more strongly correlated with species-level data than traits referring to habitat use (e.g. migration type and habitat preferences of fishes, saprobic state, and zonation of macroinvertebrates). This finding is in line with the assumption that some functional traits are phylogenetically more conserved than others (Usseglio-Polatera et al. 2000). In turn, a similar clustering of species into groups derived from both taxonomy and functional traits can be explained by the phylogenetic conservation of traits, e.g. through similar morphologic and physiologic characteristics related to the feeding type within one genus or family.

The applicability of functional diversity as surrogate for species diversity

Functional diversity (richness and Shannon Index) that can be calculated from species-level data aggregated to functional groups strongly depends on the number of trait states. For instance, the functional diversity of migration types of fishes (only 3 categories) is lower than species diversity, whereas other traits including many categories (e.g. 10 feeding types of macroinvertebrates) typically reveal higher diversity values. This finding is supported by Bêche & Statzner (2009), who pointed out the weakness of trait richness as a measure of functional diversity in stream macroinvertebrates. In contrast to richness, Evenness and Simpson's Index for single traits and the combination of all investigated functional traits per taxonomic group resulted in high diversity values for all data sets investigated herein. The functional diversity of All Traits (representing the functional trait niches of

the investigated taxa according to Poff et al. 2006) measured by Simpson's Index sometimes even exceeded species diversity, regardless of whether the combination of all traits caused a decrease or an increase in richness. Consequently, this approach appears to be more comprehensive for analyzing functional diversity than the consideration of single traits.

The detection of environmental gradients applying functional surrogates

Because other authors hypothesized that functional traits represent evolutionary responses to environmental selective forces (e.g. Southwood 1977, Poff et al. 2006, Heino 2008*b*), alternative groupings according to functional characteristics of species were expected to be more suitable for detection of environmental gradients than species data. Moreover, testing the effects of the environment on the environmental relations of taxa (functional traits) can theoretically be prone to circular reasoning, resulting in high correlation values. Surprisingly, environmental correlations with data aggregated to functional groups, taxonomic groups, and functional traits were generally lower than for species-level data. This low correlation may be a result of the combination of traits and environmental variables investigated or the fact that functional traits are strongly affected by biotic interactions (Tonn 1990, Poff 1997, Carey & Wahl 2011) and habitat complexity (Heino 2008*b*). Consequently, it seems to be most reasonable to choose a combination of functional traits from different trophic levels that should preferably reveal low statistical and phylogenetic correlations (Mouillot et al. 2005). Following the results of our study, this set of functional traits could, for instance, be a combination of trophic state of periphyton and life form of macrophytes (primary producers), saprobic state of macroinvertebrates (primary and secondary consumers), and migration type or habitat preferences of fishes (secondary consumers). This approach assures the inclusion of all important foodweb components but avoids autocorrelation.

Effects of numerical resolution

Data transformation could have strong effects on the results of ecological analyses that even exceeded the effects of taxonomic resolution for almost all data sets and taxonomic groups herein. The same observation has been made in aquatic environments by other authors (Olsgard et al. 1997, Anderson et al. 2005, Heino 2008*a*), so it is likely that this is a general phenomenon, at least in aquatic ecosystems. Especially, reductions of quantitative information (relative abundances

or presence–absence data) proved to strongly alter multivariate community patterns. This finding suggests that quantitative information can be more important than taxonomic detail if there are gradients in the productivity of the system under study, e.g. in weir-influenced river stretches (data set 1). In contrast, untransformed data or $\sqrt{x}$-transformed data contain the most information about productivity of habitats. However, classical monitoring protocols are often based on nonquantitative sampling techniques (e.g. periphyton sampling for the European Water Framework Directive; Schaumburg et al. 2007) or according to the Rapid Bioassessment Protocols of the US Environmental Protection Agency (Barbour et al. 1999). A lack of standardization in sample size makes the use of % abundance data or presence–absence the only choice for data analyses. Consequently, a rethinking of currently applied monitoring techniques may be required. Because financial resources for monitoring programs are typically limited, effort expended in taxonomic detail often could be better spent in quantitative sampling techniques and the consideration of multiple taxonomic groups, at least if the main objective is the monitoring of ecosystem changes rather than the conservation of specific rare species. Moreover, the effects of numerical resolution on the applicability of TS have to be considered. Because of the more pronounced effects of taxonomic aggregation on presence–absence data and % abundance data (Fig. 2.3), the application of TS may be less appropriate if quantitative data are not available.

Conclusions

Our study for the first time provides statistical threshold levels for the application of TS for ecological analyses in 4 different freshwater groups. The results of our study suggest that TS can be applied up to family- or order-level for macroinvertebrates and periphyton (Δ^+ species–phylum > 0.77), whereas fishes and macrophytes (Δ^+ species–phylum < 0.68) should be identified to genus- and species-level. However, for investigating the effects of environmental changes based on species-specific tolerances (e.g. water-quality determination; Lenat & Resh 2011), the use of species-level data appears generally advantageous. Because the applicability of TS was higher in data sets from impaired systems and with large spatial scale in our and in other studies (e.g. Bevilacqua et al. 2012), TS may be of great relevance for environmental-impact assessments, monitoring, and efficiency control of restoration measures. The strong impact of numerical data resolution on the outcome of ecological analysis suggests investing effort in quantitative data and number of spatial and temporal replicates rather than in

taxonomic detail. The consideration of functional traits as additional descriptive variables, e.g. plotted on taxonomic data as vectors in multivariate statistics (Mueller et al. 2011), is a more integrative approach to analyze interactions between taxonomic composition, environmental conditions, and ecosystem functions than using functional traits as input variables for NMDS.

2.3 Simultaneous consideration of multiple taxonomic groups in ecological monitoring: from single indices to multivariate indication of ecosystem change

A similar version of this section is in preparation for publication:

Mueller, M., Pander, J. & Geist, J. (2013) Simultaneous consideration of multiple taxonomic groups in ecological monitoring: From single indices to multivariate indication of ecosystem change. *In prep*.

Abstract

During the last two decades, the holistic assessment of responses to environmental disturbance simultaneously considering several biotic groups (ecosystem approach) has become increasingly important in ecological monitoring. The most common solution to combine data of different taxonomic groups is the calculation of compound indices comprising several individual indicators. However, these indices run the risk of cancelling out underlying trends when single components change in different directions. In contrast, multivariate community analyses are supposed to be more sensitive to detect environmental responses, since information on the abundance of multiple species is not reduced to a single dimension. In this study we propose a standardized procedure for merging species abundance data from different taxonomic groups, aiming at multivariate community analyses on ecosystem scale. The indicative power of multivariate analyses is compared with two single score indices integrating data from all involved taxonomic groups (Ecological Quality Class according to the European Water Framework Directive and overall Shannon Index). The results revealed that multivariate indication of ecosystem change is much more sensitive for the detection of environmental impacts and restoration effects than single numeric score indices. Compared to common monitoring systems based on compound indices, the multivariate analysis of multiple taxonomic groups presented herein is feasible with the same sampling effort, and is independent of the investigation scale and the occurrence of certain indicator taxa. Since ecological community data are structured similarly throughout freshwater, marine and terrestrial ecosystems, the presented methods for data combination and multivariate indication can be analogously applied in any other habitats and can improve data integration across ecosystem borders.

Introduction

In the context of increasing degradation of natural resources, knowledge on the current status and future change of ecosystems is crucial to maintain the ecosystem services they provide (Millennium Ecosystem Assessment 2005). Twenty years ago the United Nations proclaimed the ecosystem approach as the primary framework of the Convention on Biological Diversity (CBD, United Nations 1992), which proposed a holistic way to assess and manage ecosystems considering all plant, animal and bacterial communities and their non-living environment. From 1992 onwards, the objectives of the CBD have been gradually incorporated into international environmental legislation, considering the ecosystem approach by declaring the inclusion of multiple taxonomic groups into environmental monitoring mandatory (e.g. Republica de Panama 1998, European Parliament 2000, Republica de Argentina 2002*a*, Republic of Namibia 2004). Consequently, there is an increasing need to be able to evaluate change across multiple taxonomic groups when considering overall ecosystem state. To date the European Water Framework Directive (WFD) is a rare example of environmental regulation that gives concrete instructions on how data from different taxonomic groups should be integrated. The index-based WFD data analysis concept, similar to other multi-metric indices of biotic integrity (IBIs, first published for the assessment of fish communities by Karr et al. 1987; e.g. Barbour et al. 1999, Birk et al. 2012, Vačkář et al. 2012, Wilson & Bayley 2012) or diversity indices, is based on the general assumption that all considered metrics and taxonomic groups have to be fitted into single numeric scores. However, the strong reduction in information content makes this approach prone to levelling out valuable information in the final overall assessment (e.g. if two groups react in an opposite way) and can lead to erroneous conclusions about the real ecological status (Reynoldson et al. 1997, Millennium Ecosystem Assessment 2005, Caroni et al. 2013). Caroni et al. (2013) recently proposed that keeping all the information supplied by different taxonomic groups would produce a better overview of the entire ecosystem than calculating single score indices, but did not suggest a precise solution for data analysis and the presentation of the results. In the few scientific studies in which several taxonomic groups are considered ecological community analyses are typically run separately for each group (e.g. Heino 2001, Paavola et al. 2006, Johnson et al. 2006). The outcome of such complex multi-group studies is likely to be an assemblage of numerous results plots from multivariate or univariate statistics (e.g. Heino et al. 2005, O'Connor et al. 2000) and it remains difficult to get a holistic and at the same time clear picture of the ecosystem as a whole.

Despite of the availability of multivariate methods that allow for the simultaneous inclusion of a large number of variables without reducing information to single numeric scores (e.g. multidimensional scaling, principle components analysis, correspondence analysis), which all proofed to be very effective in the analysis of single taxonomic groups in the past (Reynoldson et al. 1997, Mueller et al. 2011; RIVPACS-like macroinvertebrate evlautation systems: Wright 2000, Smith et al. 1999), these techniques are currently not applied to combine species abundance data from several levels of biological organisation within one analysis. This may be due to difficulties in combining species abundance data from taxonomic groups that require different sampling methods and investigation scales, resulting in different data structure. Consequently, the integration of multiple taxonomic groups needs standardized and statistically appropriate combination rules.

The aim of this study was to develop a standardized procedure for the generation of a combined data matrix merging species abundance data from different taxonomic groups that can be used in common multivariate community analysis. Specifically, we hypothesize that (I) the generation of a combined species abundance data matrix from multiple taxonomic groups based on different sampling techniques and investigation scales is possible, but needs some standardisation/normalisation to account for differences in species numbers and numerical scale. (II) The combination of multiple taxonomic groups does not reduce the capability of detecting environmental gradients and differences between treatments compared to single taxonomic groups if multivariate statistics are applied. (III) Combining information from multiple taxonomic groups using multivariate analysis is advantageous for the monitoring of environmental changes compared to the calculation of single score indices (i.e. WFD Ecological Quality Class and Shannon Index) integrating multiple groups.

Material and Methods

Data sets

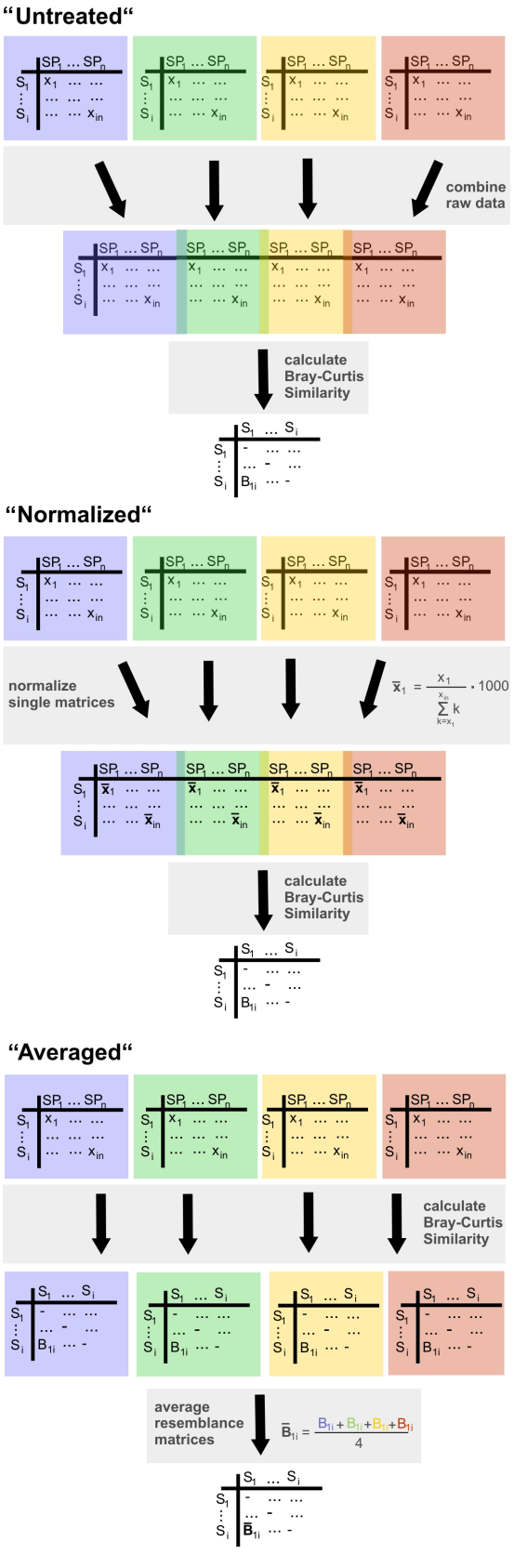
All analyses were carried out exemplarily for three large datasets from freshwater ecosystems focused on the monitoring of environmental impacts and restoration success. Each data set includes the taxonomic groups periphyton, macroinvertebrates, macrophytes and fishes. Data structure was partially different between taxonomic groups and data sets concerning quantitative or semiquantitative data, sampling methods, as well as number of sampled river stretches and treatments. Data set (1) was focused on a pairwise comparison of upstream and downstream

sides of weirs in five different rivers (Mueller et al. 2011), data set (2) was collected from different freshwater habitat types (Stammel et al. 2012) before and after floodplain restoration at the German section of the Danube River and data set (3) is focused on the effects of four substratum restoration treatments in six rivers distributed throughout the German federal state Bavaria (for a characterisation of the study rivers see Braun et al. 2012). Details on the sampling methods and sampling design are presented in Mueller et al. (2013*a*). For data sets 2 and 3 one additional sampling period (one year after restoration) applying the same sampling design as described in Mueller et al. (2013*a*) was included in this study.

Methods for merging raw data

Three principal different methods for combining species abundance data from different taxonomic groups were applied (Fig. 2.8) and compared by multivariate Spearman rank correlations and 2^{nd}-stage NMDS of the resulting resemblance matrices (based on Bray-Curtis Similarity) in PRIMER v6 (Clarke & Gorley 2006). This procedure is commonly used to compare multivariate community patterns derived from different taxonomic groups, data transformations or taxonomic levels and was first published by Somerfield & Clarke (1995). As first and most simple method, raw species abundance data matrices were combined by inserting the species of each group as additional columns (Fig. 2.8). This method is referred to as "untreated" in the following text. "Untreated" data were used as reference to analyse effects of differences in species numbers and numerical scale between taxonomic groups. Secondly, data of periphyton, macrophytes, macroinvertebrates and fishes were normalized prior to the combination of data matrices by dividing each value by the sum of all abundance values throughout

Figure 2.8 ***(on the next page)*****:** Schematic of the three different combination methods for raw species abundance data "Untreated", "Normalized" and "Averaged". S = sample, SP = species/taxon, x = abundance per sample, B = Bray-Curtis Similarity, $\bar{x}$ = normalized abundance, $\bar{B}$ = averaged Bray-Curtis Similarity, blue square = taxa abundance data from taxonomic group 1 (e.g. fishes), green square = taxa abundance data from taxonomic group 2 (e.g. periphyton), yellow square = taxa abundance data from taxonomic group 3 (e.g. macrophytes), red square = taxa abundance data from taxonomic group 4 (e.g. macroinvertebrates)


"Untreated"
combine raw data
calculate Bray-Curtis Similarity
"Normalized"
normalize single matrices
calculate Bray-Curtis Similarity
"Averaged"
calculate Bray-Curtis Similarity
average resemblance matrices

the matrix and multiplying with 1,000 (Fig. 2.8). The multiplication with the factor 1,000 was applied to avoid very small numbers and to enhance legibility. The division by the sum of the matrix was used for normalization, i.e. to ensure that each taxonomic group had the same weighting in the subsequent analyses without losing quantitative information (which would happen by applying relative abundances, i.e. the sum of each row). The respective R script for automatic matrix normalisation and combination is provided in Fig. 2.9. This method is referred

```
# Create an array containing all files to be processed, pattern is a regular
# expression, so .csv$ means "all files ending in .csv"
# We store all input files in a subdirectory to avoid processing old data in
# repeated runs
norma.files <- dir(path="input", pattern=".csv$")

# Delete old variable from previous runs
if (exists("norma.data"))
    rm(norma.data)

# Process each file
for (currfile in norma.files) {
    # Read in data, csv2 uses semicolons as separator, which is what you get by
    # Excel's csv-export; row.names=1 defines the first column as name vector
    # for all entries
    currdata <- read.csv2(paste("input/", currfile, sep=""), row.names=1, fileEncoding=
    "iso-8859-1")
    # normalize data accordingly
    currdatanorm = currdata/sum(currdata) * 1000
    # Concatenate normalized result to previous ones columnwise, if we're in the
    # first loop, just assign the data
    if (exists("norma.data"))
        norma.data <- cbind(norma.data, currdatanorm)
    else
        norma.data <- currdatanorm
}
# Write data to file; encoding and line endings compatible to Excel
# ATTENTION: Overwrites existing files without notice, so use an unique filename!
write.csv2(norma.data, "normalized-data-r-output.csv", fileEncoding="iso-8859-1", eol =
"\r\n")
```

Figure 2.9: R script for matrix normalization. Save single abundance data matrices as .csv files in a folder named "input" (rows = samples, columns = taxa; abbreviations should not contain more than 8 characters). Save "norm-app-csv.r" from Fig. 2.9 as text file in same directory as "input". Open R (available at http://www.r-project.org) and change directory to the folder where "input" and "norm-app-csv.r" are located. Run the script by typing "source(norm-app-csv.r)". A combined and normalized output file is automatically generated and saved in the folder where "norm-app-csv.r" is stored

to as "normalized" in the following text. Third, resemblance matrices were calculated separately for each taxonomic group based on Bray-Curtis Similarity

in PRIMER v6, and averaged in Microsoft® Excel (Fig. 2.8). The resulting overall resemblance matrix was also expected to contain the same weighting for each group. This method is called "averaged" in the following text and the averaged resemblance matrix was re-imported in PRIMER v6 to be compared in 2^{nd}-stage NMDS analysis with resemblance matrices from untreated and normalized data as well as the resemblance matrices of single taxonomic groups.

Evaluation of the statistical performance of combined data

PERMANOVA (permutational MANOVA, Anderson et al. 2008) in PRIMER v6 (Plymouth Marine Laboratory, Plymouth, UK) was used to analyse differences between treatments (i.e. sampling time points and restoration treatments in data sets 2 and 3). PERMANOVA is a routine for testing the multivariate response to one or more factors on the basis of any resemblance measure. It computes Pseudo-F values (the larger the value of F, the more likely it is that the null hypothesis of no differences among the group means is false, Anderson 2001) and permutational P-values, which were used to compare the discriminatory power of "overall abundance data" and single taxonomic groups. To evaluate the capability to detect community responses to environmental gradients of "overall abundance data", BEST BV-STEP analyses were carried out in PRIMER v6 for combined data and for single taxonomic groups. The BEST-procedure performs a stepwise search and Spearman rank correlation to find a minimum combination of environmental variables which results in maximum possible correlation with the biotic data. Following Olsgard et al. (1997), the strength of Spearman rank correlation was used to evaluate the goodness of description of community patterns resolved from different resemblance matrices (overall, periphyton, macrophytes, macroinvertebrates, fishes).

Comparison of multivariate indicators and single numeric score indices

To compare the sensitivity of multivariate analysis on the overall data matrix and integrative single score indices, the ecological status according to the WFD and overall Shannon Index (Shannon & Weaver 1949) were calculated. The ecological status was determined applying the European/German evaluation systems for the biological quality elements fishes (European fish index EFI, Pont et al. 2007), macroinvertebrates (PERLODES, AQEM Consortium 2002) as well as diatoms, phytobenthos and macrophytes (PhyLib, Schaumburg et al. 2007) using pooled data from all replicates from each treatment (upstream and downstream sides of

weirs in data set 1, pre- and post-restoration status in data sets 2 and 3). The usage of pooled data was necessary to reach minimum abundances for index calculation and equals the required multihabitat sampling technique of the WFD protocols. Since the calculation method for combining the results from the different biological quality elements can have a major impact on the results, we applied two different combination methods. First, the currently practiced "one-out-all-out" combination rule was used, where the overall Ecological Quality Class is determined by the biological quality element with the lowest value. Second, the arithmetic mean of the index values from all biological quality elements (periphyton (including diatoms and remaining phytobenthos), macrophytes, macroinvertebrates, fishes) was calculated. Additionally, overall Shannon Index was calculated as further single score index from the normalized overall abundance data (integrating periphyton, macrophytes, macroinvertebrates and fishes) using the DIVERSE procedure in PRIMER v6. Ecological status scores where compared descriptively between treatments and the overall Shannon Indices were analysed by univariate statistics (Mann-Whitney *U*-Test since all data were not normally distributed). Results from the two single numeric score indices were compared with those from multivariate analysis of overall community composition (Bray-Curtis Similiarity and PERMANOVA *P*-values).

Results

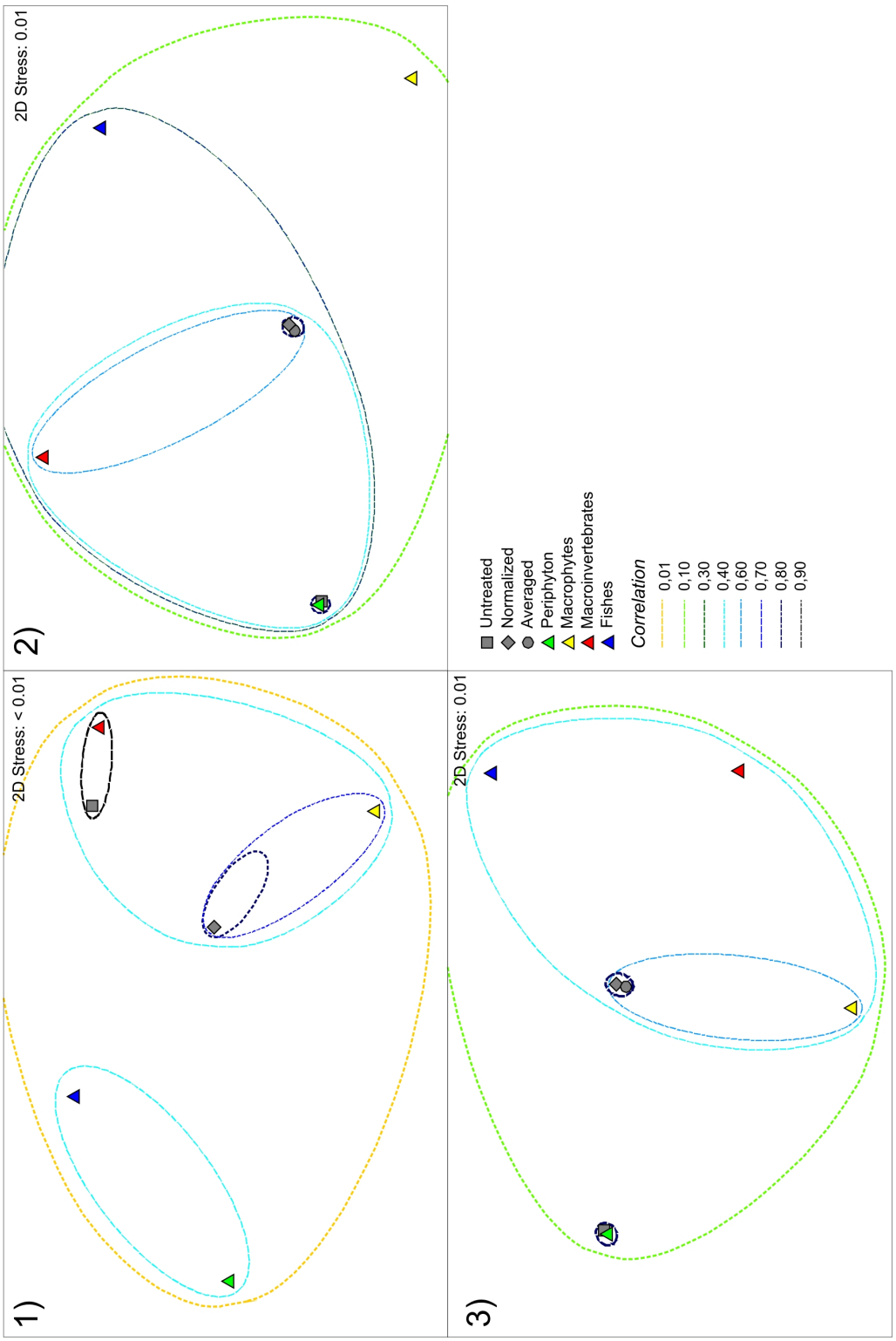
The ecosystem level multivariate community pattern derived from the method "untreated" was strongly dominated by one of the four taxonomic groups in all three data sets (Fig. 2.10). The numerically dominant group in terms of individuals and species usually was periphyton. Only in data set 1, where periphyton data structure was different (presence-absence data), the "untreated" pattern was dominated by the macroinvertebrates. The contribution of single taxonomic groups to the overall community pattern ranged from 0.07 to 1.00 for the method "untreated" (0.43 ± 0.34) and was more evenly distributed between taxonomic groups for the methods "averaged" (range = 0.18-0.86; mean = 0.59 ± 0.16) and "normalized" (range = 0.29–0.69; mean = 0.57 ± 0.11). The results from the methods "averaged" and "normalized" were similar to each other, with a mean Spearman rank correlation coefficient of 0.90 (SD = 0.01, Fig. 2.10).

PERMANOVA main tests revealed intermediate Pseudo-*F* values for combined data (data set 2: Pseudo-*F* (restoration treatments and time points) = 6.08, Pseudo-*F* (habitats) = 5.27; data set 3: Pseudo-*F* (restoration treatments and time points = 2.41, Pseudo-*F* (habitats) = 12.35) compared to single taxonomic groups

(data set 2: Pseudo-F (restoration treatments and time points) = 1.87-7.73, Pseudo-F (habitats) = 2.50-15.75; data set 3: Pseudo-F (restoration treatments and time points) = 0.96-6.52, Pseudo-F (habitats) = 6.52-19.79). No differences were detected between P-values from PERMANOVA main tests on combined data and single taxonomic groups ($P = 0.001$), except for macrophytes in data set 3, where P-values were much higher and indicated no significance ($P = 0.53$). Test results for single habitats within the data sets also differed among taxonomic groups. The subset of habitats with a significant difference in community composition revealed by analysis of combined data represented a synthesis of the results from single taxonomic groups. However, for the analysis of single taxonomic groups, several samples had to be excluded from fishes in data set 2 and macrophytes in data set 3 due to all-zero-values, limiting statistical power. Only for combined data all samples could be integrated into the analyses for each data set.

BEST analyses revealed highest correlation with environmental variables for macrophytes ($\rho = 0.67 \pm 0.16$), fishes ($\rho = 0.61 \pm 0.08$) and combined data (ρ "normalized" $= 0.60 \pm 0.04$). Lowest correlation occurred for periphyton ($\rho = 0.42 \pm 0.21$). The number of included variables ranged between 1 and 6 for single taxonomic groups and between 1 and 3 for combined data ("normalized"). Shannon Index of combined data did not significantly differ between habitats or restoration treatments for data sets 2 and 3, while multivariate analyses indicated a significant change of overall community composition after restoration in river (R) and oxbow (O) habitats of data set 1 and 3 of 6 study rivers from data set 2 (Table 2.9). In data set 1, univariate statistics revealed significant differences in Shannon Index between upstream and downstream sides of weirs in three of five rivers, while multivariate analysis of combined species abundance data could detect weir effects on overall community composition for all study rivers at different levels of significance (Table 2.9). The ecological quality class (EQC) values could not be analysed with statistics due to the need for pooling data over all replicates to reach minimum abundances for index calculation. The descriptive comparison of

Figure 2.10 ***(on the next page)*****:** 2^{nd}-stage non-metric multidimensional scaling (NMDS) of resemblance matrices from the taxonomic groups periphyton, macrophytes, macroinvertebrates and fishes as well as resemblance matrices from a combination of all four groups, generated from data sets 1), 2) and 3) with three data combination methods (description of methods see Fig. 2.8)


2D Stress: 0.01
2)
2D Stress: < 0.01
1)
2D Stress: 0.01
3)
Untreated
Normalized
Averaged
Periphyton
Macrophytes
Macroinvertebrates
Fishes
Correlation
0,01
0,10
0,30
0,40
0,60
0,70
0,80
0,90

EQC values between treatments generally had a lower sensitivity to detect differences in the overall community than multivariate community analysis (Table 2.9). Multivariate analyses also allowed a finer graduation of effect size than EQC values. Furthermore, the resulting EQC was strongly dependent on the method of combining the indices from single taxonomic groups. The calculation according to the "one-out-all-out" rule of the WFD evaluation system revealed more differences in EQC between treatments than averaged values of the multi-metric indices from single taxonomic groups. However, the results from both calculation methods often were not in line with those from multivariate community analysis and univariate analyses of single parameters (e.g. productivity, species richness, Shannon Index). For instance, R and O habitats of data set 2 showed a highly significant change in overall community composition after restoration, while the EQC value did not change. The rivers P and MG of data set 3 did not change according to PERMANOVA analysis and univariate analysis of Shannon Index, while the EQC value even indicated degradation after restoration. However, single parameter analysis indicated a slightly increased productivity and species richness which is generally not considered as degradation. For the detection of weir effects (data set 1) the "one-out-all-out" calculated EQC was reliable, but did not allow a differentiation of effect size which is possible in multivariate analysis by comparing Bray-Curtis Smiliarties and PERMANOVA *P*-values.

Discussion

This study presents and evaluates standardized methods for the implementation of simultaneous multivariate analyses of community structure integrating multiple taxonomic groups, applying Bray-Curtis Similarity and NMDS. Since ecological community data usually are available as "species" x "sites" matrices, with a type of abundance in each cell, independent of habitat type and ecosystems, the presented methods for data combination may be universally applied to integratively assess community change in multiple taxonomic groups. The main advantage of this multivariate approach is the high sensitivity to stressors at multiple scales and the universal applicability across ecosystem borders and geographic regions.

Representativeness of different methods for merging data sets

The methods "normalized" and "averaged" were similarly suitable to produce a representative overall community pattern with a balanced contribution of each taxonomic group. As expected, the simple combination of "untreated" data cannot

Table 2.9: Comparison of multivariate analysis using multi-group abundance taxa with overall Shannon Index and Ecological Quality Class values. River/Habitat = Codes for the studied rivers/habitats; Bray-Curtis = Bray-Curtis Similarity between treatments; *P* PERMANOVA = *P*-values derived from PERMANOVA analysis between treatments; Δ H = difference in Shannon Index between treatments; *P U*-test = *P*-value derived from Mann-Whitney *U*-Test between treatments; Δ EQC = difference in Ecological Quality Class according to Water Framework Directive, calculated following "one-out-all-out" combination rule; Δ EQC (av) = difference in Ecological Quality Class according to Water Framework Directive, calculated by averaging ECQ values from diatoms, phytobenthos, macrophytes, macroinvertebrates and fishes. Significant *P*-values are printed in bold

Data set	River/ Habitat	Bray-Curtis	*P* PERMANOVA	Δ H	*P U*-test	Δ EQC	Δ EQC (av)
1	W	61	< **0.01**	0.25	< **0.05**	1	0
	S	48	< **0.01**	0.31	< **0.01**	1	1
	M	47	< **0.01**	-0.04	> 0.05	1	0
	L	74	< **0.05**	-0.11	> 0.05	-1	0
	G	48	< **0.01**	0.80	< **0.001**	0	0
2	R	29	< **0.001**	0.32	> 0.05	0	0
	O	33	< **0.001**	0.13	> 0.05	0	1
	D	31	> 0.05	-0.16	> 0.05	0	0
3	AG	34	< **0.01**	-0.40	> 0.05	2	2
	GO	61	< **0.05**	0.01	> 0.05	0	1
	MG	50	> 0.05	0.31	> 0.05	-1	0
	P	67	> 0.05	0.17	> 0.05	-1	0
	SR	28	< **0.05**	0.07	> 0.05	0	0
	W	79	> 0.05	0.14	> 0.05	0	0

be recommended, since the outcome is strongly dominated by the taxonomic group with highest numbers of species and individuals. For instance, in central European rivers algal species richness and cell numbers are usually much higher than numbers of fish species and individuals, as also evident in data sets 2 and 3. Compared to the use of percent-abundance data, which would result in a sum of 100% for each study site, the "normalizing" and "averaging" methods suggested herein preserve differences in total abundance between sites. Since the total number of individuals per site is an important proxy for ecosystem productivity (Karr et al. 1987, Oberdorff et al. 2002), it can be of great relevance not to lose this information in environmental impact assessments (Mueller et al. 2011). Since presence-absence data (e.g. periphyton in data set 1), percent-abundance data (e.g. macrophytes in data set 3) and quantitative data (fishes and macroinvertebrates in all data sets) generated using strongly different sampling methodology (e.g. electrofishing, surber sampling, estimation of coverage) had similar contributions to the overall picture after standardisation by "averaging" and "normalizing", these methods can be performed irrespective of data structure and sampling method, provided that all matrices include the same set of study sites. This can be advantageous if heterogenic data structures arising from different research fields, such as vegetation science (plant coverage is described as frequency of occurrence on a predefined scale) and zoology (single individuals are counted), have to be combined in large monitoring programs. The major disadvantage of the "averaging" method is that sites where one of the taxonomic groups is absent cannot be included in the analyses due to undefined similarity values for all-zero-samples. Since data from different taxonomic groups are combined in the form of abundance data prior to the calculation of resemblances in the procedure of "normalizing", all-zero-samples for single taxonomic groups do not have to be excluded provided that individuals of any other taxonomic group occurred in the sample. The normalized multi-group abundance data can also be used for the calculation of other similarity measures and multivariate statistics than those applied herein.

Comparison of multivariate indicators and single numeric score indices

The power of PERMANOVA and BEST to distinguish between treatments was not reduced for combined data and the results were reasonable compared to single taxonomic groups, indicating that the multivariate consideration mirrored restoration-induced changes in aquatic communities and the most important physicochemical drivers. In general, the significance level provided by multivari-

ate statistics allowed a very subtle quantification of effect size independent of the spatial scale of the data set (Table 2.9). In contrast, index-based ecological indicators are likely to be more suitable for the detection of broad-scale trends of change or major impacts (e.g. differences between upstream and downstream sides of weirs in data set 1) than for the detection of local scale stressors (Dahm et al. 2013) or habitat change (e.g. effects of restoration measures in data sets 2 and 3; Jähnig et al. 2011). The major advantage of multivariate methods is that the information content is not reduced to a single numerical score, which is more prone to levelling out adverse effects of single taxonomic groups. For instance, in data set 3, a strong decrease in macrophyte diversity disguised the restoration-induced increase in macroinvertebrate diversity in univariate analyses, making the EQC and overall Shannon Index less sensitive or even misleading indicators compared to multivariate overall community patterns. The WFD evaluation system and similar indices strongly depend on the presence of distinct indicator taxa at minimum abundances from each group included, which reduces their applicability in ecosystems with high anthropogenic pressure and dominance of neobiota (Arndt et al. 2009). This was evident in all of the studied data sets, resulting in uncertain values or even impossible index calculation for single taxonomic groups in many rivers although up to 25 replicate samples were pooled. Multivariate community analysis of overall data sets is independent of indicator taxa and minimum abundances and can be carried out with any set of species that may occur using the full number of replicates. Characteristic species for certain habitats can be determined case-specific and comprehensively within one analysis for all taxonomic groups, using e.g. SIMPER (Clarke & Gorley 2006) or TITAN (Baker & King 2010) analyses. This procedure can be applied across ecosystem types. Measures of productivity (e.g. biomass, numbers of individuals, length-frequency distributions) can be better accounted for in multivariate community analyses than in the WFD evaluation system, which for instance only allows a differentiation of fishes smaller and larger than 15 cm and delivers the same EQC value for a site with 5 individuals of the same species as for a site with 500 individuals. A further limitation of the multi-metric index system used in the WFD evaluation is its dependence on reference conditions, which are mostly based on expert estimation and are often hampered by knowledge gaps. Moreover, reference conditions are missing for many habitat types, such as for oxbows, lakes or artificial fish bypass channels (which differ in discharge and size from the main river) in aquatic ecosystems. Multivariate analyses can be applied more flexible, e.g. for relative comparisons between different habitats or for comparisons between treated sites and untreated reference sites that could be considered as "reference

to move away from" (Palmer et al. 2005). If existing reference conditions of single taxonomic groups were combined, the species abundance data combination and normalisation method proposed herein could also provide the basis to extend well established multivariate evaluation tools from their current application for single taxonomic groups (e.g. RIVPACS: Wright 2000, AUSRIVAS: Smith et al. 1999, BEAST: Rosenberg et al. 2000 for river macroinvertebrates) to more holistic models integrating several levels of biological organisation.

Conclusions

The combination of "normalized" taxa abundance data from multiple taxonomic groups proofed to be an easy applicable and representative method, which is a prerequisite for statistically verifiable multivariate indication of ecosystem change. Compared to common monitoring systems based on multimetric indices, the multivariate analysis of multiple taxonomic groups presented herein, based on current statistical methods, is feasible with the same sampling effort but constitutes a much more sensitive indicator. This approach can also be applied for taxonomic surrogates (e.g. coarser taxonomic levels, functional groupings, Mueller et al. 2013*a*) and can be performed independent of ecosystem type, sampling strategy and the occurrence of certain species. Existing global indices in aquatic systems (e.g. Water Framework Directive, Rapid Bioassessment) are restricted to certain ecoregions and tend to be insensitive to local scale stressors. In contrast, the methods proposed herein may be universally applied at multiple scales. The presented multivariate approach can help to improve data integration across borders of adjacent habitats and ecosystems that are exposed to similar stressors (e.g. alterations of groundwater level in aquatic and terrestrial floodplain habitats), thus allowing comparisons of effect size between ecosystem types. The R script for automatic matrix normalization and combination provided herein can be a useful tool for future ecosystem monitoring in freshwater, marine and terrestrial systems.

3 Validation of new methods for the ecosystem approach

3.1 The effects of weirs on structural stream habitat and biological communities

A similar version of this section is published:
Mueller, M., Pander, J. & Geist, J. (2011) The effects of weirs on structural stream habitat and biological communities. *Journal of Applied Ecology* **48**(6), 1450-1461.

Abstract

Most of the world's rivers are affected by dams and weirs. Information on the quantitative and qualitative effects of weirs across biological communities is crucial for successful management and restoration of stream ecosystems. Yet, there is a lack of comprehensive studies that have analysed the serial discontinuity in direct proximity of weirs including diverse taxonomic groups from algae to fish. This study compared the abiotic stream habitat characteristics upstream and downstream of weirs as well as their effects on the community structure of periphyton, aquatic macrophytes, macroinvertebrates and fish at five different study rivers. Physicochemical habitat characteristics discriminated strongly between upstream and downstream sides of weirs in terms of water depth, current speed, substratum composition and the transition between free-flowing water and interstitial zone. Accordingly, abundance, diversity, community structure and functional ecological traits of all major taxonomic groups were indicative of serial discontinuity, but the discriminative power of individual taxonomic groups strongly differed among rivers. The simultaneous inclusion of abiotic habitat variables, taxonomic diversity and biological traits in multivariate non-metric multidimensional scaling (NMDS) was most comprehensive and powerful for the quantification of weir effects. In some cases, the intra-stream discrimination induced by weirs exceeded the variation between geographically distant rivers of different geological origin and drainage systems. Community effects were generally detectable on high levels of taxonomic resolution such as family- or

order-level. River sections in spatial proximity to weirs are affected seriously and should be included in the ecological assessments of the European Water Framework Directive. Multivariate models which include several taxonomic groups and physicochemical habitat variables provide a universally applicable tool for the ecological assessment of impacts on serial discontinuity and other stressors on stream ecosystem health.

Introduction

The introduction of weirs into rivers is considered a major threat to aquatic biodiversity (Bunn & Arthington 2002). Alterations of hydraulic components can change the availability of habitat space, habitat quality and the structure of aquatic communities (Brunke et al. 2001, Almeida et al. 2009). The "Serial Discontinuity Concept" (Ward & Stanford 1983) describes the effects of physical barriers such as weirs and dams on biotic and abiotic components of lotic systems in a hypothetical framework, but experimental studies into the ecological effects of weirs have mostly focused on single rivers and single taxonomic groups. Habit et al. (2007) and Santos et al. (2006) could detect changes in the fish community at upstream sides of hydropower plants, Zhou et al. (2008) showed effects of a small dam on riverine zooplankton composition and Bredenhand & Samways (2009) recorded a serious decline in macroinvertebrate diversity below a dam in a small river. For a comprehensive assessment of the weir-induced serial discontinuity, it is essential to compare upstream and downstream sides of weirs in their abiotic and biotic habitat characteristics including all major taxonomic groups. This is important since there is recent evidence that cross-taxon congruence in diversity and community composition of aquatic organisms is typically low (Heino 2010). Consequently, comprehensive studies which assess the effects of human impacts on stream ecosystem health (i.e. on aquatic habitat quality and multiple biotic assemblages) are urgently needed.

The main objective of the study presented here was to analyze how abiotic stream habitat characteristics and biotic community effects in the taxonomic groups of periphyton, macrophytes, macroinvertebrates and fishes differ among upstream and downstream sides of weirs, located within carbonate and silicate streams in the three major drainage systems Elbe, Main/Rhine and Danube. Specifically, we hypothesize that upstream and downstream sides of weirs within one river differ in abiotic habitat characteristics, biodiversity and community composition and test if different taxonomic groups (of different trophic levels) differ in their response to weirs. Furthermore, we hypothesize that multivariate

methods which include abiotic (water depth, current speed, substratum composition, water chemistry) and biotic effects on different taxonomic levels (community composition, abundance, biomass, functional groups) are more suitable for the quantification of weir effects compared to the univariate consideration of single taxonomic groups.

Material and Methods

Study area

The study was carried out between May and July 2009 at five different study rivers distributed throughout major geological units in Bavaria, Germany (Fig. 3.1). All

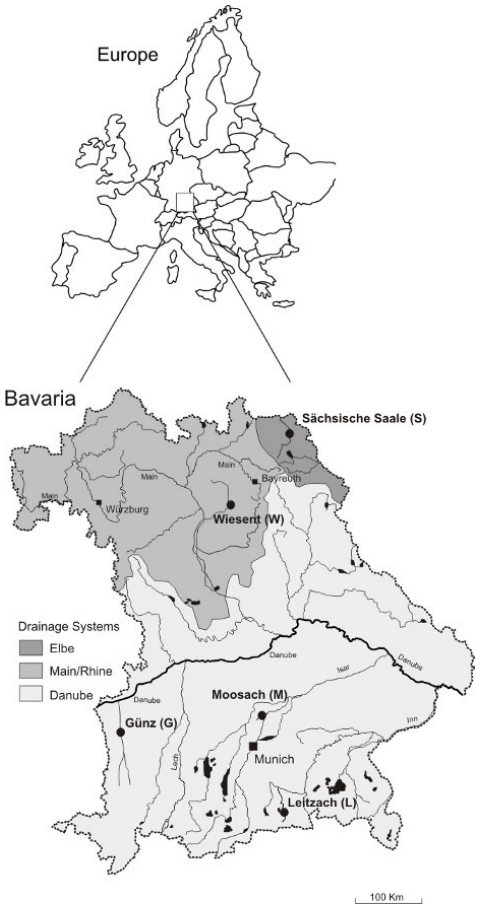


Figure 3.1: Location and map of the study area. The three major drainage systems (Elbe, Main/Rhine, Danube) are shown in different shades of grey. Study rivers are marked with black dots. For study river details see Table 3.1

rivers are located in an area of similar climatic conditions and have similar flow regimes which are governed by snow melt-induced peak flows during spring (Table 3.1). All rivers were altered by weirs for hydroelectric power production, which form barriers for fish migration. In this study, the term weir refers to a style of dam which is routinely overtopped by water. The sections above dams (referred to as upstream sides, U) reveal strongly altered velocity distributions while downstream sides (D) more resemble the natural flow. None of the study stream sections is affected by hydropeaking regimes. In each river, U and D sides were compared using a standardized sampling design comprising 15 replicates in each side (Fig. 3.2). The length of the sampled river sections on each side

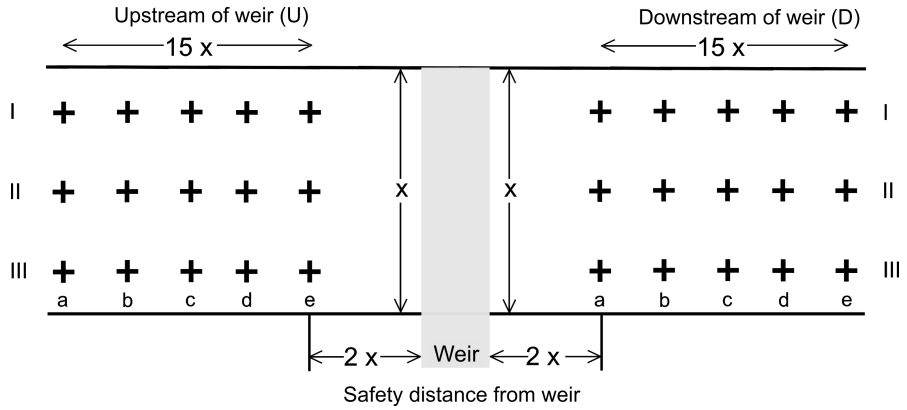


Figure 3.2: Schematic of the sampling design with: I–III = tracks (for the fish sampling), a–e = labelling for sampling points of each track, + = sampling points (for the sampling of physicochemical habitat variables, periphyton, macroinvertebrates and macrophytes), x = average width of the river measured 50 m upstream (U) and downstream (D) of the weir, 15 x = length of the sampling area, 2 x = area excluded from the assessment for safety reasons

was adjusted to the fifteen-fold river width at respective weir sides, resulting in study sections of 150 m to 420 m (Fig. 3.2). For safety reasons, the area in direct proximity of the weirs (two-fold stream width distance) was excluded. This study was designed to evaluate the effects of serial discontinuity in direct proximity of weirs, since the underlying effectors would be disguised with increasing distance (Ward & Stanford 1983). We are aware that the effects of weirs can exceed those observed in the study area.

Table 3.1: Characterization of the five study streams: catchment characteristics, geology, discharge, weir construction details, water chemistry (mean values from field sampling dates)

	Günz (G)	Leitzach (L)	Moosach (M)	Sächsische Saale (S)	Wiesent (W)
Catchment area [km^2]	526	112	175	523	432
Drainage	Danube	Danube	Danube	Elbe	Main
Geology	Molasse	Limestone	Moraine	Basement	Chalkstone
Mean annual discharge [$m^3\,s^{-1}$]	8.35	4.65	2.64	5.41	7.48
Year of construction	1945	1899	17 th. c.	a 1905	1924
Heigth of weir [m]	4.0	4.2	1.3	1.5	1.8
Average river width [m]	24	14	20	14	20
pH value	7.8	8.0	7.7	7.2	7.9
Specific conductance [$\mu S\,cm^{-1}$]	556	470	762	292	635
Dissolved oxygen [$mg\,L^{-1}$]	9.3	10.9	9.6	8.3	10.0
Temperature [°C]	18.5	9.6	13.5	17.8	14.3
Redox potential [mV]	580	470	520	490	460

Physicochemical habitat characteristics

Since substratum characteristics exert significant control on the quality of streambed habitat and benthic community structure (e.g. Geist & Auerswald 2007), the composition of the stream substratum was investigated at 15 points in each U and D site (Fig. 3.2). Substratum was sampled with a steel corer of 8 cm diameter (riverside corer, Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands). Grain sizes were fractioned with a wet-sieving tower (Fritsch, Idar-Oberstein, Germany) of decreasing mesh sizes (63 mm, 20 mm, 6.3 mm, 2.0 mm and 0.85 mm). The fractions retained on each sieve were dried at 100 °C and weighed to the nearest gram. The percentage of each grain fraction was determined and the geometric mean particle diameter (dg) was calculated according to Sinowski & Auerswald (1999) . For a hydraulic characterization, water depth, current speed below surface and 15 cm above ground were measured at each sampling point using a HFA flow-measuring-instrument (Höntzsch Instrumente, Waiblingen, Germany). Dissolved oxygen, temperature, specific conductance, redox potential and pH were measured in the hyporheic zone in 10 cm depth and in the free-flowing water. Water extractions from the hyporheic zone and redox potential measurements were carried out as described in Geist & Auerswald (2007). Dissolved oxygen, temperature, specific conductance and pH were measured using handheld Multi-340i equipment (WTW, Weilheim, Germany).

Periphyton

As most of primary production in medium-sized streams is related to the algal biomass associated with epilithal biofilms (Müllner & Schagerl 2003), periphyton can play an important role for the assessment of the functionality of stream ecosystems. At each sampling point, periphyton was scraped off a 1-4 cm^2 total surface area from all available substratum types (stones and dead wood) using a kitchen knife and a flexible plastic tablet to determine surface area. The sampled periphyton mass was dissolved in 200 ml of water and preserved with 20 mL of acidified Lugol's iodine solution (80% Lugol's iodine solution, 10% glacial acetic acid, 10% methanol). The Utermöhl technique (Utermöhl 1931, DIN EN 15204 2006) was applied before cell numbers were counted using an inverted microscope. Periphyton samples were left to settle for at least 24 h and the sample volume for sedimentation was adjusted to 1-50 mL depending on particle concentration in the sample. Algae were determined according to Ettl et al. (1978-1999) and Cox (1996).

Macrophytes

At each sampling point, all aquatic macrophytes and macroalgae were collected from a surface area of approximately 20 m^2 using a garden rake according to the methodology described in Deppe & Lathrop (1993). Sampling was continued until no additional species was found (typically 15 min). Species were determined according to van de Weyer & Schmidt (2007). Macroalgae were determined to genus-level according to John et al. (2002). The dominance of macrophyte species was calculated as percentage of sampling points at which the particular species was present.

Macroinvertebrates

Macroinvertebrate samples were collected with a Surber-Sampler (Surber 1930) at 15 sampling points for each U and D side. The substratum inside the sampling area of 0.096 m^2 at each sampling point was vigorously disturbed for two minutes to a depth of 10 cm using a metal fork. Makrozoobenthos was then collected in the net (mesh size 0.25 mm) and preserved in 30% ethanol. Macroinvertebrates were classified according to Nagel et al. (1989) using a binocular microscope. Classification was performed on species-, family- (Chironomids, some Trichoptera and Ephemeroptera) or order-level (few Diptera).

Fishes

Fish sampling was conducted using a boat-based electrofishing generator (EL 65 II, Grassel, Schoenau, Germany). Each D and U side was subdivided into three separate tracks (I-III in Fig. 3.2) which were sampled from downstream to upstream direction by the same electrofishing crew. A single anode was used and stunned fish were collected with a dipnet. Fish of each track were kept in separate plastic tanks with oxygen supply. The total length of all specimens was measured to the nearest 0.5 cm. Fish of 10 cm or more were individually weighed to the nearest gram. For smaller specimens, a representative number of at least 15 fish was weighed to determine the condition factor and to determine the total biomass as described in Pander & Geist (2010*b*). After sampling all three tracks, the fish were released.

Univariate data analysis

In order to assess the exchange between the free-flowing water and interstitial zone, the difference of dissolved oxygen concentration, temperature, pH and redox potential was calculated per sampling point. The catch per unit effort (CPUE) of fish (abundance per 100 m^3, fish biomass in g/100 m^3), macroinvertebrate abundance, number of periphyton cells per cm^2 and species richness of each taxonomic group per sampling point (for all groups except fishes) / track (fishes) and arithmetric means for each U and D side for each river were calculated. Normality of data was tested with the Shapiro-Wilk-Test and the homogeneity of variances was tested with the Levene-Test. Since data were not normally distributed, Mann-Whitney *U*-Tests were performed for comparisons between pooled U and D sides over all rivers. For multiple comparisons between sides and rivers, Kruskal-Wallis-Test and – in case of significance – post-hoc Mann-Whitney *U*-Tests were carried out. Bonferroni correction was applied for multiple testings. All statistical analyses were performed in the open source software R (http://www.r-project.org). Shannon Index (H), maximum diversity (H_{max}) and Evenness (E) were computed for all taxonomic groups at each U and D side using the R-package vegan (Oksanen et al. 2009). The Saprobic Index (SI) (DIN 38410-1 2004), the FRI (Fish Region Index, Dußling et al. (2005)) and the following ecological traits were determined for pooled data of all sampling points/tracks per side: Fishes were assigned to categories of flow current preference, feeding type and structural requirements (Jungwirth & Haidvogl 2003). Macroinvertebrates were assigned to functional feeding groups (FFG) (Merritt & Cummins 1995) , locomotion types (Moog 1995) and flow current preference (Schmedtje & Colling 1996). The FFGs "filtering collectors" and "gathering collectors" were grouped as "collectors" and the locomotion types "swimming/diving", "borrowing/boring" and "sprawling/walking" were grouped as "active moving". The percentage of individuals from each functional trait was calculated per study side and additionally compared over pooled U and D sides from all study rivers. Additionally, the percentage of Ephemeroptera, Plecoptera and Trichoptera (EPT%) was calculated for each side. Characteristic indicator species for U and D sides were determined using one-way SIMPER analysis in PRIMER v6 (Plymouth Marine Laboratory, United Kingdom). Untransformed species count data of all taxonomic groups, pooled for each U and D side, was used as input data, with Bray-Curtis Similarity for the resemblance matrix and a cut off value for low contributions of 90%.

Multivariate data analysis

In order to detect differences in the response of different taxonomic groups, non-metric multidimensional scaling (NMDS) was performed using taxa abundance data of each of the four groups as input variables for the function metaMDS of the R-package vegan (Oksanen et al. 2009). For a comprehensive assessment, NMDS was performed with the input matrix containing physicochemical habitat characteristics and functional traits of each taxonomic group. The resemblance matrix was calculated in PRIMER v6 based on Euclidian distances of the sampling sides for the variables FRI, saprobic index, FFGs, EPT%, percentage of active moving taxa, percentage of rheophilic macroinvertebrates, species richness (for all taxa), eveness (for all taxa), CPUE of fish and macroinvertebrate abundance, cell number of periphyton, abundance of macrophytes, fish biomass, water depth, and current speed below surface. For homogenizing different measurement scales before calculating the distance matrix, raw data were normalized using the pre-treatment normalization function in PRIMER v6 For a validation of this NMDS method, regular NMDS and detrended correspondence analysis (DCA) based on commonly used taxa abundance data of all taxonomic groups were performed using functions metaMDS and decorana of the R-package vegan (Oksanen et al. 2009). In order to test the discrimination of U and D sides at different levels of taxonomic resolution, NMDS and DCA was compared for all taxa on the species-, family- and order-level. Environmental fitting on all NMDS plots was performed with 1,000 permutations. Only environmental variables with significant ($P <= 0.05$) correlation with the NMDS were considered as ordination plot vectors. In addition, β-diversity was calculated as species turn-over (β_t) between U and D in each river for fishes, macroinvertebrates, aquatic macrophytes, periphyton and for all taxa with the function *betadiver* (R-package vegan) using index g (Koleff et al. 2003).

Results

Physicochemical habitat characteristics

Physicochemical habitat characteristics discriminated strongly between upstream (U) and downstream (D) sides of the weirs (Table 3.2). Water depth was significantly higher (mean depth U = 1.55 m, D = 0.83 m, $P < 0.01$) and current speed below surface and above ground was significantly lower (mean v a U = 0.15 m s^{-1}, D = 0.24 m s^{-1}, mean v b U = 0.28 m s^{-1}, D = 0.36 m s^{-1}, $P < 0.01$, respectively) in U than in D sides. Substratum composition differed significantly between U

and D sides as measured by geometric mean particle diameter (dg), percentage of fines and the fraction >63 mm. Mean particle size dg in D was nearly twice the value of U ($P < 0.05$). The percentage of fines in D was 9% lower than in U (mean D = 28%; mean U = 37%, $P = 0.029$) and the fraction <63 mm was 7% higher in D compared to U (mean D = 10%, mean U = 3%, $P < 0.05$). The differences in substratum composition were also reflected in the water chemical gradients between free-flowing water and interstitial zone. For instance, differences in the concentrations of dissolved oxygen between free-flowing water and the interstitial zone were 20% higher in U than in D ($P < 0.05$). Similarly, gradients in temperature (0.3 °C higher in U than in D, $P < 0.05$), and in pH (0.1 higher in D than in U, $P < 0.05$) were observed. Differences in redox potential and specific conductance were least discriminative between U and D due to high standard deviations (Table 3.2).

Periphyton

A total number of 129 periphyton taxa was identified. Species richness was significantly lower in the river S than in all other study rivers ($P < 0.01$) and cell numbers per cm^2 differed significantly between the five study rivers ($P < 0.01$, Fig. 3.3). Over all study rivers a consistent trend towards higher species richness and cell numbers in D sides could be observed (Fig. 3.3), with two additional periphyton species in D compared to U (mean U = 10; mean D = 12, $P < 0.05$), and the number of cells per cm^2 being 40% lower in U than in D (mean U = 611,406; mean D = 993,605, $P < 0.01$). Significant differences in cell numbers per cm^2 between the U and the D side of single study rivers were found in the rivers G and S, with 17-fold higher cell counts in the D than in the U side of river G ($P = 0.05$) and 2-fold higher cell counts in the D than in the U side of river S ($P < 0.01$) (Fig. 3.3). Beta diversity between U and D was very similar between study rivers, ranging from 0.22 to 0.33 (Table 3.4). Characteristic periphyton taxa according to SIMPER analysis were Chlorophyceae and Cyanophyceae for U sides and Diatoms from the genera *Navicula* and *Gomphonema* for D sides.

Table 3.2: Physicochemical habitat characteristics. D = downstream; U = upstream; v a = current speed 15 cm above ground; v b = current speed 10 cm below water surface; *dg* = geometric mean particle diameter; Eh = redox potential; sc = specific conductance; Δ = difference between the free-flowing water and interstitial zone; bold numbers show significant differences between respective U and D sides; mean values are given with ± standard deviation; % * = percent significant differences

River	Side	Depth [m]	v a [m s^{-1}]	v b [m s^{-1}]	*dg* [mm]	Δ O2 [mg l^{-1}]	Δ T [°C]	Δ pH	Δ Eh [mV]	Δ sc [μS cm^{-1}]
G	U	**2.22** ± 0.27	**0.11** ± 0.03	**0.19** ± 0.06	25 ± 7	4.6 ± 1.8	-1.0 ± 0.7	0.2 ± 0.2	95 ± 36	16 ± 22
	D	**1.44** ± 0.23	**0.29** ± 0.09	**0.42** ± 0.08	24 ± 7	4.5 ± 1.4	-0.7 ± 0.4	0.3 ± 0.2	147 ± 93	6 ± 75
L	U	**0.93** ± 0.21	0.43 ± 0.20	**0.73** ± 0.28	45 ± 16	5.6 ± 2.7	-0.8 ± 0.3	0.2 ± 0.2	54 ± 41	-39 ± 51
	D	**0.31** ± 0.14	0.40 ± 0.47	**0.45** ± 0.52	24 ± 6	5.8 ± 2.0	-0.8 ± 0.4	0.3 ± 0.1	67 ± 46	-33 ± 71
M	U	**1.42** ± 0.17	**0.11** ± 0.05	**0.22** ± 0.05	3 ± 1	7.1 ± 2.4	-2.7 ± 1.1	0.3 ± 0.2	127 ± 72	-13 ± 78
	D	**1.04** ± 0.33	**0.20** ± 0.14	**0.32** ± 0.15	8 ± 2	6.1 ± 2.4	-2.3 ± 1.3	0.6 ± 0.3	82 ± 53	-27 ± 72
S	U	**1.57** ± 0.35	**0.02** ± 0.01	**0.05** ± 0.02	**9** ± 2	4.4 ± 1.9	**-0.8** ± 0.5	0.2 ± 0.2	195 ± 24	-172 ± 297
	D	**0.67** ± 0.25	**0.13** ± 0.05	**0.20** ± 0.09	**30** ± 8	4.1 ± 1.7	**-0.1** ± 0.3	0.2 ± 0.1	160 ± 55	-289 ± 317
W	U	**1.58** ± 0.44	**0.07** ± 0.08	**0.20** ± 0.11	**10** ± 3	4.8 ± 2.1	-1.1 ± 0.5	0.4 ± 0.3	**135** ± 32	**-39** ± 69
	D	**0.69** ± 0.45	**0.19** ± 0.14	**0.40** ± 0.21	**50** ± 19	2.4 ± 2.1	-1.1 ± 0.6	0.4 ± 0.3	**66** ± 47	**-16** ± 25
Pooled	U	**1.55** ± 0.51	**0.15** ± 0.17	**0.28** ± 0.27	**6** ± 9	5.3 ± 2.3	-1.3 ± 1.0	**0.2** ± 0.2	117 ± 65	-49 ± 153
	D	**0.83** ± 0.48	**0.24** ± 0.24	**0.36** ± 0.27	**11** ± 12	4.5 ± 2.3	-1.0 ± 1.0	**0.3** ± 0.2	105 ± 72	-65 ± 188
% *		100	80	100	60	20	20	20	20	20

Macrophytes

Species richness of aquatic macrophytes was generally low and strongly variable among study rivers. Overall, 18 species of macrophytes from 13 families were found. Numbers of species were almost balanced in U and D (total number of species U: 15; D: 16) with a slightly higher mean species richness in D (species richness U: mean = 5; D: mean = 6, Fig. 3.4). Species richness, Shannon Index and Evenness were not significantly different between U and D. Macrophyte dominance was higher in U sides of three rivers in comparison to the corresponding D sides (U-G 67%, D-G 53%; U-L 100%, D-L 93%; U-M 100%, D-M 80%), lower in U-S (20%) than in D-S (40%) and equal in U-W and D-W (100%). The Shannon Index of macrophytes was higher in D than in U sides by a factor of 1.4 (mean U = 0.87; mean D = 1.18). Beta diversity values also indicated great variability among rivers, with the greatest differences between U and D found in the river S and the lowest difference in the river G (Table 3.4). Only one characteristic species for D, *Fontinalis antipyretica* HEDW., and no characteristic species for U was identified by SIMPER analysis.

Macroinvertebrates

A total of 11,921 specimens from 93 species of macroinvertebrates comprising 51 families were identified. The most common taxa were Diptera (23%), Amphipoda (16%), Ephemeroptera (15%), Plecoptera (10%), Coleoptera (5%) and Trichoptera (4%). The most abundant functional feeding groups were collectors (52%) and shredders (36%), whereas predators (2%), scrapers (2%) and all other groups (8%) were less abundant. Macroinvertebrate abundance was significantly lower in the river G than in all other study rivers ($P < 0.01$, Fig. 3.3). Differences in abundance between the U and D within one study river were most pronounced for the rivers W, S and G ($P < 0.01$, Fig. 3.3). Species richness differed significantly among study rivers ($P < 0.05$) except W-L and S-M. Significant differences in species richness between U and the D were found in the rivers S and W. Pronounced differences between U and D (U: 64 species, mean = 18.2, and D: 81 species, mean = 25.2, $P < 0.01$) occurred, whereas Shannon Index and Evenness were less discriminative (except for river S with a 2.3 times higher Shannon Index and a 1.6 times higher Evenness in D-S than in U-S, Fig. 3.4). Beta diversity as a measure of similarity indicated strong differences in community composition between U and D (Table 3.4). EPT%-values were up to four-fold higher in D sides, with differences varying strongly between streams (Table 3.3). Characteristic macroinvertebrate

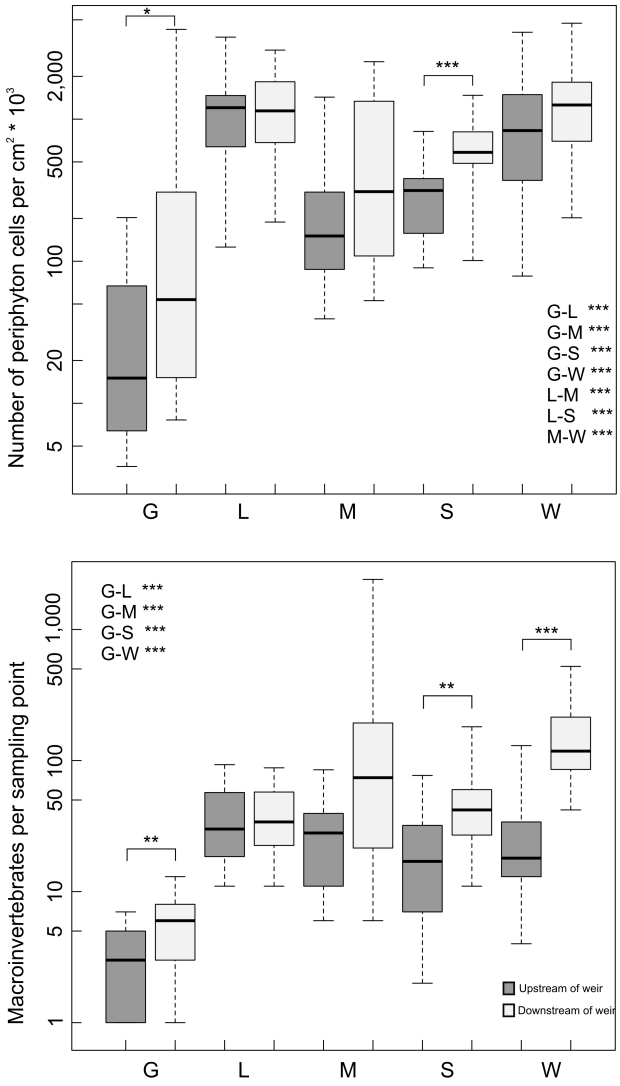


Figure 3.3: Characterization of periphyton and macroinvertebrate abundance in U and D sides (15 replicates each) of the five study streams: G, L, M, S and W refer to the different study streams, as described in Table 3.1. Box: 25% quantile, median, 75% quantile; whisker: minimum, maximum values. Square brackets between boxes show significant differences in single comparisons within one study river. Significant differences between study rivers are given as text. Significance levels are indicated as follows: $0.01 < P \leq 0.05$*, $0.001 < P \leq 0.01$**, $P \leq 0.001$***

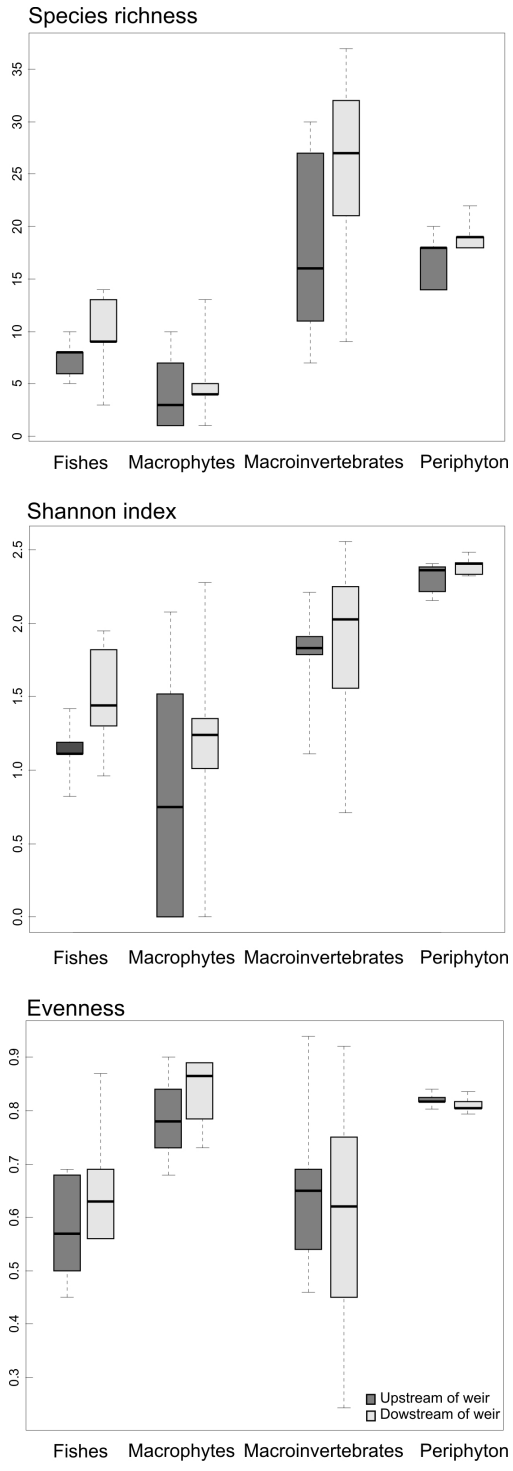


Figure 3.4: Comparison of species richness and diversity indices of the investigated taxonomic groups in U and D sides: data are pooled for U and D sides in each study river, resulting in 5 replicates each box except for Shannon Index and Evenness of macrophytes (4 replicates). Box: 25% quantile, median, 75% quantile; whisker: minimum, maximum values

taxa according to SIMPER analysis were Oligochaeta and Chironomidae for U and the rheophilic taxa *Leuctra nigra* (Plecoptera) and *Rhyacophila spp.* (Trichoptera) for D. The observed differences in abundance, species richness and diversity were even more pronounced considering the functional traits of these groups. Concerning the flow current preference, differences in the percentage of rheophilic taxa of up to 64% between respective D and U sides were observed, with a trend towards higher abundance of rheophilic taxa in D compared to U in three of the streams (Table 3.3). Accordingly, the percentage of active moving taxa was lower in U sides than in D sides for all study rivers (U: 32%, D: 49%). A classification according to functional feeding groups indicated greater abundance of collectors in U sides (U: 59%, D: 49%) and of shredders in D sides (U: 23%, D: 41%). The saprobic index was higher in U sides compared to the respective D sides (except for L, Table 3.3).

Fishes

Overall, 27 species from 9 families and one lamprey species (*Lampetra planeri*) were sampled, comprising a total of 2,508 specimens and a total biomass of 244 kg. Species richness was higher in D than in U over all study rivers (U: 19 species, mean = 7.4, D: 23 species, mean = 9.6, Fig. 3.4). The CPUE per number of specimens was significantly higher in D than in U (mean U = 4.9 per 100 m^3, mean D = 5.8 per 100 m^3, $P < 0.05$). The CPUE biomass was three times higher in D than in U (mean U = 270 g/100 m^3, mean D = 851 g/100 m^3, $P = 0.01$). Fish diversity was higher and more even in D than in U (Shannon D: 2.37, Evenness D: 0.74, Shannon U: 2.05, Evenness U: 0.68, Fig. 3.4). Species richness was most discriminative between U-S (8) and D-S (13) and between U-G (10) and D-G (14). In contrast to the other study rivers, there were two more species in U-L (5) than in D-L (3). Fish diversity was higher in D-G, D-M, D-S and D-W than in the corresponding U sides and more even in D-W, D-G and D-L than in the corresponding U sides. Beta diversity values ranging from 0.40 to 0.64 indicated pronounced differences in species composition between U and D (Table 3.4). In addition to the differences in abundance, species richness, diversity and community composition among D and U, the highly different fish region index (FRI) between U (5.89) and D (4.91) sides (stream-specific difference between U and D of 0.02-2.15) indicated pronounced weir effects on fish community structure and the availability of ecological niches for rheophilic specialists (Table 3.3).

Table 3.3: Ecological traits of macroinvertebrates and fishes.Values refer to the percentage ratio of the number of individuals in relation to all specimens. EPT = Ephemeroptera, Plecoptera and Trichoptera; FRI = Fish Region Index; D = downstream; U = upstream

		Macroinvertebrates						Fishes			
River	Side	SI	Shredders [%]	Collectors [%]	Rheophilic [%]	EPT%	Active moving [%]	FRI	High structural requirements [%]	Benthivor [%]	Rheophilic [%]
G	U	2.20	2	73	33	7	5	6.39	25	0	0
	D	2.08	4	72	10	26	18	6.09	39	29	29
L	U	1.54	27	62	72	45	63	4.06	69	71	71
	D	1.63	40	52	72	48	72	4.05	77	77	77
M	U	1.87	32	34	22	6	42	6.56	31	4	4
	D	1.85	91	7	86	7	96	4.41	79	57	57
S	U	2.11	16	45	0	19	18	6.96	4	0	0
	D	1.91	3	65	3	34	19	6.10	11	11	11
W	U	1.91	13	70	43	37	34	3.96	87	98	96
	D	1.77	13	76	66	38	39	4.16	80	96	95
Pooled	U	1.96	23	59	34	23	32	5.89	43	35	24
	D	1.90	41	49	47	31	49	4.91	57	54	59

The difference in the FRI mostly results from the higher abundance of rheophilic species such as *Salmo trutta* L., *Thymallus thymallus* L., *Cottus gobio* L., *Gobio gobio* L., *Barbatula barbatula* L. and *Barbus barbus* L. in D (59%) than in U (24%). The most characteristic fish species according to SIMPER analysis were *Rutilus rutilus* L. for U sides and *S. trutta*, *C. gobio*, *G. gobio* and *Squalius cephalus* L. for D sides. In addition to flow current preference, the fish community composition of U and D also represented different feeding types and structural requirements, with lower abundance of benthivoric and habitat structure-specialized species in U than in D sides in all rivers except the river W (Table 3.3).

Table 3.4: Beta diversity (β_t) as measure of similarity between U and D sides

	β_t				
River	Periphyton	Macroinvertebrates	Macrophytes	Fishes	All taxa
G	0.33	0.77	0.00	0.47	0.53
L	0.30	0.36	0.60	0.40	0.43
M	0.32	0.58	0.29	0.58	0.49
S	0.22	0.64	0.75	0.60	0.52
W	0.22	0.44	0.23	0.64	0.36

Multivariate data analysis

The consideration of single taxonomic groups instead of comprehensive community response analysis in NMDS revealed strong river-specific patterns (Fig. 3.5, Table 3.5). For instance, there was a strong separation between U and D in the river M for fishes and macroinvertebrates, but weak to no separation for periphyton and macrophytes, respectively. In contrast, differences in the river S were greatest for periphyton and macrophytes, but less pronounced for macroinvertebrates and fishes. The simultaneous inclusion of abiotic habitat variables and biological traits of all taxonomic groups in NMDS resulted in a more comprehensive and universally applicable assessment (Fig. 3.6). Both normalized distance-matrix based NMDS (including ecological traits and physicochemical variables of U and D as input variables) as well as classical NMDS and DCA (based on taxa abundance data, DCA not shown) revealed similar discrimination patterns of sides and rivers, indicating a strong linkage of ecological traits, community composition and habitat characteristics. For instance, the strongest separation of U and D was in both NMDS input scenarios found in the river M, and the weakest in the river

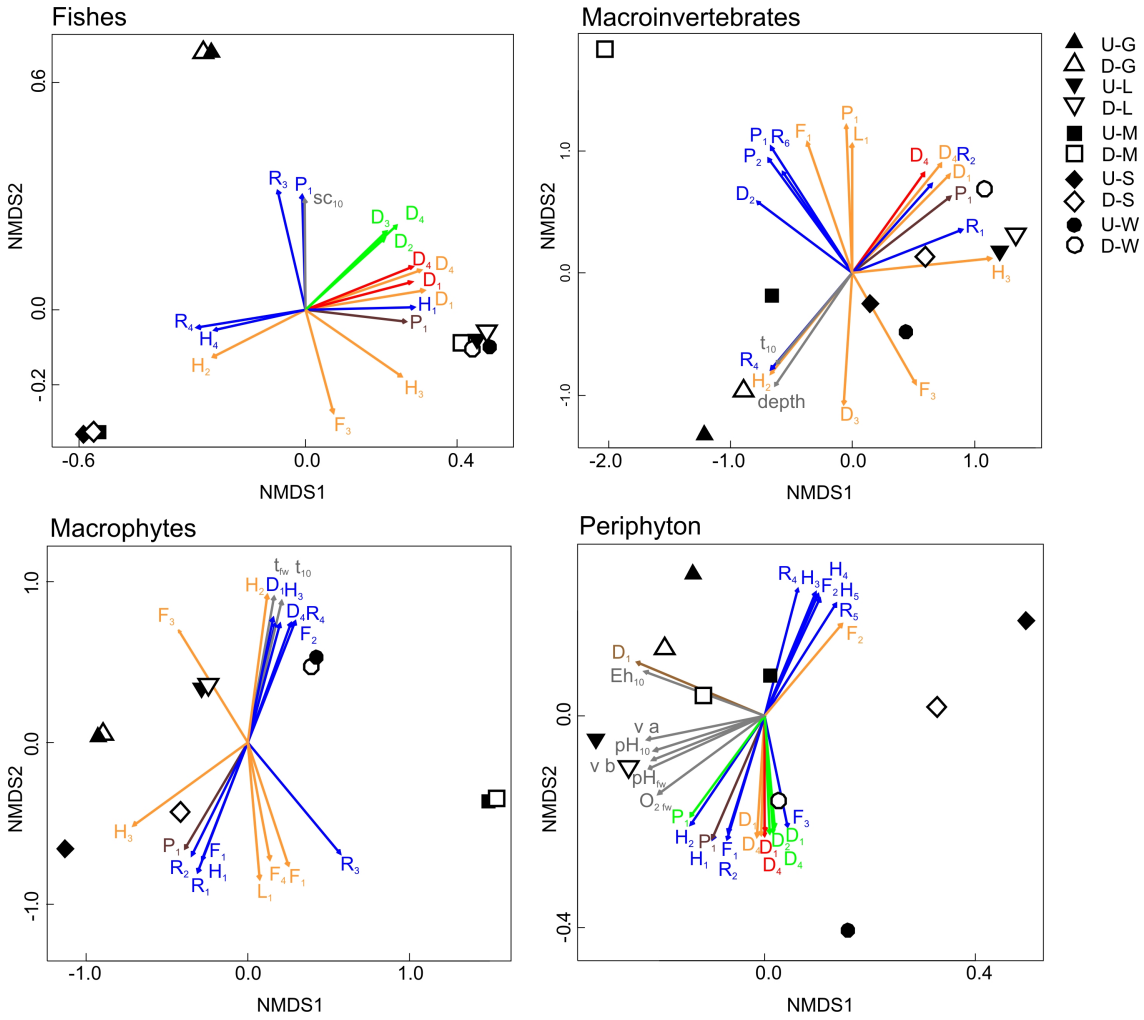


Figure 3.5: Non-metric multidimensional scaling (NMDS) performed for different taxonomic groups, based on taxa abundance data and Bray-Curtis Similarity. Fishes: non-metric stress = 0.06 * 10^{-4}; Macroinvertebrates: non-metric stress = 0.03; Aquatic macrophytes: non-metric stress = 0.02; Periphyton: non-metric stress = 0.06. Study rivers are displayed with different pictograms, upstream sides (U) with dark grey and downstream sides (D) with bright grey. Environmental variables and metrics ($P \leq 0.05$ based on 1,000 permutations) are displayed as vectors and can be distinguished by colour according to their relatedness to physicochemical habitat characteristics (grey), fishes (blue), macroinvertebrates (orange), macrophytes (green), periphyton (brown) and all taxa (red) as well as by capital letters according to their relatedness to feeding type (F), locomotion type (L), reproductive strategy (R), productivity (P), diversity (D) and habitat requirements (H). Codes and coefficients of variance (r^2) of the environmental variables are shown in Table 3.5

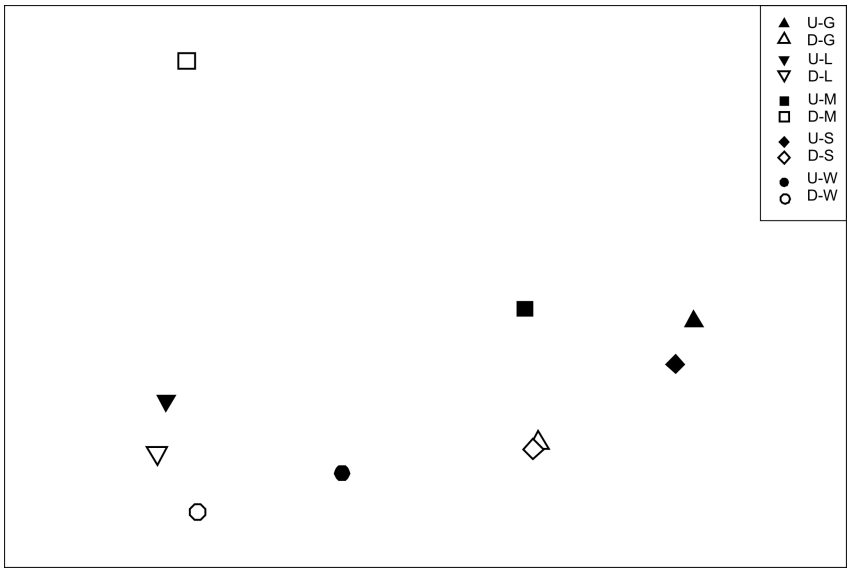


Figure 3.6: Non-metric multidimensional scaling (NMDS) of the U and D sampling sides based on Euclidean distances resulting from biological traits and physico-chemical habitat characteristics. Study rivers are displayed with different pictograms, upstream sides with dark gray and downstream sides with bright gray, non-metric stress = 0.03

L. Generally, community effects were not only detectable on the species-level, but also on higher levels of taxonomic resolution such as family- and order-level (Fig. 3.7, Table 3.5). Remarkably, differences upstream and downstream of weirs at adjacent sides within the same river were often greater than the differences observed among rivers from different geological units and drainage systems. For instance, differences on all levels of taxonomic resolution were greater between adjacent U and D sides at the river M than between the river M and the rivers G, S and W (Figs 3.6 & 3.7) which belong to different drainage systems (G, M: Danube, S: Elbe, W: Main/Rhine) and which are geographically separated by more than 200 km (Fig. 3.1). On the other hand, the differentiation between the rivers L and W (Figs 3.6 & 3.7) as well as between G and S was remarkably low in comparison to the other study rivers (Fig. 3.6), although these rivers are geographically separated by 300 km (W-L) and 400 km (G-S), belong to different drainage systems (G, L: Danube; W: Main/Rhine, S: Elbe) and to different geological units (G: molasses, L: limestone alps; W: chalkstone, S: basement).

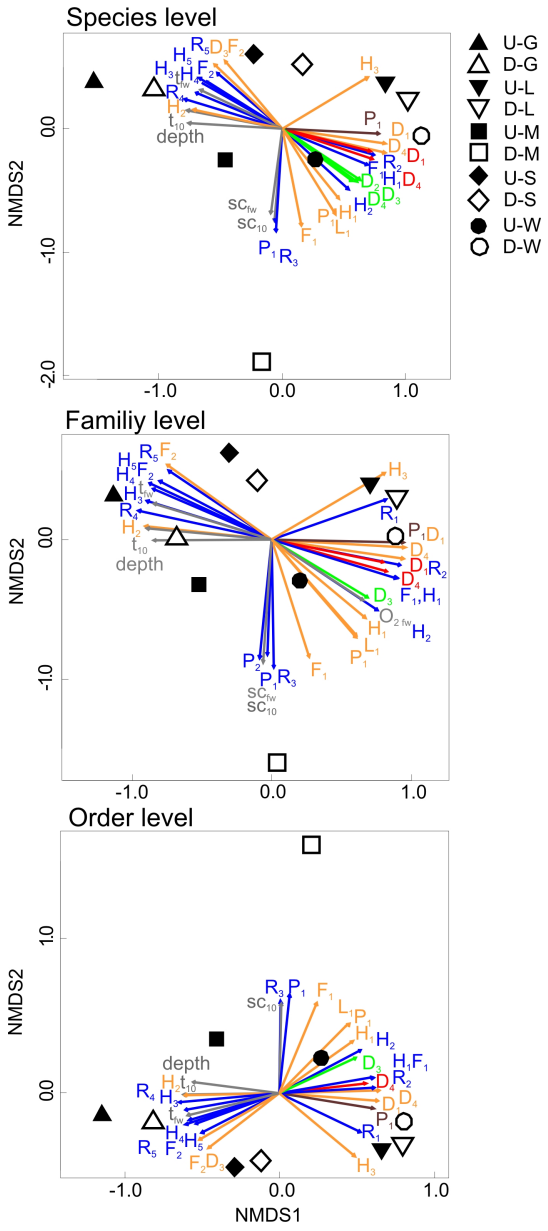


Figure 3.7: Non-metric multidimensional scaling (NMDS) performed for different levels of taxonomic resolution, based on taxa abundance data and Bray-Curtis Similarity: species level = including all specimen that could be identified on species-level, non-metric stress: 0.05; Family level = including all specimen that could be determined to family-level or further, summarized on family level, non-metric stress: 0.06; Order level = including all specimen summarized to order-level, non-metric stress: 0.06. Environmental variables and metrics ($P \leq 0.05$ based on 1,000 permutations) are displayed as vectors. For codes of study rivers, sides and environmental variables see legend Fig. 3.5 and Table 3.5

Table 3.5: Codes of the environmental variables displayed in Figs 3.5 & 3.6 with full names and coefficients of variance (r^2) for environmental fitting in the NMDS for taxonomic groups (Fig. 3.5) and for taxonomic levels (Fig. 3.6). P = periphyton; MP = macrophytes; MIV = macroinvertebrates; F = fishes; EV = environmental variables. Significance correlations are indicated as follows: $0.01 < P \leq 0.05$*, $0.001 < P \leq 0.01$**, $P \leq 0.001$***

			r^2 taxonomic groups (Fig. 3.5)				r^2 taxonomic levels (Fig. 3.6)		
Group	Code	Full name	P	MP	MIV	F	Species-level	Familiy-level	Order-level
All taxa	D1	Species richness	0.64*			0.75**	0.66*	0.61*	
	D4	H_{max}	0.68*		0.63*	0.84**	0.73**	0.68 *	0.64*
F	D1	Species richness		0.65*					
	D2	Shannon Index			0.59*				
	D4	H_{max}		0.61*					
	F1	Benthivor %	0.83**	0.64*			0.69*	0.81**	0.76 *
	F2	Euryophag %	0.80**	0.61*			0.76**	0.87**	0.82**
	F3	Filtering %	0.60***						
	H1	Rheophilic %	0.84**	0.64*			0.69*	0.81**	0.75*
	H2	High structural requirements %	0.82**				0.64*	0.77*	0.72*
	H3	Indifferent %	0.84**	0.66 *			0.70*	0.82**	0.77*
	H4	Fish Region Index	0.84**			0.56*	0.69*	0.80**	0.74*
	H5	No structural requirements %	0.80**				0.65*	0.76**	0.67*
	P1	Total length [cm]			0.63*	0.83**	0.68*	0.67*	0.71*
	P2	Biomass [kg 100/m^3]			0.84*			0.71*	
	R1	Speleophilic %		0.78*	0.58 *		0.60.	0.70**	0.68**
	R2	Rheopar %	0.79**	0.64*	0.59*	0.74*	0.74*	0.82**	0.77***

Group	Code	Full name	r^2 taxonomic groups (Fig. 3.5)				r^2 taxonomic levels (Fig. 3.6)		
			P	MP	MIV	F	Species-level	Familiy-level	Order-level
	R3	Pelagophilic %		0.85***		0.94***	0.84***	0.78***	0.83**
	R4	Euryopar %	0.82**	0.69*	0.66*	0.77*	0.83**	0.89***	0.85*
	R5	Phytolithophilic %	0.84**				0.59.	0.73**	0.64**
	R6	Lithopelagophilic %			0.93***				
MP	D1	Species richness	0.58*						
	D2	Shannon Index	0.64*			0.71*	0.56*		
	D3	Evenness				0.97*	0.67*	0.59*	0.60*
	D4	H_{max}	0.62*			0.79*	0.61*		
	P1	Abundance per side %	0.74**						
MIV	D1	Species richness	0.69*		0.79**	0.91***	0.87**	0.84**	0.83*
	D3	Evenness			0.72**		0.63*		0.67**
	D4	H_{max}	0.67*		0.82**	0.93***	0.90**	0.84**	0.84*
	F1	Shredders %		0.68**	0.79*		0.78*	0.72*	0.80*
	F2	Undefined %	0.68*				0.70*	0.79**	0.72*
	F3	Collectors %		0.71**	0.67*	0.72*			
	F4	Scrapers %			0.57*				
	H1	Rheophilic %					0.63*	0.71*	0.69**
	H2	Saprobic Index		0.90**	0.69**	0.70*	0.75**	0.77**	0.77**
	H3	EPT %		0.81**	0.80**	0.86**	0.80**	0.81**	0.81**
	L1	Active moving taxa %		0.75*	0.69*		0.77**	0.78**	0.82
	P1	Individuals per sampling point			0.90***		0.81**	0.78**	0.79**
P	P1	Cell count cm^{-2}	0.70*		0.64*	0.64*	0.76**	0.83**	0.76**

Group	Code	Full name	r^2 taxonomic groups (Fig. 3.5)				r^2 taxonomic levels (Fig. 3.6)		
			P	MP	MIV	F	Species-level	Familiy-level	Order-level
	D1	Species richness	0.91***	0.61*					
EV	depth	Depth [m]			0.78**		0.70*	0.67*	0.64*
	O_{2fw}	Dissolved oxygen [mg L^{-1}]	0.82**					0.56*	
	sc_{fw}	Specific conductance free-flowing water [μS cm^{-1}]					0.57*	0.72**	
	sc_{10}	Specific conductance substratum [μS cm^{-1}]				0.77*	0.68*	0.63*	0.69*
	t_{10}	Temperature substratum [°C]		0.87**	0.58*		0.66*	0.75*	0.75**
	t_{fw}	Temperature free-flowing water [°C]		0.90**			0.66*	0.73*	0.75**
	Eh_{10}	Redox potential substratum [mV]	0.77*						
	v a	Current speed above ground [m s^{-1}]	0.68*						
	v b	Current speed below water surface [m s^{-1}]	0.68*						
	pH_{fw}	pH free-flowing water	0.77**						
	pH_{10}	pH substratum	0.63*						

Variables which correlated with the ordination distances between study sides based on taxa distribution mostly refer to habitat preferences and functional feeding groups, as well as to diversity characteristics and physicochemical variables. The comparison of beta diversity (including all taxa) between U and D of individual rivers and between the rivers showed that the difference between U and D equals more than half of the differences between rivers (beta diversity between rivers: mean = 0.68, beta diversity between sides: mean = 0.35, Table 3.4).

Discussion

The pronounced weir effects detected in this study suggest that damming strongly alters community structure, productivity and the diversity of stream ecosystems. These alterations are supposed to originate in an interruption of the natural gradient of physical habitat conditions and the biotic responses from the headwater to the mouth of river systems (Ward & Stanford 1983), as originally described in the River Continuum Concept (RCC) by Vannote et al. (1980). To our knowledge, this is the first study that comprehensively assesses the ecological effects of weirs on the serial river discontinuity including physicochemical habitat characteristics as well as community effects on all major taxonomic groups. The most important finding is the overriding influence of weirs on biological communities compared to other variables including geology or drainage system. This finding was unexpected, since several authors suggest strong relatedness of rivers of the same or similar geological origin (Mykrä et al. 2007, Stendera & Johnson 2006, Kim et al. 2007) or drainage system (Corkum 1989, Richards et al. 1996, Robinson 1998, Schaefer & Kerfoot 2004). Consequently, the different geochemical conditions of the rivers included herein were expected to result in entirely different community structures. Remarkably, small scale effects of heterogeneity in *dg*, water depth and current speed introduced into adjacent sites of the same stream by weirs greatly exceeded the large scale effects of geology and geographic isolation.

Differences between taxonomic groups and rivers

This study shows that none of the single taxonomic groups (periphyton, macrophytes, macroinvertebrates, fishes) alone is a universally suitable indicator of the overall discrepancy in community structure upstream and downstream of weirs, yet they are widely used as indicators for the ecological status of aquatic ecosystems (e.g. macrophytes for the trophic status (Schneider & Melzer 2003); fishes for the ecological status in context of the WFD (Dußling et al. 2005); periphyton for

the ecological condition (Stevenson et al. 2009); macroinvertebrates for freshwater monitoring (Menezes et al. 2010)). The low congruence between the responses of different taxonomic groups to weirs is also supported by their individual and distinct responses to environmental gradients in other freshwater ecosystem studies (Heino 2010). For instance, Declerck et al. (2005) showed that different taxonomic groups in shallow lakes react individualistically to environmental gradients and Heino et al. (2005) revealed similar results for running waters. Based upon similarity values, diversity measures, functional traits and multivariate community composition analyses, none of the four taxonomic groups studied was a more integrative and sensitive indicator of weir effects than others. The signal strength of weir effects on biological communities turned out to be not only dependent on the taxonomic group investigated, but also differs strongly between rivers within taxonomic groups. This is mostly due to the stream-specific habitat structure, community composition, diversity and productivity which have strong influence on the discriminative power of different taxonomic groups (Heino 2010).

Periphyton

Periphyton, which constitutes the basis of aquatic food webs (Vannote et al. 1980, Szabó et al. 2008), strongly depends on physical habitat characteristics (Soininen 2002, Müllner & Schagerl 2003). This is supported by our data where most physicochemical variables revealed significant correlation with periphyton community composition. Along with the different abiotic habitat conditions observed in the study streams, this finding can explain the differing suitability of periphyton as an indicator of weir effects in different rivers. For instance, in the comparatively deep and slow flowing river Günz, cell counts of periphyton were 17-fold higher in the more shallow and high-current D compared to the U side. In the shallow and fast flowing Leitzach, periphyton cell counts were on average three-fold higher than in the Günz and only differed by a factor of 1.01 between D and U (Table 3.2).

Macrophytes

In contrast to periphyton, macrophytes only occurred in some streams and only one species discriminated between D and U, which limits their use as a general indicator for weir effects. Only in two of the rivers (W, M) macrophyte diversity and abundance was high enough to detect differences between U and D, while differences in community composition were evident for the river S. However, as

aquatic macrophytes can play an important role for habitat structure (Balanson et al. 2005), weir-induced alterations of the macrophyte community could affect the entire ecosystem in streams with high macrophyte dominance. This is evident from the strong correlation of macrophyte diversity measures with community composition of fishes and periphyton and of all taxonomic groups on species-level.

Macroinvertebrates

Macroinvertebrate community structure (diversity indices, functional feeding groups, saprobial index, flow current preference, locomotion types) strongly discriminated between U and D sides, which probably results from different flow conditions up-and downstream of weirs. Whereas the effect of the different flow velocities on flow current preference and locomotion types of macroinvertebrates is obvious (i.e. rheophilic and actively moving taxa being most characteristic for high-current D sides), current also affects the availability and ratio of CPOM to FPOM, which can explain the differences in functional feeding groups up- and downstream of the weirs. For instance, the higher abundance of the filter-feeding collectors Simulidae and Chironomidae at U sides with lower current may be explained by higher sedimentation rates of FPOM, and consequently higher FPOM/CPOM ratios. Accordingly, shredder organisms which are considered highly sensitive to perturbation (Rawer-Jost et al. 2000) were more abundant at D sides. Analogously, these effects on the distribution of functional feeding groups and organic matter seem to be also true for the general texture of the stream substratum which was much finer in U than in D. Fine-textures substrata typically reduce the availability of voids and consequently the abundance and diversity of benthic organisms in the hyporheic zone (Gayraud & Philippe 2003, Geist & Auerswald 2007, Rice et al. 2010), which can explain the lower abundance and diversity of Plecoptera, Ephemeroptera and Trichoptera at U sides.

Fishes

The observed differences of up to two fish faunal regions (according to the classification by Dußling et al. 2005) between U and D mirror community structures with entirely different ecological requirements and represent habitat conditions typical for rhithron vs. potamon regions within entire stream ecosystems. Weir-associated fish habitat modifications mostly result from changes of water depths, current speed and substratum composition, which compared to the natural status are more pronounced in U than in D. In most cases, U sides cannot fully meet

the habitat requirements of species with high structural requirements but are tolerated by indifferent species (Kruk 2007). For instance, the rheophilic species *S. trutta*, *C. gobio*, *G. gobio* and *S. cephalus* occurred at higher densities in D sides, whereas the generalist species *R. rutilus* occurred in higher densities in U sides. In rivers with occurrence of high numbers of specialized (e.g. rheophilic, lithophilic or benthivoric) fish species (e.g. M), the most pronounced differences in fish community structure between U and D were observed, while differences in rivers with a high number of tolerant species where generally low (e.g. G).

Implications for management

The continuity of river systems is a hydromorphological quality element in the European Water Framework Directive (WFD) which requires the evaluation of human impacts on water bodies (European Parliament 2000). However, river sections in spatial proximity of weirs are currently excluded from assessments in context of the WFD. As most European rivers today are a mosaic of upstream and downstream sides of weirs that succeed each other in short geographical distances, information on the qualitative and quantitative effects of weirs on these river sections is crucial for representative assessment of their ecological status and for conservation and restoration management. For example, restoration measures which form a variety of shallow overflowed habitats could improve the overall biodiversity in weir-regulated rivers with increased and uniform water depths and reduced current speed (Freeman et al. 2001, Kemp et al. 1999). The normalized NMDS based on physicochemical variables and ecological traits is highly suitable for a comprehensive quantification of weir effects in different rivers on the ecosystem level as well as for the monitoring of restoration measures. Additionally, this method provides the possibility to assign indicator weights to specific target taxa or ecological traits to account for conservation management prioritization.

Due to river-specific differences, the univariate consideration of single abiotic parameters and of single taxonomic groups is not suitable as a generally applicable indicator for the detection of weir effects. Multivariate methods which simultaneously include different taxonomic groups and physicochemical variables produce a more complete and coherent picture of the serial discontinuity and may serve as a comprehensive and universal indicator of ecosystem health.

Community effects and the underlying effectors were generally detectable at high levels of taxonomic resolution such as family- and order-level. This illustrates that the effects of the interruption of the river continuum caused by weirs are

not only restricted to a few sensitive species or taxonomic groups but affect the entire aquatic community structure. Therefore, a classification on the family- or even order-level may be sufficient for most taxonomic groups. This finding is particularly relevant for the applicability of this methodology in other regions with different community composition. Typically, funding for the ecological monitoring of weir effects and of other impacts on aquatic ecosystems is limited. The results of this study suggest that the inclusion of multiple taxonomic groups at low levels of resolution is advantageous compared to the inclusion of few groups at high levels of taxonomic resolution in ecological monitoring.

3.2 The ecological value of stream restoration measures: an evaluation on ecosystem and target species scales

A similar version of this section is published:

Mueller, M., Pander, J. & Geist, J. (2014) The ecological value of stream restoration measures: An evaluation on ecosystem and target species scales. *Ecological Engineering* **62**, 129-139.

Abstract

Stream restoration is widely applied for conservation of freshwater ecosystems, but systematic comparisons on the effects of different techniques are rare. In this study, we systematically evaluated two types of gravel introduction, substratum raking and the placement of boulders in six streams. We compared indicator-based and multi-scale approaches that simultaneously assess effects on target species, different taxonomic groups and on ecosystem scale. Gravel introduction had by far the strongest effects on macroinvertebrates (increase of species density and numbers of individuals), periphyton (increase of cell numbers) and macrophytes (decrease of coverage, species numbers and biomass), followed by substratum raking. The placement of boulders had no significant long-term effects on aquatic communities. Over all investigated restoration treatments, fish community composition only changed significantly in 50% of the study rivers depending on the occurrence of species sensitive to the structures introduced by the restoration treatments. These were lithophilic, rheophilic and invertivorous fishes, comprising several species listed in the Red List of endangered species which used the added 16-32 mm gravel as juvenile habitat. Areas with introduced gravel were also most frequently used by spawning *Salmo trutta*, *Thymallus thymallus* and *Phoxinus phoxinus*. In contrast, active bioindication using *Salmo trutta* eggs indicated that none of the restoration treatments was sufficient to enhance habitat conditions in deeper substratum layers throughout the egg incubation period. Our results suggest that instream restoration measures can contribute to freshwater biodiversity conservation, but reproductive success of species depending on long-term improvement of interstitial water quality cannot be achieved without considering catchment effects and natural substratum dynamics.

Introduction

Stream ecosystems comprise about 10% of global biodiversity, even though they cover less than 1% of the earth's surface (Strayer & Dudgeon 2010). At the same time, freshwaters provide the most essential ecosystem services for human existence (Vitousek et al. 1997, Geist 2011). Consequently, there is high anthropogenic pressure on these ecosystems, causing a strong depletion of riverine species that runs even faster than the loss of terrestrial biodiversity in tropical rain forests (Bernhardt et al. 2005). To mitigate the proceeding degradation of aquatic ecosystems, river restoration has recently become a widely applied management strategy (Søndergaard & Jeppesen 2007). Since stream-bed habitats are well known to play a key role in the ecological functioning of rivers (e.g. Bretschko 1995, Boulton et al. 1998, Sternecker et al. 2013*b*, Mueller et al. 2013*c*), improving substratum quality has become a core target in stream restoration. For instance, in the restoration of salmonid stocks, huge financial effort is invested to improve the availability and quality of spawning gravel (Kondolf et al. 1996). While the requirements on substratum quality are well documented for many riverine species (e.g. fishes: Sternecker et al. (2013*b*); freshwater molluscs: Geist & Auerswald (2007); macroinvertebrates: Bretschko (1981)) with a large body of scientific literature on the adverse effects of stream bed degradation (e.g. fine sediment input: Jones et al. (2012); alteration of natural substratum transport: Habersack (2013); gravel mining: Brown et al. (1998)), it still remains widely unknown how to effectively restore non-favourable stream-bed conditions to favourable conditions. This is mostly due to the trial- and error-based approach practiced in restoration management (Jansson et al. 2005, Pander & Geist 2013) and the limited availability of systematic studies on this topic following scientific standards. Most studies evaluating stream restoration are either based on geomorphologic effects (e.g. Zeh & Dönni 1994, Rubin et al. 2004, Meyer et al. 2008) or focus on indicator species to determine potential improvements (e.g. Pretty et al. 2003, Miller et al. 2009, Cooksley et al. 2012, Lorenz et al. 2012, Pulg et al. 2011). The applied indicators are usually riverine flagship species of high socioeconomic value (Muotka et al. 2002, Geist 2010). According to the indicator species concept (Primack et al. 1993, Reynolds & Souty-Grosset 2012), all other species co-occurring with the indicator species are also supposed to benefit from measures that improve habitat quality for the target species. This includes important food web components such as primary producers (e.g. algae and macrophytes) and invertebrates (e.g. aquatic insects, molluscs and crayfishes), which may also comprise critically threatened organisms (e.g. Plecoptera: Fochetti & de Figueroa 2006). However, several authors highlighted

that different species or taxonomic groups do not react congruently to environmental changes (e.g. anthropogenic impacts: Mueller et al. (2011); Heino (2010); restoration measures: Pander & Geist (2013)). Consequently, an improvement or failure for target species does not necessarily predict similar responses on ecosystem level. Since target species are not the only taxa to contribute to the conservation value of stream restoration, the evaluation of restoration success needs a holistic approach which integrates the consideration of several endpoints on different scales (target species, indicator groups, ecosystem scale), as recently proposed by Geist (2011) and Pander & Geist (2013). To date, such approaches have rarely been systematically tested due to their high time- and cost intensity, despite the recognition of the need for such studies expressed by restoration ecologists (e.g. Muotka et al. 2002, Arlettaz et al. 2011).

Herein, we evaluate the success of four different stream substratum restoration treatments at multiple scales, from single target species (lithophilic and rheophilic fish species) and single taxonomic groups to communities (algae, macroinvertebrates, macrophytes, fishes), and the entire ecosystem (changes in overall aquatic community composition and diversity). Specifically, we hypothesize that the four substratum restoration treatments differ in their effects on reproductive success and population structure of lithophilic and rheophilic fishes as well as in their ecosystem scale effects. The focus of this study was on biological endpoints since these are the typical targets of any restoration action. Based on the outcome of the study, we compare the conservation value of the investigated restoration measures. In this context, we evaluate the suitability of the indicator species concept for determining restoration success and investigated how the choice of endpoint affects the results. Particularly, we hypothesize that there is low congruency between restoration success for target species and ecosystem scale effects.

Material and Methods

Study rivers and restoration treatments

The study was conducted between June 2010 and June 2011 in six rivers from three major central-European drainage systems (Danube, Main/Rhine, Elbe) within Germany. The rivers represented small streams in cool, humid, sub-oceanic climate, with mean annual discharges ranging between 0.42 and 0.88 $m^3 s^{-1}$. They differed in their river morphology, fluvial dynamics and bedrock geology, comprising three calcareous (Günz (G): 48°16'10.23" N, 10°19'29.77" E, Mühlangergraben (M): 48°23'33.59" N, 11°43'31.82" E, Wiesent (W): 49°54'30.63" N, 11°19'11.62"

E) and three siliceous rivers (Große Ohe (O): 48°43'48.32" N, 13°15'14.73" E, Perlenbach (P): 50°13'33.74" N, 12°05'02.50" E, Südliche Regnitz (R): 50°17'13.04" N, 12°00'03.18" E). A detailed description of their physicochemical properties is presented in Braun et al. (2012). The study section in each river was located within headwater reaches, without confluence of tributaries directly before or within the study section. The fish community in these sections was formerly dominated by rheophilic specialists (brown trout *Salmo trutta* L., European grayling *Thymallus thymallus* L., nase *Chondrostoma nasus*, barbel *Barbus barbus*). In each study section, four different restoration treatments were completed and investigated for their biological effects. The treatments were gravel introduction of the grain sizes 16-32 mm (16/32) and 8-16 mm (8/16), substratum raking (SR) and the placement of boulders as sickle-formed current constrictor (referred to as "sickle-formed constrictor" SC in the following text). The treatment sites were arranged downstream of an untreated control site (CR) in each river. The SR site was located at the bottom end of the site to avoid high fine sediment deposition on other treatment sites caused by raking (Sternecker et al. 2013*a*); the 8/16, 16/32 and SC sites were arranged randomly in between CR and SR sites (Fig. 3.8). The length of the studied river sections incorporating all treatments ranged between 330 m and 3,520 m. The minimum distance between treatments was 70 m. For both gravel introductions, 20 m^3 of gravel were inserted into each river by an excavator, resulting in a homogenous area of 50 m^2. The treatment "substratum raking" was carried out by mechanically relocating 50 m^2 of clogged gravel with an excavator operating from the river bank, similar to the methodology described in Sternecker et al. (2013*a*). The sickle-formed constrictor was constructed according to Sindelar & Mende (2009), using chalkstone boulders with a void size of 0.6-0.8 m placed in the shape of two opposed sickles on both bank sides (reducing the river width to 35%). All treatments were carried out within five weeks in June/July 2010.

Study design

Ecosystem assessment All treatment and control sites were sampled one day before the implementation of the restoration treatments (to establish baseline condition), within one day after restoration (t = 0), three months later (t = 1) and after one year (t = 2) (Fig. 3.8). This approach was chosen to distinguish natural river dynamics, stochastic effects, and restoration-induced changes. At each sampling period, five replicate sampling points per treatment and control site were investigated. Due to the two distinctive habitats created by the construction of the sickle-formed constrictor, six sampling points comprising three between (SCI)

and three outside the sickles (SCA) were evaluated (Fig. 3.8). The study design resulted in a total of 618 samples: 6 rivers x (4 treatment sites + 1 control site) x 5 sampling points x 4 sampling periods = 600 samples + 18 additional samples at SC sites. In order to avoid spatially autocorrelated data and to ensure sampling representativeness, the distance and number of sampling points were determined according to assessments of the spatio-temporal heterogeneity in the same rivers (Braun et al. 2012).

Periphyton Periphyton constitutes an important food source for riverine meio- and macrofauna (Bunn et al. 1999). Consequently, periphyton was scraped off from the substratum surface at five sampling points per treatement at each timepoint, preserved with 20 mL of acidified Lugol's iodine solution and further processed as described in Mueller et al. (2011). Algae were determined according to Cox (1996), John et al. (2002) and Hofmann et al. (2011).

Macroinvertebrates Macroinvertebrates were included in the ecosystem assessment of the restoration treatments in this study since they are commonly considered good indicators for the ecological status of running waters (Wallace & Webster 1996). Macroinvertebrate samples were collected with a Surber-Sampler (Surber 1930, ; sampling area of 0.096 m^2, mesh size 0.50 mm) according to Mueller et al. (2011). Macroinvertebrates were preserved in 50% ethanol and classified according to Bauernfeind & Humpesch (2001), Bellmann (1993), Eggers & Martens (2001), Glöer & Meier-Brook (2003), Sundermann & Lohse (2006), Waringer & Graf (1997) and Zwick (2004) using a binocular microscope. Classification was performed at species, family (Chironomids, some Trichoptera and Ephemeroptera) or order-level (few Diptera).

Macrophytes Macrophytes are known to structure aquatic habitats (Braun et al. 2012) and to provide important habitat for macroinvertebrates and fishes (Balanson et al. 2005). To characterize restoration-induced changes in macrophyte abundance and species composition, total macrophyte cover as well as the coverage of each single species was estimated according to Braun-Blanquet (1932) . Species were determined according to van de Weyer & Schmidt (2007). Macroalgae were determined to genus-level according to John et al. (2002). Additionally, macrophyte biomass was estimated according to Downing & Anderson (1985) by quantitative harvest of all macrophytes contained in a metal quadrat (0.096 m^2) placed at each sampling point and subsequent determination of dry weight

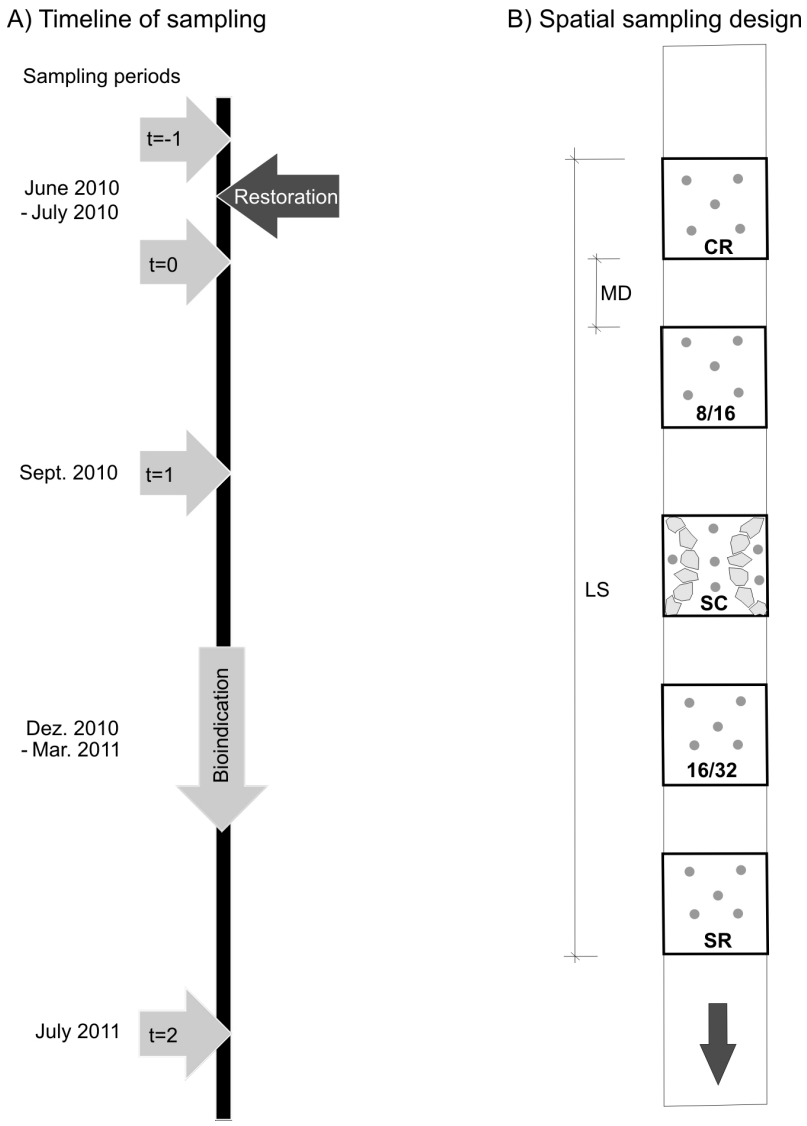


Figure 3.8: Schematic of the sampling concerning A) timeline and B) spatial sampling design. A) dark grey arrow = implementation of the restoration measures; bright grey arrows=sampling periods. B) MD = minimum distance between sampling sites (70 m); LS = length of sampling stretch (330 m-3,520 m); CR = upstream control site; 8/16 = gravel introduction of the particle size 8 mm-16 mm, position randomly assigned in each study river; 16/32 = gravel introduction of the particle size 16 mm-32 mm, position randomly assigned in each study river; SC = sickle-formed constrictor, position randomly assigned in each study river; SR = substratum raking, downstream site in each river; filled grey circles indicate sampling points

per m^2.

Target species assessment

Rheophilic and lithophilic fish species In order to evaluate the effects of restoration treatments on the populations of rheophilic and lithophilic fishes, the community was assessed one day prior to the implementation of the restoration treatments (-1) and after one year (2) using a electrofishing generator (EL65II 11kW, Grassel, Schoenau, Germany). Since fishes are very mobile, an area of 10 m upstream and downstream of each treatment site and control site was included, resulting in sampling stretches of 30 m length, previously identified as suitable to characterize fish habitats following restoration (Grossman et al. 1987, Pander & Geist 2010*b*). Fishes were sampled with a single anode from downstream to upstream direction according to the depletion method by (de Lury 1951). Fish of each stretch were kept in separate aerated plastic tanks. The total length of all specimens was measured to the nearest 0.5 cm. Fishes were released after all three stretches were sampled.

Natural spawning redds To evaluate the acceptance of the treatment sites as spawning habitat, the number of redds was counted in all treatment and control sites in late autumn 2010 (*Salmo trutta*), spring 2011 (*Thymallus thymallus*) and summer 2011 (*Phoxinus phoxinus*). Due to low turbidity and low water depth, redds were easily detectable by walking along the banks after the known completion of the spawning. While this method does not allow interpretations of egg and fry survival (see following point), it provides an adequate estimate of the acceptance of sites for spawning.

Bioindication with *Salmo trutta* eggs Active bioindication with fish eggs is highly suitable for evaluating the success of spawning-ground restoration for specific target species (Pander et al. 2009, Sternecker et al. 2013*a*). We applied the "egg sandwich" (Pander et al. 2009) for exposing eggs of *Salmo trutta* in the substratum of the treatment and control sites. Fertilized *Salmo trutta* eggs were obtained from hatchery fishes and were incubated in "egg sandwiches" (30 eggs per "egg sandwich") at the eyed-egg stage. For details on the origin and characteristics of the broodstock, see Sternecker et al. (2013*b*). Three "egg sandwiches" were exposed in the substratum of each study site in all study rivers (90 "egg sandwiches" in total) in December 2010. Additionally, 3 x 30 eggs were

exposed in the free-flowing water at each treatment site and control site using the same system as a control for each river. As an additional control, 3,000 eggs were exposed in "salmonid egg floating boxes" (Pander & Geist 2010*a*) under laboratory conditions at the Aquatic Systems Biology Unit (Technische Universität München, Freising, Germany). The "egg sandwiches" remained in the river substrate until January 2011 (river Moosach), February 2011 (rivers Wiesent, Alte Günz and Große Ohe) or March 2011 (rivers Südliche Regnitz and Perlenbach), depending on river-specific water temperature and the resulting time span until hatch in the free-flowing controls. Survival rates were calculated after removing the "egg sandwiches" from the substratum.

Data analysis

To evaluate the success of the four restoration treatments in comparison to the control sites and the baseline condition, a set of endpoints at multiple scales was used. These included multivariate changes of aquatic community composition and effects on aquatic biodiversity at the ecosystem scale, increased abundances of specialized taxa, certain functional/ecological traits for single taxonomic groups, and effects on target species (functional groups, population structure, acceptance as spawning ground, reproductive success).

Ecosystem level effects For the evaluation of ecosystem level effects we applied taxonomic sufficiency according to Mueller et al. (2013*a*). Pre-assessments indicated that multivariate community patterns were strongly correlated (spearman rank correlation coefficient > 0.91) and no differences in the discriminative power of permutational analysis of variance (PERMANOVA) analysis between treatments could be found up to the threshold levels of resolution identified in Mueller et al. (2013*a*). Consequently, we combined species-level data of macrophytes, family-level data of macroinvertebrates and order-level data of periphyton and calculated overall Bray-Curtis Similarities in the statistical software program Primer v6 (Plymouth Marine Laboratory, Plymouth, United Kingdom). Based on the Bray-Curtis resemblance matrix, PERMANOVA was carried out for each restoration type separately to test for significant differences between treatment sites and control sites at each sampling period (factor study site nested into sampling period) as well as between sampling period for each single restoration type (factor sampling period nested into study site). Sampling period (-1, 0, 1, 2) and treatment site (16/32, 8/16, SC, SR, CR) were defined as fixed factors. To visualize changes of the aquatic community, non-metric multidimensional scaling (NMDS) was performed for each

restoration type (combined with all control sites) separately. Additionally, univariate statistics based on species-level data were used to detect changes in numbers of individuals, species density, diversity and the composition of functional groups for periphyton, macroinvertebrates, macrophytes and fishes. Numbers of individuals, species density, Shannon Index and Evenness were calculated for each group using the DIVERSE procedure in the PRIMER v6 software. Macroinvertebrates were assigned to functional groups using the software program ASTERICS (available http://www.fliessgewaesserbewertung.de), fishes were assigned to functional groups according to the German fish evaluation system (Fischbasiertes Bewertungssystem FiBS, available at http://wrrl.flussgebiete.nrw.de/Ziele_und_Chancen/f__r_die_Gew__sser/__kologischer_Zustand/Fischfauna/FIBS/index.jsp). For periphyton and macrophytes the functional traits used in Mueller et al. (2013*a*) were applied. All data were tested for normality using the Shapiro-Wilk-Test and for homogeneity of variances using the Levene-Test. Since correlation coefficients and diversity indices were not normally distributed, the Kruskal-Wallis-Test and post-hoc pairwise Mann-Whitney *U*-Test were used to test for differences between aggregation levels. Bonferroni correction was applied to correct for multiple comparisons. Significance was accepted at $P \leq 0.05$. All univariate statistics were carried out in the software program R (http://www.r-project.org).

Effects on target species General changes in fish community composition were analysed via one-way analysis of similiarities (ANOSIM) based on Bray-Curtis Similiarity calculated from abundance data. To test if the restoration treatments influence the population structure of the target species, length-frequency data of all occurring lithophilic fish species were imported in PRIMER v6. Bray-Curtis coefficients between length-frequency distributions before the restoration (-1) and one year after the restoration (2) were calculated separately for each species and tested for significant differences between sampling period via PERMANOVA (species occurring in several rivers) and ANOSIM (species occurring in one river) analysis. For a visualisation of restoration effects on length-frequency distributions of different species at different sampling periods, a NMDS plot was computed with data from all species which differed significantly in their population structure between pre- and post-restoration status.

Relative survival rates of *Salmo trutta* eggs from the bioindication experiment were calculated by dividing the proportional survival of eggs exposed in the substratum by the survival rates of the respective free-flowing controls (Sternecker et al. 2013*b*). Relative survival rates were compared using univariate statistics

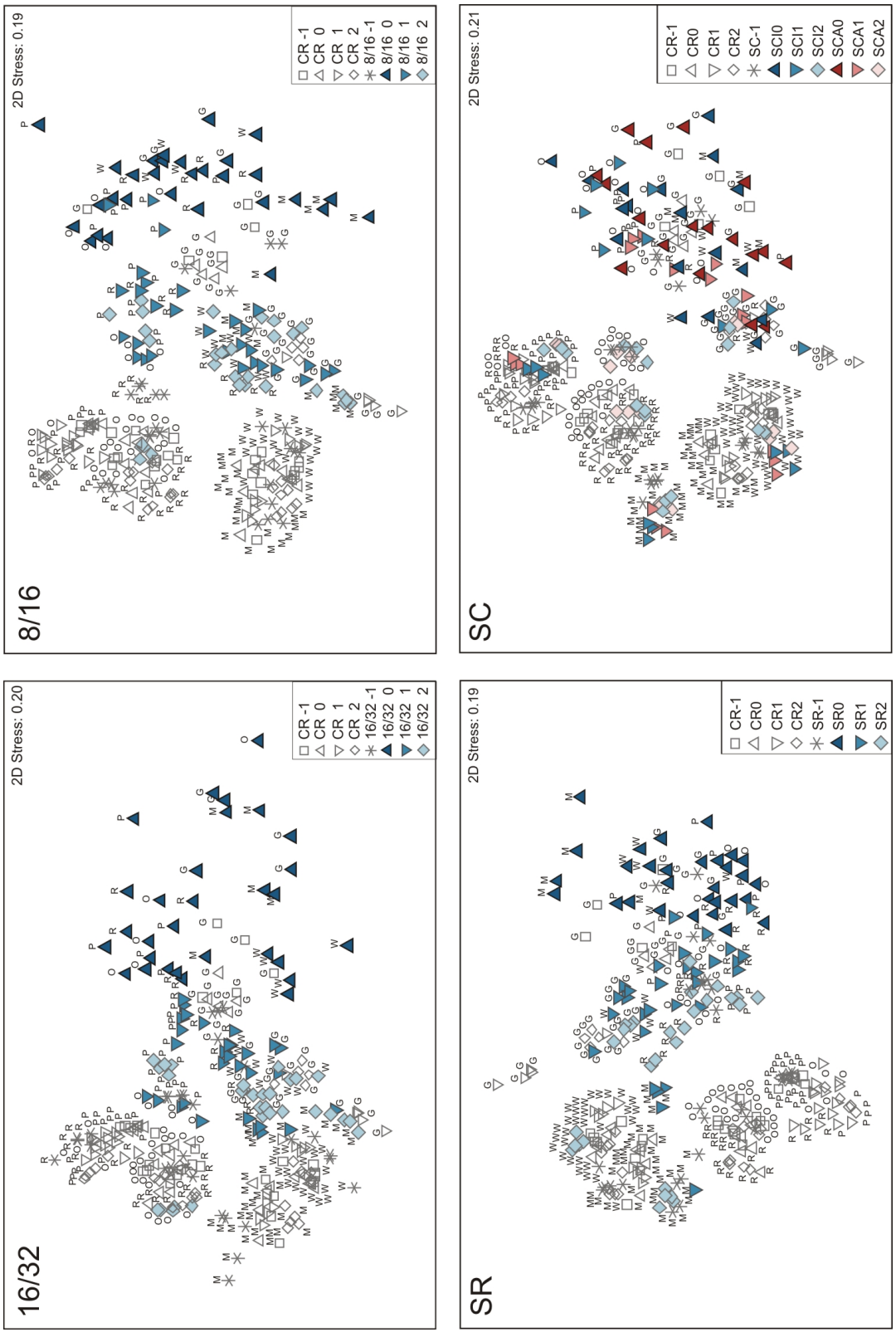
analogously to the analysis of diversity and functional traits.

Results

Integrative analysis of periphyton, macroinvertebrates and macrophytes

Restoration effects on overall aquatic community structure For all rivers, the combined multivariate analysis of periphyton, macrophyte and macroinvertebrate abundance data indicated that restoration-induced effects on aquatic community composition were most pronounced and long-lasting at 16/32 sites, followed by 8/16 sites and SR sites (Fig. 3.9). In contrast, the sickle-formed constrictor had only short-term biological effects after construction, as indicated by higher Bray-Curtis Similarities than control sites and baseline conditions (Fig. 3.9, Table 3.6). For both gravel introductions, aquatic community composition changed significantly one day after the introduction of the gravel compared to the baseline conditions and was still significantly different after three months and one year (PERMANOVA: Pseudo-$F = 7.03$; $P < 0.001$). A comparison between control sites and baseline conditions revealed that aquatic community composition at 16/32 and 8/16 sites already differed from the control sites at the baseline conditions (PERMANOVA: Pseudo-$F = 6.08$; $P < 0.01$), but the difference became more pronounced in the remaining sampling periods (PERMANOVA: Pseudo-$F = 6.08$;

Figure 3.9 ***(on the next page)*****:** Non-metric multidimensional scaling of abundance data from combined data of periphyton (order-level), macrophytes (species-level) and macroinvertebrates (family-level) based on Bray-Curtis Similarity, including all study rivers and timepoints. Open symbols, x and + show control sites and baseline conditions, filled symbols show treatment sites. G = River Alte Günz; M = River Mühlangergraben; O = River Große Ohe; P = River Perlenbach; R = River Südliche Regnitz; W = River Wiesent; 16/32 = gravel introduction of the particle size 16 mm-32 mm; 8/16 = gravel introduction of the particle size 8 mm-16 mm; SR = substratum raking; SC = sickle-formed constrictor at baseline conditions; SCI = sickle-formed constrictor: area between the two sickles; SCA = sickle-formed constrictor: area outside the sickles; CR = control sites; -1 = baseline conditions; 0 = 24 h after restoration; 1 = three months after restoration; 2 = one year after restoration


8/16
2D Stress: 0.19
CR -1
CR 0
CR 1
CR 2
8/16 -1
8/16 0
8/16 1
8/16 2
SC
2D Stress: 0.21
CR-1
CR0
CR1
CR2
SC-1
SCI0
SCI1
SCI2
SCA0
SCA1
SCA2
16/32
2D Stress: 0.20
CR -1
CR 0
CR 1
CR 2
16/32 -1
16/32 0
16/32 1
16/32 2
SR
2D Stress: 0.19
CR-1
CR0
CR1
CR2
SR-1
SR0
SR1
SR2

$P < 0.001$). The overall effects of substratum raking were very similar to those from the two gravel introductions, with significant differences within SR sites between all sampling periods (PERMANOVA: Pseudo-$F = 5.62$; $P < 0.001$) and the control site at each sampling period (PERMANOVA: Pseudo-$F = 8.50$; $t(-1) = 1.5$, $P(-1) < 0.05$; $t(0) = 3.6$, $t(1) = 3.6$, $t(2) = 2.5$, $P(0,1,2) < 0.001$). A comparison of Bray-Curtis Similarities between timepoints -1 and 2 at 16/32, 8/16 and SR sites indicates that the effect size was lower for SR than for 16/32 and 8/16 (Table 3.6). SC sites changed strongly after construction work (PERMANOVA: Pseudo-$F = 3.02$, $t(\text{SCI-CR}) = 2.7$, $P(\text{SCI-CR}) < 0.001$; $t(\text{SCA-CR}) = 2.8$, $P(\text{SCA-CR}) < 0.001$) and were still different from CR sites after three months (PERMANOVA: Pseudo-$F = 3.02$, $t(\text{SCI-CR}) = 1.8$, $P(\text{SCI-CR}) < 0.01$; $t(\text{SCA-CR}) = 2.0$, $P(\text{SCA-CR}) < 0.01$). After one year, SCI and SCA sites had the same community composition as CR sites. SCI and SCA sites did not differ significantly at any sampling period.

Within rivers, Bray-Curtis Similarities between treated and untreated sites (control sites as well as baseline conditions) indicated more pronounced effects of the gravel introductions 16/32 and 8/16 after one year in the rivers M, P, R and W (16/32: 1-46, 8/16: 1-42) than in the rivers G and O (16/32: 7-41, 8/16: 6-87). In contrast to the gravel introductions, substratum raking caused the strongest change of aquatic community composition after one year in the rivers O and P (Bray-Curtis Similarity between treated and untreated sites ranging between 2 and 25), while effects were less clear in the rivers G, M, W and R (Bray-Curtis Similarity between treated and untreated sites ranging between 1 and 82). The effect size of the sickle-formed constrictor on aquatic community composition after one year was highest in the rivers G, P and O (Bray-Curtis Similarity ranging between 4 and 86) and comparatively low in the rivers M, R and W (Bray-Curtis Similarity ranging between 19 and 89).

Restoration effects on overall aquatic biodiversity When periphyton, macroinvertebrate and macrophyte data were combined, none of the investigated restoration treatments caused a significant change of diversity measures lasting longer than three months. For both gravel introductions, overall numbers of taxa decreased significantly immediately after the restoration (compared to control sites and baseline conditions) and reached the level of baseline conditions after three months (Table 3.6). Overall Evenness increased significantly after three months for both gravel introductions (compared to control sites and baseline conditions), but decreased again to the level of baseline conditions after one year (Table 3.6). Overall Shannon Index was significantly higher at 16/32 and 8/16 sites than at

CR sites after three months, but did not differ from the baseline conditions at any sampling period for 16/32 sites. For 8/16 sites overall Shannon Index was significantly reduced immediately after construction work (Table 3.6). Overall abundance also decreased strongly at 16/32 and 8/16 sites compared to -1 and CR sites after construction work (0) and was still reduced at sampling period 1. After one year overall abundance increased slightly but not significantly higher than baseline condition values at 16/32 sites. At 8/16 sites, overall abundance was still reduced compared to the baseline conditions (Table 3.6). At SR sites, diversity descriptors developed similarly as in the gravel introductions (Table 3.6). At SC sites overall richness, abundance and Evenness changed significantly after construction work and reached between 80% and 100% of baseline conditions within three months. Overall Shannon Index did not change significantly throughout the study period (Table 3.6).

Table 3.6: Changes in diversity, productivity, functional/ecological guilds, protected species and Bray–Curtis Similarity for the four investigated substratum restoration treatments (16/32 = gravel introduction of the particle size 16–32 mm; 8/16 = gravel introduction of the particle size 8–16 mm; SR = substratum raking; SC = sickle-formed constrictor). The degree of change after one day (0), three months (1) and one year (2) is given as a factor in relation to the baseline conditions (-1). Accordingly, values smaller than 1 indicate a decrease compared to the baseline conditions and values higher than 1 an increase, while 0 means that the respective variable decreased to 0. Values significantly different to baseline conditions (-1) are printed in bold, significant differences to the control sites are indicated by * (Kruskal–Wallis–Test and post-hoc Mann-Whitney *U*-Test, Bonferroni corrected, $P < 0.05$; Bray–Curtis Similarities: PERMANOVA $P < 0.05$). Diatoms = cell numbers of diatoms, Biomass = macrophyte dry mass per m^2; Plecoptera = number of individuals from the order Plecoptera; Rhithral species = percentage of macroinvertebrates adapted to rhithral habitats; Akal, Lithal, Psammal = percentage of macroinvertebrates adapted to akal, lithal and psammal habitats; Rheophilic fishes = number of specimen with a high current preference; Lithophilic fishes = number of specimen with a preference for gravel habtiats for spawning; Invertivorous fishes = number of specimen feeding on invertebrates; Red List/FFH fishes = number of specimen protected according to the German Red List of threatened Species and/or the European Habitats Directive. Overall view = synthesized data from periphyton, macroinvertebrates and macrophytes. NA = value not calculable

	16/32			8/16			SR			SC		
	0	1	2	0	1	2	0	1	2	0	1	2
Periphyton												
Species richness	**0.5***	0.9	0.9	**0.4***	**0.7**	**0.7**	**0.6***	1	0.9	**0.6**	**0.7**	0.8
Cell numbers	**0.04***	**6.4**	**10.3**	**0.02***	4.3	7.3	**0.03***	1.9	2	0.6*	1	1
Shannon Index	0.9	0.9	0.8	0.9	1.1	0.8	1	1	0.9	0.8	1	0.9
Evenness	**1.3**	0.9	0.8	**1.5**	1.2	0.9	**1.2**	1	1	1.1	1.2	1
Diatoms	**0.04***	10.4	7.3	**0.03***	15.9	2.7	**0.05***	6.2	4.2	0.3	2.8	1.7
Bray-Curtis Similarity	**0.07***	**0.16**	**0.16**	**0.03***	**0.36**	**0.2**	**0.06***	**0.6***	**0.44**	**0.71***	0.45	**0.68**

	16/32			8/16			SR			SC		
	0	1	2	0	1	2	0	1	2	0	1	2
Macrophytes												
Species richness	**0***	0.1	0.4*	**0***	**0.1**	**0.1**	**0***	0.3	0.8	**0***	0.3	0.5
Coverage	**0***	0.04	0.8	**0***	**0.01**	0.1*	**0***	0.1	0.9	**0***	0.8	0.7
Shannon Index	NA	NA	0.4	NA	0.1	0.1*	NA	0.4	1.1	NA	0.4	0.7
Evenness	NA	NA	1	NA	1.3	1.1	NA	1.4	1.5	NA	1.2	1.2
Biomass	**0***	**0.03***	**0.5***	**0***	**0***	**0.6***	**0***	**0.03***	**0.5**	**0***	0.6	1.2
Macroinvertebrates												
Species richness	**0.3***	1.1	**1.6**	**0.4***	**1.4**	**1.8**	**0.4***	1	**1.4**	**0.5***	0.9	1.4
Abundance	**0.1***	1	**3.8***	**0.1***	1.2	**3.6***	**0.1***	0.5	**2.1**	**0.2***	0.4	1.3
Shannon Index	**0.5***	1	1.1	**0.5***	1.1	**1.2**	**0.7***	1.1	1.1	**0.8**	1	1.1
Evenness	1	0.9	**0.9**	**1.1**	1	0.9	**1.3***	1.1	0.9	**1.2***	1	1
Bray-Curtis Similarity	**0.17***	**0.48***	**0.32***	**0.17***	**0.5***	**0.34**	**0.11***	**0.38***	**0.39***	0.25*	**0.45**	**0.38**
Plecoptera	0*	0.7	**14***	**0***	**2***	**15***	**0.3**	0.5	**4.5**	**0.5**	0.5	**2.8**
Rhitral species	**0.8**	**1.3**	**1.3***	1	1	1.1	1	1.3	1.1*	0.8	1	1
Akal, lithal, psammal	0.9	**1.1***	**1.3**	1.1	**1.3***	**1.4**	1.1	**1.2**	**1.5**	1.1	1.1	**1.5**
Fishes												
Species richness			1.2			1			1.8			1.2
Abundance			4.6			1.8			2.6			2.4
Shannon Index			1			1			1.4			1
Evenness			0.9			1			1			0.8
Bray-Curtis Similarity			0.32			0.65			0.55			0.53
Rheophilic fishes			4.5			1.6			2.1			1.4
Lithophilic fishes			4			2			3			2.3

	16/32			8/16			SR			SC		
	0	1	2	0	1	2	0	1	2	0	1	2
Invertivorous fishes			5.5			1.7			2.4			2.4
Red List/FFH fishes			4,1			1.8			2.7			2.4
Overall view												
Species richness	**0.4***	0.9	1.1	**0.4***	0.8	0.9	**0.5***	0.9	1	**0.5***	**0.8**	0.9
Overall abundance	**0***	**0.4***	1.4	**0***	**0.3***	0.6	**0***	**0.2***	1*	**0.05***	0.8	0.8
Shannon Index	1	1.3*	1.1	**0.7***	1.2*	1	1	**1.3***	1.3*	1	0.9	0.8
Evenness	1.4	**1.4***	1	1	**1.2***	1	**1.3***	**1.4***	1.2*	**1.4***	1	0.8
Bray-Curtis Similarity	**0.02***	**0.18***	**0.39***	**0.02***	**0.16***	**0.26***	**0.02***	**0.2***	**0.57***	**0.09***	**0.55***	0.72

Effects on diversity and functional/ecological guilds of single food web components

Periphyton Changes in periphyton community composition were most pronounced at 16/32 sites, followed by 8/16 sites (Table 3.6). Accordingly, at 16/32 sites overall cell numbers and diatom cell numbers increased most strongly after a temporary decrease at sampling period 0. At SR and SC sites, no increase of cell numbers compared to CR sites was observed (CR: 2.2-fold, SR: 2-fold, SC: 1-fold). Periphyton species density decreased significantly to 40%-60% after construction work for all treatment sites, reaching 70%-90% of the baseline conditions after one year (Table 3.6). At CR sites species density was also 20% lower in June 2011 (2) than in June 2010 (-1). Except a short-lasting increase in Evenness at timepoint 0, no changes in Shannon Index and Evenness could be detected for periphyton (Table 3.6).

Functional characteristics of the periphyton community (trophic state, life form, motility, habitat preference) did not change for any restoration treatment.

Macrophytes Macrophyte community composition changed strongly after restoration, with most species disappearing in all treatments except for the reference. This resulted in all-zero-samples at many treatment sites, particularly immediately after restoration. Macrophyte species density and coverage at 8/16 and 16/32 sites was 0% after construction work. After one year, macrophyte species density was still 90% reduced for the gravel introduction 8/16 and 60% reduced for the gravel introduction 16/32. Macrophyte coverage after one year was 80%-90% reduced in both gravel introductions. At SR and SC sites, macrophyte coverage was 10%-30% reduced after one year (Table 3.6), but was still low compared to the CR sites (2.1-fold increase from -1 to 2). Analogously, macrophyte biomass was significantly reduced compared to the baseline conditions and control sites for all sampling periods at 8/16 and 16/32 sites. The reduction of macrophyte biomass at SR sites was similar to the gravel introductions (Table 3.6). At SC sites, macrophyte biomass reached the level of control sites and baseline conditions after one year (Table 3.6). For Shannon Index and Evenness, no significant changes were detected after one year. However, Evenness was generally increased after restoration, while Shannon Index decreased at most treatment sites according to the strongly reduced species density and coverage (Table 3.6).

Macroinvertebrates Macroinvertebrate community composition changed significantly immediately after restoration for both gravel introductions and substratum

raking (Table 3.6). At 16/32 and SR sites, community composition was still significantly different to control sites and baseline conditions after three months and one year, while community composition at 8/16 and SC sites were only significantly different to baseline conditions (Table 3.6). The increase in macroinvertebrate species density after one year was most pronounced for the gravel introduction 8/16 (1.8-fold) and the increase in number of individuals was most pronounced for the gravel introduction 16/32 (3.8-fold, Table 3.6). This increase was less pronounced at SR sites (species density: 1.4-fold, number of individuals: 1.3-fold). At SC sites, no significant changes in species density and number of individuals could be detected after one year. Except for a 20% increase in Evenness at 8/16 sites, Shannon Index and Evenness were not influenced one year after restoration at all treatment sites (Table 3.6). Functional feeding groups (FFG) indicated no significant change in the availability of coarse and fine particular organic matter (percentage of shredders) and predator-prey relationships within macroinvertebrates (percentage of predators vs. other feeding types) for all restoration treatments. A comparison of the Saprobic index between rivers and treatment sites revealed no significant differences between sampling periods. In contrast, the analysis of functional/ecological groups referring to habitat structure, revealed significant changes for species with a preference for gravel habitats (akal, lithal, psammal) for all restoration treatments and for species with a preference for rhithral conditions at 16/32 sites (Table 3.6). Considering species of high conservation relevance, the strongest change was found for the order of Plecoptera, where numbers of individuals increased 5-fold stronger at 8/16 and 16/32 than at SC and CR sites. For substratum raking, this increase was 2-fold stronger than at SC and CR sites (Table 3.6).

Effects on target species

Rheophilic and lithophilic fish species Fish community composition differed strongly between rivers (ANOSIM: Global $R = 0.82$, $P < 0.001$) but did not change significantly between sampling periods -1 and 2 over all rivers (PERMANOVA: Pseudo-$F = 1.29$, $P > 0.05$). Accordingly, species density, Shannon Index and Evenness also stayed constant (Table 3.6). Numbers of individuals significantly increased one year after restoration in all rivers and treatments (Mann-Whitney U-Test: $P < 0.01$). Within rivers, a significant change in community composition could also be observed for rivers G (PERMANOVA: $t = 1.5$, $P < 0.05$), O (PERMANOVA: $t = 2.7$, $P < 0.05$) and R (PERMANOVA: $t = 2.0$, $P < 0.01$). This was mainly caused by the significant increase in abundance of rheophilic, lithophilic

and insectivoric fish species in these rivers (Mann-Whitney U-Test: $P < 0.05$). A slight but non-significant increase of these species was also observed in the rivers P and W, while the fish community of the river M was unchanged from the restoration treatments, comprising a high number of ubiquitous species (90%). The strongest increase of rheophilic, lithophilic and invertivorous species occurred at 16/32 sites, followed by SR sites (Table 3.6). Enhanced abundances were also found at 8/16, SC and CR sites (Table 3.6), but this increase was much lower than for the gravel introduction 16/32 and substratum raking. The numbers of federally protected fish species according to the German Red List (Haupt et al. 2009) and the European Habitats Directive (European Parliament 1992) increased accordingly at 16/32 and SR sites (Table 3.6). A comparison of length-frequency data from all occurring lithophilic species (*Salmo trutta*, *Chondrostoma nasus*, *Barbus barbus*, *Phoxinus phoxinus*, *Cottus gobio*, *Barbatula barbatula*, *Alburnoides bipunctatus*) between the electrofishing survey before and one year after the restoration revealed significant changes for *Phoxinus phoxinus* (PERMANOVA: Pseudo-$F = 3.79$, $P < 0.001$), *Cottus gobio* (PERMANOVA: Pseudo-$F = 1.57$, $P < 0.05$), *Barbatula barbatula* (PERMANOVA: Pseudo-$F = 2.62$, $P < 0.001$) and *Alburnoides bipunctatus* (ANOSIM: Global $R = 1$, $P < 0.01$). For all of these species, the most pronounced change of length-frequency distributions occurred at 16/32 sites and were mainly caused by an increase of juvenile abundance (Fig. 3.10). No significant change in the multivariate length-frequency distribution was detected for *Salmo trutta*, *Chondrostoma nasus* and *Barbus barbus*. However, the abundance of *Salmo trutta* < 10 cm increased 18 fold at 8/16 sites and 6 fold at 16/32 sites. This increase mainly resulted from the catch numbers in the rivers O, P and W, while the numbers of juvenile *Salmo trutta* stayed constantly very low in the rivers G, M and R. The catch of *Chondrostoma nasus* and *Barbus barbus* was generally restricted to few individuals in the river G.

Natural spawning redds By far the most spawning redds (68%) were found in the 16/32 sites, comprising 21 *Salmo trutta* (M, W and P) redds, 10 *Thymallus thymallus* (W) redds and 12 *Phoxinus phoxinus* (R) redds. The second highest percentage of spawning redds (27%) was found in the 8/16 sites, with a total of 14 *Salmo trutta* redds (M, W and R) and 3 *Thymallus thymallus* (W) redds. Only 5% of all spawning redds were found in the control sites (three spawning redds of *Salmo trutta* in river M). No natural spawning redds could be detected in SR and SC sites. From two of the study rivers (G, O), spawning redds were excluded from the analyses due to their limited size and uncertainty in identification (Fig. 3.11).

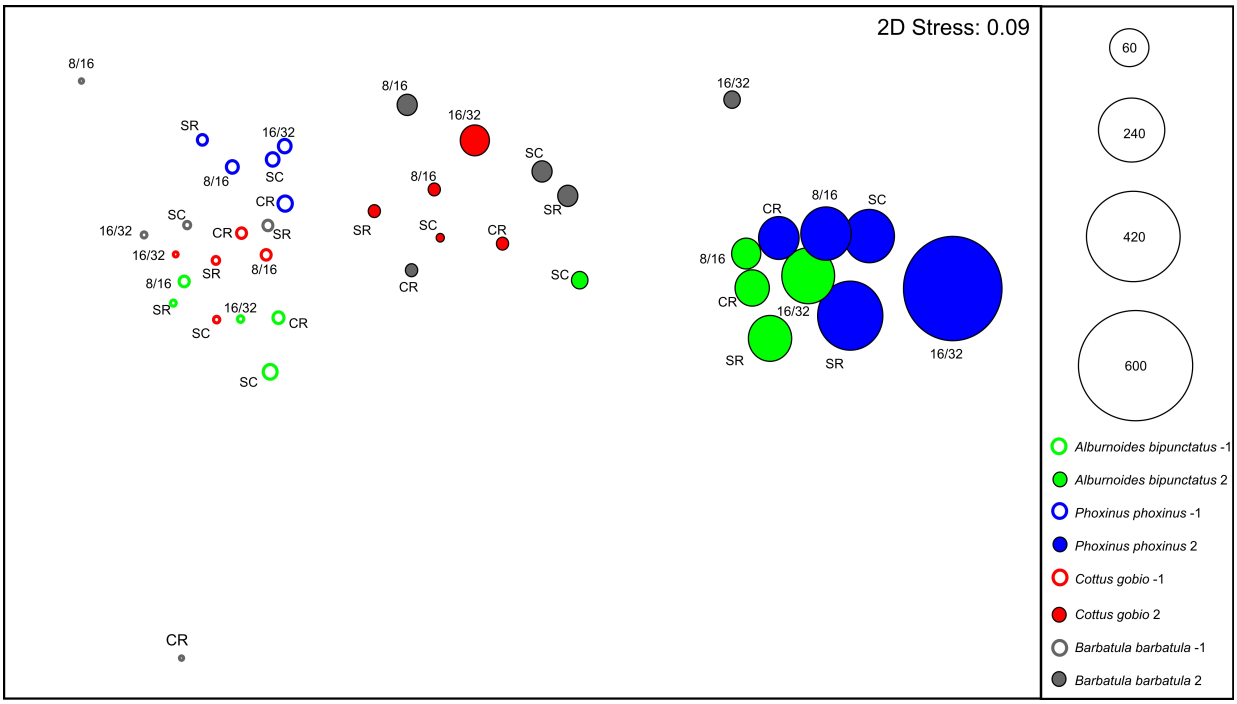


Figure 3.10: Non-metric multidimensional scaling of length-frequency data from lithophilic fishes. Open circles indicate sampling sites of the baseline conditions in 2010, filled circles indicate sampling sites one year after restoration in 2011. The size of the circles visualizes the number of juvenile individuals for each fish species. Juveniles were classified according to Patimar et al. (2012) for *Alburnoides bipunctatus*, (Kováč et al. 1999) for *Barbatula barbatula*, (Davey et al. 2005) for *Cottus gobio* and (Mills & Eloranta 1985) for *Phoxinus phoxinus*. 16/32 = gravel introduction of the particle size 16 mm-32 mm; 8/16 = gravel introduction of the particle size 8 mm-16 mm; SR = substratum raking; SC = sickle-formed constrictor; -1 = baseline conditions; 0 = 24 h after restoration; 1 = three months after restoration; 2 = one year after restoration

Bioindication with *Salmo trutta* eggs None of the investigated substratum restoration treatments significantly improved survival rates of *Salmo trutta* eggs and larvae compared to the untreated control sites (Fig. 3.11), with variability within and between sites being very high. At 16/32 sites the average survival rate was 7% higher than at the CR sites (16/32: 53 ± 50%, CR: 46 ± 49%), while at SR, SC and 8/16 sites survival rates were even lower than at the control sites (SR: 45 ± 63%, SC: 42 ± 45%, 8/16: 41 ± 43%). Survival rates in the free-flowing water ranged between 39% and 70%, with lowest survival in the rivers W and G and best survival in the rivers M and R. Maximum survival rates in the free-flowing water of the study rivers were similar or even higher (62%-81%) than in the laboratory control (77%).

Discussion

Throughout the world, increasing effort is being directed to stream restoration for conservation of aquatic biodiversity (Geist 2011, Bernhardt et al. 2005). The major goal of these efforts should be to restore the ecological integrity of river ecosystems (Poudevigne et al. 2002). As many authors emphasized before, an assessment of the ecological integrity of stream ecosystems needs to include multiple levels of biological organisation (e.g. Heino 2010, Geist 2011, Pander & Geist 2013) and the consideration of an adequate number of replicates (e.g. Pik et al. 1999, Braun et al. 2012). In contrast to the systematic investigation of biological effects of different restoration techniques presented herein, most studies of restoration success are still single case studies in specific rivers for only one measure and neither synthesize comparisons of different techniques nor responses of multiple taxonomic groups (e.g. Merz et al. 2005, Sarriquet et al. 2007, Palm et al. 2007). As the results of the present study indicate, different ecosystem components respond to restoration in different ways. For instance, the assessment of juvenile fish abundances and macroinvertebrates revealed high restoration success for the gravel introduction 16/32, while the mere consideration of egg survival of the target species *Salmo trutta* would have indicated no restoration success. In addition, restoration measures can affect each river differently. In the river M, macroinvertebrate and periphyton abundance strongly increased at 16/32 sites, but there was no improvement in juvenile abundances of lithophilic fishes. Restoration success of substratum raking largely depends on the characteristics of the autochthonous river substratum and consequently strongly differed between rivers. In light of the strong influence of site- and river-specific baseline conditions on single indicators, an integrative assessment of restoration success in several

rivers is essential to avoid misleading conclusions based on outliers. The simultaneous inclusion of multiple evaluation scales applied herein (spawning and egg development of target species; population structure of lithophilic fishes; food web components on different trophic levels: algae and macrophytes as primary producers, macroinvertebrates as primary consumers, macroinvertebrates and fishes as secondary consumers) allowed a holistic and representative evaluation of restoration success in the context of aquatic conservation. Despite the high complexity of results inherent to comprehensive investigations, it is possible to deduce a combination of easily accessible endpoints (such as those presented in Fig. 3.11: factors of change for overall beta diversity, macroinvertebrate, macrophyte and periphyton abundance, length-frequency distributions, *Salmo trutta* egg survival, acceptance for spawning) that allow practitioners to focus on specific aspects or individually weight different factors according to the conservation target. This synthesized view indicates that the gravel introduction 16/32 had the most positive effects on primary and secondary production as well as on the acceptance as spawning ground, while none of the restoration treatments investigated herein could achieve a sustainable improvement of reproductive success for salmonids (Fig. 3.11). Since the set of restoration treatments evaluated herein represents the most commonly applied in-stream measures addressing riverine key habitats, it is likely that the long-term efficiency of in-stream measures in general is strongly limited.

Integrative analyses of multiple taxonomic groups

The multivariate comparison of Bray-Curtis Similarities, calculated from the combined data of multiple taxonomic groups, was a highly suitable tool for quantification of the effect size between restoration types and rivers. This method still discriminated between treatment sites and sampling periods when the taxonomic resolution was reduced to family-level for macroinvertebrates and to order-level for periphyton. The general results were in line with those revealed from single taxonomic groups and target species, indicating that the effect size on overall aquatic community was highest for the gravel introduction of the grain size 16-32 mm and lowest for the sickle-formed constrictor. However, if species numbers, abundances and diversity indices from several taxonomic groups are synthesized and considered in univariate analysis, effects of high conservation relevance may be overlooked when different taxonomic groups have opposite reactions to environmental changes. This was evident for overall abundances in the present study, where a strong decline of macrophytes at 16/32 and 8/16

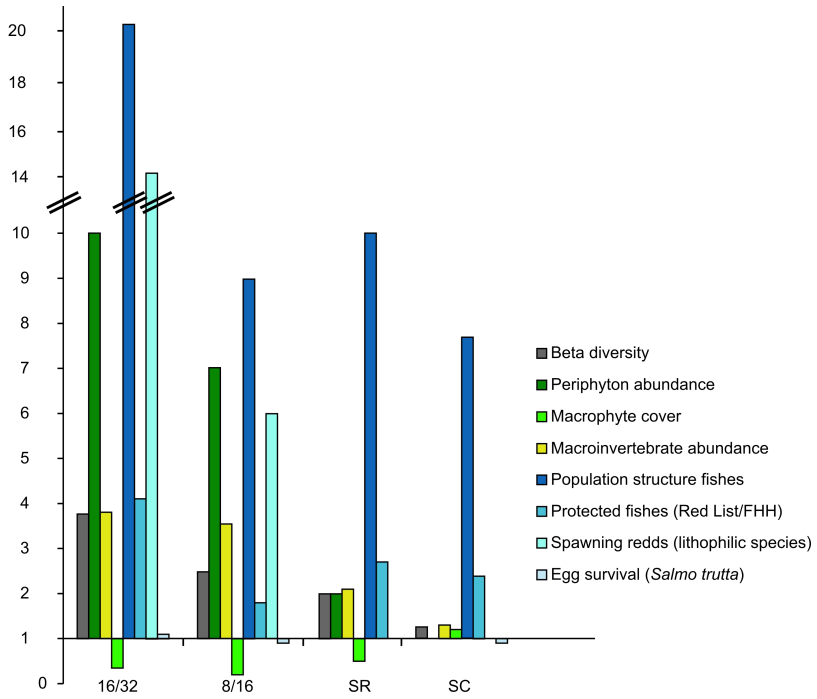


Figure 3.11: Bar plot of selected ecosystem and target species based endpoints for an integrative and comparative evaluation of restoration success. The length of the bars indicates the factor of change one year after the implementation of the restoration treatments compared to the baseline conditions. A factor of 1 indicates no change, factors < 1 a decrease and factors > 1 an increase compared to baseline conditions. Beta diversity = change of Bray-Curtis Similarity (including periphyton, macrophytes and macroinvertebrates); Periphyton abundance = change of periphyton cell numbers; Macrophyte cover = change of macrophyte cover; Macroinvertebrate abundance = change of macroinvertebrate numbers of individuals; Population structure fishes = change of Bray-Curtis Similiarity in length-frequency distributions of lithophilic target species; Protected species (Red List/FFH) = change of numbers of individuals of protected fish species according to the German Red List and the European Habitats Directive; Spawning redds (lithophilic species) = change of number of spawning redds of lithophilic fishes compared to the control sites; Egg survival (*Salmo trutta*) = change of survival rates of *Salmo trutta* eggs compared to the control sites. 16/32 = gravel introduction of the particle size 16 mm-32 mm; 8/16 = gravel introduction of the particle size 8 mm-16 mm; SR = substratum raking; SC = sickle-formed constrictor; CR = control sites

sites disguised the strong increase in macroinvertebrates, resulting in only minor overall increase (16/32) or even decrease (8/16) of abundances and species density. For this reason, the general view should ideally be accompanied by selected criteria based on important food web components and critical life stages of target species.

Restoration effects on the integrity of aquatic food webs

As the saprobic and trophic status did not differ significantly between treatment sites and rivers, it is highly likely that observed differences in the overall evaluation of the investigated treatments are founded in the structural characteristics of the created habitats. The coarse gravel introduction (16/32) provided clean and light-exposed surface area for benthic algae to grow on and reduced suitable substrate area for macrophyte growth. This shift from macrophytes to periphytic algae can be a desired conservation target, e.g. when the lack of riparian shade leads to increased macrophytes growth followed by sediment trapping, changes in habitat and water quality as well as a decline in periphyton food quality (Bunn et al. 1999, Jones et al. 2012). The increased cell numbers of diatoms constitute an ideal food source for many herbivoric macroinvertebrates, such as Ephemeropterans from the genus *Baetis* (Willoughby 1988). In addition, the introduction of coarse gravel generated large interstitial spaces in the uppermost substratum layer which constitute important habitat space for many macroinvertebrates of high conservation value, such as the order Plecoptera with a high proportion of species that prefer cobble and gravel habitats (Duan et al. 2009). The less pronounced effects of the gravel introduction 8/16 and substratum raking suggest that available surface area for peripyhton (SR, 8/16) and habitat space for macroinvertebrates (SR) in this treatment were reduced as compared to 16/32 sites, due to the smaller grain size of the 8-16 mm gravel and the remaining proportion of fine sediments left behind after substratum raking. In contrast to coarse gravel, boulders obviously did not constitute a crucial habitat structure in the investigated river stretches. This finding was unexpected since an increase of habitat variability is generally considered to be beneficial and desirable in stream restoration and conservation (Hughes et al. 2005). Presumably, the arrangement of the sickle-formed constrictor did not sufficiently improve substratum structure and the large-sized boulders themselves were not a limiting structural element in this study. Consequently, the conservation value of stream restoration for the integrity of stream ecosystems is, in contrast to the suggestions of other authors (Sarriquet et al. 2007, Miller et al. 2009, Pander et al. 2013), less dependent on an

increase (observed at SC sites) or decrease (observed at 16/32 and 8/16 sites) of habitat variability than on the introduction of the most limiting habitat structures. A high variability of habitat structures introduced by restoration may by chance include some important key habitats. However, to ensure a high effectivity of restoration measures it is of great importance to identify the most limiting deficits in the river of interest prior to the restoration (Pander & Geist 2013).

Restoration effects on population structure of target species

A more detailed consideration of fish population structure revealed that the 16-32 mm gravel not only provides additional habitat space for macroinvertebrates and periphyton, but is also valuable spawning and juvenile habitat for lithophilic fishes. Most of these species are protected according to national (German Red List) or international (European Habitats Directive) regulations and therefore are of high conservation value. The increase of the percentage of invertivorous fishes suggests that the 16/32 sites also provided an optimal feeding habitat. The comparatively low increase at 8/16 sites confirms the assumption that the observed changes were mostly caused by structural effects, since interstitial spaces near the surface of 8-16 mm gravel are probably too small to provide habitat structure for fishes beyond very early life stages, while food supply for invertivorous fishes was also strongly improved at 8/16 sites. Generally, the effects on population structure were restricted by species being already present at a minimum viable population size prior to the restoration (*Phoxinus phoxinus*, *Cottus gobio*, *Barbatula barbatula* and *Alburnoides bipunctatus*). Populations of species that already occurred in very low numbers prior to the restoration (*Chondrostoma nasus*, *Barbus barbus*, *Salmo trutta*, *Thymallus thymallus*) and have comparably long generation times could not significantly benefit from the restoration yet.

Restoration induced effects on critical life stages of target species

The acceptance of the artificially constructed gravel habitats as spawning habitat by different species (*Salmo trutta*, *Thymallus thymallus* and *Phoxinus phoxinus*) implies a general suitability of both of the introduced gravel mixtures for lithophilic fishes. The comparatively low number of natural spawning redds at the remaining treatment sites and control sites confirms the assumption that gravel bar habitats are limited. However, the low survival rates of *Salmo trutta* eggs in the hyporheic zone of all treatment sites compared to the free-flowing and laboratory controls indicate that interstitial water chemistry may continue to be unsuitable. These

deficits probably result from a rapid degradation of hyporheic exchange rates due to high fine sediment loads from the catchment area in the agriculturally dominated landscape (Davies et al. 2009) and missing fluvial dynamics (e.g. high peak flows and substratum transport). Consequently, reproductive success of species depending on long-term improvement of interstitial water quality cannot be solely enhanced with the investigated substratum restoration treatments. In contrast to the results of our study, positive effects of substratum restoration for salmonid egg survival were observed by Sternecker et al. (2013*a*). However, in that study restoration took place immediately before the spawning season and effects were only evaluated within a short time span of three months. The results after six months of the bioindication monitoring in our study imply that the positive effects observed by others may be restricted to short term improvements only. A sustainable mitigation of the remaining deficits, which should be the overall goal of restoration, can only be achieved by a catchment scale restoration approach.

Conclusions

In light of the fact that the restoration treatments investigated herein are generally considered as effective management strategies in restoration practice (e.g. River Restoration Centre 2002, Hanfland et al. 2009), the biological effects observed in our study were surprisingly small and restricted in time. Enhanced habitat variability turned out to have no effect on stream biota as long as the most limiting habitats for the target species of conservation are not addressed. Taking into account the adverse effects of substratum raking on downstream sites and the marginal substratum improvement achieved by the sickle-formed constrictor, the introduction of gravel is the only technique that can be recommended as in-stream restoration measure for immediate short-term improvements of substratum quality. The limited success of all treatments concerning a long-term improvement of interstitial water chemistry suggests that sustainable restoration needs to move beyond the in-stream scale and instead needs to integrate the catchment scale (fine sediment input, alteration of natural fluvial dynamics).

In general, restoration measures should be systematically tested on a scientific basis before being widely applied or recommended as management strategies. An optimum way to realize this is to carry out restoration explicitly for scientific investigation. In this study, restoration success was determined using a combination of biological endpoints from different levels of biological organisation, and a comparative evaluation of different measures in relation to untreated control sites. Such an approach allows to explore ecological mechanisms of improvement and

to identify deficits that cannot be mitigated. We suggest applying the multi-scale evaluation concept presented herein (with specifically adapted target species and ecosystem components) to establish a scientific background for a broader field of restoration types and ecosystems. For further monitoring of single projects a clear conservation objective is necessary to select the most appropriate endpoints, since the indicator concept is of limited use for the determination of general ecosystem improvements.

4 Strategies for ecosystem monitoring

In the past decades, investigations of biodiversity have mainly focused on species richness of certain, intensively studied biotic groups (e.g. birds, butterflies, stream macroinvertebrates) and systematically neglected and oversimplified the complexity of ecosystems and functional interactions between different components (de Leo & Levin 1997, Fischer et al. 2004). This is probably one of the reasons for the failure in achivieng the biodiversity conservation targets set by international conventions in time (Sachs et al. 2009, Rands et al. 2010). This thesis synthesizes scientific knowledge from five case studies on ecosystem scale ecological research and a literature review, aiming at the development of a holistic and integrative approach to ecosystem monitoring. In this chapter, methods of data analysis and monitoring components used are evaluated in concern to their ecological relevance, statistical credibility, cost-effectiveness, and transferability to other systems as being the most important criteria for successful ecological monitoring programs (Caughlan & Oakley 2001).

4.1 Feasibility of a holistic ecosystem approach to freshwater monitoring at different assessment stages

4.1.1 Sampling design and implementation

An implementation of holistic and integrative ecosystem research already begins at the stage of planning the investigation. In particular, a standardized spatial and temporal sampling design for all included ecosystem components is a crucial prerequesite for the alignment of data from different taxonomic groups to a common spatial area and time frame at the later stage of data integration. However, this bears the difficulty that field sampling methods for different taxonomic groups and physicochemical characteristics do not necessarily require similar spatial extent of a single sample. For instance, fishes and macrophytes are mostly sampled from a water surface area of several m^2, while sampling of smaller organisms such as macroinvertebrates, periphyton (Mueller et al. 2011, 2014) or bacteria (Mueller et al. 2013*c*) occurs at scales of less than one m^2 to few mm^2. A possible solution for this problem is to apply a nested spatial sampling design as presented in

Mueller et al. (2011), where the sampling area of larger taxa (fishes) is covered by a collective sample of smaller taxa (e.g. macroinvertebrates sampled in different microhabitats or periphyton scraped off from several stones). Within this lowest common unit each discipline can use a sampling design and methods adopted to discipline-specific needs (Jürgens et al. 2012).

Ideally, the spatial extent of the lowest common sampling unit is chosen as small as possible to allow a high number of spatial and temporal replicates. Thereby it is important that the spatial extent of the resulting samples is still reasonable for each discipline. In case the minimum necessary sample size is unknown, the reduction of sampling area has to be statistically evaluated in a pre-assessment (e.g. using geostatistical methods, Braun et al. 2012). For fish sampling in rivers, 30 m are an optimum sample stretch (Grossman et al. 1987) that is still much smaller than the sampling stretches commonly applied in assessments for the WFD (Diekmann et al. 2005). The smaller sampling area allows a higher number of spatial and temporal replicates as well as a better spatial allocation to the sampling sites for other taxonomic groups and to specific habitat structures (e.g. pool-riffle sequences, artificial spawning grounds). The comparison of a nested sampling approach presented in Mueller et al. (2011) and a mixed approach with several replicates of macroinvertebrates and periphyton nested in a reduced sample area for fishes applied in Mueller et al. (2014), indicates that both strategies can be successfully used for later data integration (Mueller et al. 2011, 2014). However, a smaller sample size and higher replication of the overall data set strongly improves the possibilities for multivariate statistical verificiation of the results (Mueller et al. 2014). In light of the highly stream and site-specific habitat structures, community composition, diversity and productivity (Karr et al. 1987, Beisel et al. 1998, Li et al. 2001), a larger number of small samples that can be adequately spatially allocated is also advantageous. Furthermore the application of the synchronized sampling design for several taxonomic groups for the assessment of baseline conditions (temporal reference) and of control sites that are outside of the potentially impacted habitat area (spatial reference) proofed to be highly suitable for the monitoring of new projects, such as substratum restoration efforts in streams (Mueller et al. 2014).

Since the results of several studies indicate that ecosystem productivity is a very important aspect in the evaluation of ecosystem changes (Thorp & Delong 1994, Mueller et al. 2011), it is crucial that the applied sampling techniques allow the assignment of the samples to a quantitative unit. This can be realized by assigning defined sampling units, e.g. standardized sampling time, area, transect length, volume, or mass that allow the calculation of Catch Per Unit Effort (CPUE).

Standardized sampling techniques for all of the freshwater taxonomic groups considered in this thesis are suitable for quantitative sampling or can easily be adapted. The sampling of hyporheic bacteria can be standardized by the volume of the interstitial water sample filtered or by the mass of the substratum sample used for DNA extraction, depending on the sampling method applied (Mueller et al. 2013*c*). For the sampling of benthic algae, the WFD phytobenthos monitoring protocol by Schaumburg et al. (2007) suggests to scrape periphyton off from stones. This method can be standardized easily and cost-effectively by using a flexible plastic tablet to define the sampling area as an easy applicable tool (as described in Mueller et al. 2011). Macrophyte samples collected with a garden rake following the methodology of Deppe & Lathrop (1993) can be standardized by the time invested for each sample on a specific surface area (Mueller et al. 2011). A further possibility for gaining quantitative macrophyte data is to apply the coverage estimation after Braun-Blanquet (1932) on a defined surface area (Mueller et al. 2014). Kick- and Surber-Sampling (Surber 1930, Frost et al. 1971) can be used to calculate catch per unit effort by standardizing the sampling time and transect length (Kick-Sampling) or surface area (Surber-Sampling). For the sampling of freshwater fishes there are also several possibilities for standardization available, such as fishing a defined stretch length (de Lury 1951) or point-abundance sampling (Brandner et al. 2013). Similarly, sampling methods that allow for some kind of standardization are also available in other ecosystem types, such as pittfall traps or the sampling of a defined volume of leaf-litter for terrestrial invertebrates (Oliver & Beattie 1996), or transect and quadrat methods for the study of marine benthos (Eleftheriou 2013). Moreover, many of the standardization methods used in freshwaters originially come from other ecosystems (e.g. pore water sampling for bacteria in groundwater: Griebler & Lueders 2009; soil samples for DNA extraction in terrestrial and marine systems: Llobet-Brossa et al. 1998, Waldrop et al. 2000, Fierer et al. 2003, Gilbert et al. 2011; or the coverage estimation method for terrestrial vegetation after Braun-Blanquet 1932) and thus can be used across ecosystem boarders.

In addition to the need for a nested sampling design including all disciplines, a statistically appropriate number of spatial and temporal replications and quantitative sampling, it is necessary that the field sampling of different ecosystem components at one site is conducted simultaneously. Otherwise temporal effects of changing weather conditions and discharge could interfere with the targeted factors of influence and lead to erroneous conclusions. To realize the simultaneous investigation of multiple taxonomic groups within such comprehensive sampling designs, it is highly recommended to use field sampling techniques that

are optimized for easy and time effective application. The sampling techniques for freshwater taxonomic groups described above have been successfully applied for this purpose in several studies (e.g. Mueller et al. 2011, Stammel et al. 2012, Mueller et al. 2014).

The standardized approach to sampling design proposed in this chapter, which is applicable throughout ecosystem types, is thought to encourage scientists and managers from differnt disciplines to collaborate (e.g. terrestrial and aquatic ecologists, geologists, social scientists and economists, and also specialists within one discipline such as terrestrial zoologists and botanists, Szaro et al. 1998). This can further help to overcome the challenges of the EA (Redman et al. 2004).

4.1.2 Taxonomic identification

The inclusion of multiple taxonomic groups in ecosystem assessments generally involves a considerable amount of laboratory work for taxonomic identification. Especially for taxonomically difficult groups, such as bacteria, algae or some invertebrate groups (e.g. chironomids), this can strongly increase monitoring costs as well as error rates (Haase et al. 2010) and consequently reduce the feasibility of the ecosystem approach or make it even impossible in highly diverse regions where species-level knowledge is limited. For this reason different shortcuts are applied in environmental monitoring to reduce laboratory effort. While there is increasing scientific evidence that the use of single species or species groups as indicators for ecosystem change is not appropriate (Tolonen et al. 2005), the use of coarser taxonomic levels as surrogates for species-level data proofed to be highly suitable to reduce costs of the ecosystem approach to monitoring in freshwater ecosystems (Mueller et al. 2013*a*). Especially when information from algae, macroinvertebrates, macrophytes and fishes are combined, coarse taxonomic resolutions up to family- and order-level are highly adequate to quantifiy local scale stressors (i.e. interruption of the river continuum by weirs, Mueller et al. 2011) and improvements (i.e. substratum restoration success, Mueller et al. 2014), but also allow a comparison of effect size at the regional scale (Mueller et al. 2011). For bacteria the level of OTUs is obviously highly suitable to detect ecologically relevant differences between habitats (Mueller et al. 2013*c*) and can be achieved by well established molecular fingerprinting approaches (e.g. T-RFLP, ARISA). These are high throughput methods, which allow the analysis of a large number of samples, and can be ordered from industrial laboratories. Consequently, this level of detail has high potential for the integration of destruents into ecological monitoring within the framework of

the EA. However, for more specialized research questions such as the analysis of microbial activity and metabolic processes, DNA fingerprinting should be supplemented by methods that deliver species-level resolution (metagenomics, 454 sequencing).

In other ecosystem types, taxonomic sufficiency was also found to be similarly suitable for environmental monitoring, at least for the group of invertebrates (e.g. Pik et al. 1999, Timms et al. 2013, in terrestrial and marine systems). Since statistical threshold levels for the applicability of coarse taxonomic resolution seem to be rather dependent on the taxonomic group than on the type of ecosystem (Mueller et al. 2013*a*), the family- or order-level is probably equally suitable for most of the taxonomically difficult groups (e.g. invertebrates, algae, bacteria) throughout ecosystem borders. However, in other ecosystem types than freshwaters the applicability of TS so far has mostly been tested for single taxonomic groups and systematic tests within more holistic ecosystem assessments considering more than one taxonomic group simultaneously (such as Bevilacqua et al. 2013, for marine systems) are very rare. Consequently, statistical threshold values for the applicability of TS in other taxonomic groups than invertebrates are still missing for most habitat types. Recently published tools, such as calibration lines using the average taxonomic breadth (Mueller et al. 2013*a*) or the R-based tool "BestAgg" (Bevilacqua et al. 2013), allow for an easy determination of the most appropriate aggregation level in new habitats and taxonomic groups in future. This can allow the use of TS for a broader implementation of the EA across ecosystem types.

Nevertheless, scientists should still pay attention to species-level identification when it comes to studies into the effects of subtle environmental gradients, the analysis of functional characteristics that can only be assigned at fine taxonomic resolution and to allow further basic research in this and other topics (Mueller et al. 2013*a*, Heino 2014). Moreover, in monitoring context a combination of species-level identification in target groups of conservation (e.g. rheophilic fishes and macroinvertebrates for stream substratum restoration; Mueller et al. 2014) with the application of TS for the remaining ecosystem components is highly recommended to allow both an evaluation of the effects on the main conservation targets and ecosystem effects (Pander & Geist 2013).

4.1.3 Data integration and analysis

At the analysis stage, the integration of data from the multiple components of the ecosystem is an essential element of the ecosystem approach. In light of the highly diverse reaction of different taxonomic groups to environmental stressors (Heino

2010, Mueller et al. 2011, 2014), the presentation of scientific results as an integrative, overall picture is the most appropriate way for the evaluation and comparison of effect sizes from the ecosystem perspective, and consequently the management of the ecosystem as a whole. As for the sampling design and field sampling of multiple taxonomic groups, the integration of these data gained by very different methodological approaches is subject to several constraints. First, the sampling of multiple taxonomic groups produces large, multidimensional (each species constitutes one dimension) data sets. The commonly applied reduction of the multiple dimensions and the information content of these data to one single dimension (single score index, e.g. number of species, diversity index, multimetric index) has serious limitations in sensitivity (Norris 1995, Caroni et al. 2013, Dahm et al. 2013, Mueller et al. 2013*b*). Data integration methods should therefore allow multivariate analysis of the overall data set to conserve the multiple dimensions of ecosystem components (Reynoldson et al. 1997, Mueller et al. 2013*b*) and avoid the levelling out of opposite responses of different taxonomic groups (e.g. macrophytes and macroinvertebrates in Mueller et al. 2014). Multivariate ordination of combined abundance data was first applied by Guerra-García et al. (2006) and Martínez-Crego et al. (2010) and proofed to be a more sensitive indicator than fitting the complexity and dynamics of biological communities into single numeric scores in further studies (Mueller et al. 2011, 2014). However, Guerra-García et al. (2006) and Martínez-Crego et al. (2010) only combined data collected with the same sampling method and did not solve the challenge of combining data with different numerical magnitude. This can result from differences in data structure, e.g. if presence-absence data are combined with quantitative data, and from natural differences in numbers of individuals per sampling unit, such as algae with very high cell numbers and fishes with comparably low numbers of individuals per site. The most simple normalization would be the calculation of relative abundances per sample. However, if data are normalized in this way, important information on differences in productivity between habitats (Mueller et al. 2011) gets lost. In Mueller et al. (2013*b*), a multivariate data integration method is established that is based on the normalization of the entire data matrix of each group. This procedure ensures an equal contribution of each group, but at the same time conserves information on productivity. The combination of normalized abundance data from multiple groups to a large, overall abundance matrix almost eliminates common problems with all-zero-samples in multivariate analysis, since at least algae usually occur everywhere and the probability that no species of any taxonomic group occurs at a site is very low. Non-metric multidimensional scaling of the resulting normalized overall abundance matrix proofed to produce a

more complete and coherent picture than univariate analyses of single taxonomic groups and could successfully be applied for a holistic and universal indication of ecosystem health (Mueller et al. 2011, 2014). To account for conservation management prioritization, differences in weighting of specific taxa or groups are possible through the alteration of the factor applied in the normalizing procedure prior to data integration. The presented multivariate data integration approach also allows the simultaneous inclusion of multiple evaluation scales (life stage, target species, community, ecosystem) and the consideration of several covariates (Fig. 4.1, e.g. phyiscochemical factors, functional traits), which is highly recommended for e.g. detecting restoration induced changes (Geist 2011, Pander & Geist 2013, Mueller et al. 2014). The considered covariates ideally comprise case-specific endpoints based on functional processes and target species (Mueller et al. 2014). The combination of the multivariate approach with a Before After Control Impact (BACI) design (Underwood 1992) under consideration of a sufficient number of spatial and temporal replicates allows the statistical analysis of the integrated abundance data and several covariates, e.g. via ANOSIM, PERMANOVA, BEST and DistLM, which provides a highly sensitive and representative tool for the quantification of ecological effect size with high statistical power (Mueller et al. 2014). Data gained from modern molecular genetic analyses, such as T-RFLP data from bacteria or microarray data on gene expression, can be normalized and integrated with the methodology presented in Mueller et al. (2013*b*) in the same way as conventional taxonomic abundance data. Similarly, data from taxonomic surrogates (e.g. coarse taxonomic levels, functional traits or metrics) can also be used. The application of the concept of taxonomic sufficiency within the ecosystem approach still provides a powerful tool to distinguish habitats. Obviously, it is more effective for the analysis of ecological change to include several taxonomic groups from different trophic levels into multivariate analysis than to analyze single groups at a very detailed taxonomic level.

4.2 Costs and benefits of the ecosystem approach in freshwater monitoring

The greatest remaining challenge for a broad implementation of the EA are the costs: It is very expensive to monitor multiple ecosystem components at the species-level while typically access to funding is limited.

However, the rate of misleading conclusions would have been considerably high if only single taxonomic groups would have been used as indicators in the case

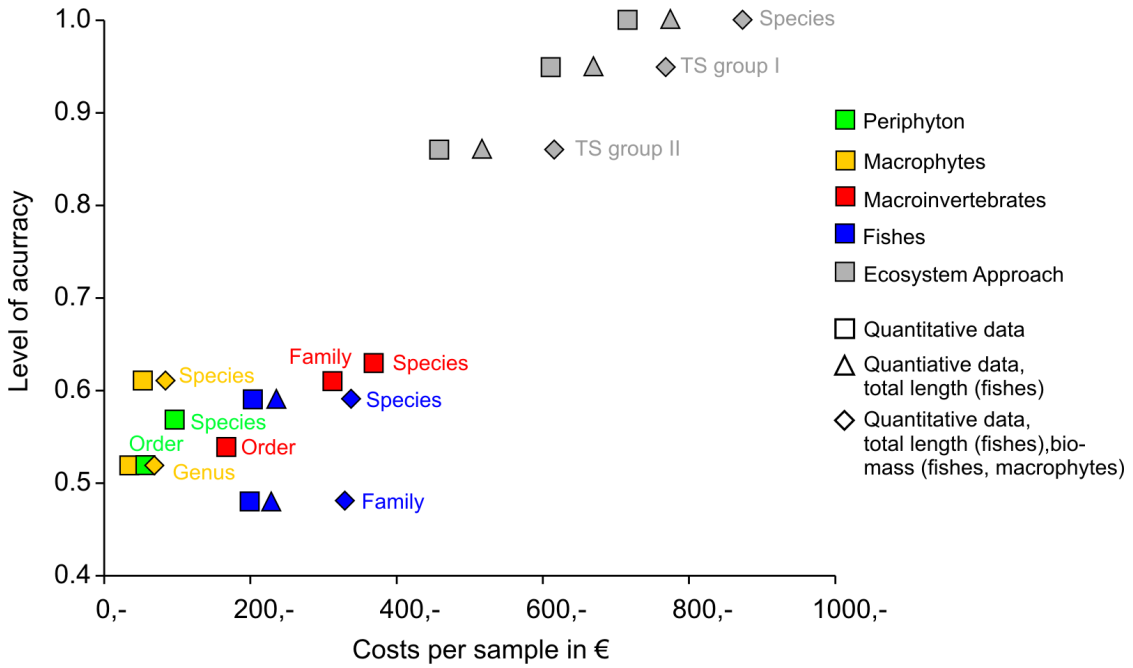


Figure 4.1: Comparison of the cost-effectiveness of single group assessments and the ecosystem approach. Different colours represent the different taxonomic groups and the simultaneous consideration of multiple taxonomic groups (ecosystem approach); different symbols indicate different levels of numerical resolution (see legend). Quantitative data = number of individuals for a standardized sample size. TS group I = statistical threshold levels of taxonomic resolution identified in Mueller et al. 2013*b*, ρ (species-level) ≥ 0.9 (periphyton: order, macrophytes: genus, macroinvertebrates: family, fishes: species); TS group II = ρ (species-level) ≥ 0.8 (periphyton: order, macrophytes: family, macroinvertebrates: order, fishes: family); note that for TS group II a significant loss of information can occur for some data sets. Costs per sample include all resources needed for data collection, taxonomic identification, data analysis and interpretation as well as laboratory equipment and chemicals. The calculation is based on an hourly wage of 65 € for scientific data analysis and interpretation, 55 € for scientific field and laboratory work, 40 € for technical assistance and 30 € for data keying etc. (suggested by the German association of professional biologists and equivalent to the invoiced personell costs for third-party funded projects at German universities, pay scale January 2013). The level of accuracy is measured as ρ to the species-level for each taxonomic resolution (Mueller et al. 2013*a*) multiplied by ρ values between the respective single taxonomic group and the overall ecosystem picture from Mueller et al. 2013*b*

studies presented herein (40 - 80% in Mueller et al. 2011 and 17 - 75% in Mueller et al. 2014). In this context, the probability of false negative results (Type II error, no reaction of biota) was higher than the probability of false positive results (Mueller et al. 2011, 2014), which strongly limits the suitability of single group assessments for deducing management recommendations. The indicator value of single groups strongly varied between study rivers and data sets. Considering both example data sets presented in Chapter 3 (Mueller et al. 2011, 2014), none of the investigated taxonomic groups can be identified to be a better indicator for ecosystem change than another. In contrast, the consideration of multiple groups strongly increased accuracy in describing ecosystem changes, even if a moderate reduction of taxonomic resolution was applied (20-55%; Fig. 4.1). Based on typical salary assumptions for Germany (for calculation details see Fig. 4.1), the costs per sample for the entire assessment (from sampling to data analysis and interpretation) are generally highest for macroinvertebtrates, followed by fishes (especially if length-frequency distribution and biomass are assessed), while periphyton and macrophytes are the most cost effective groups (Fig. 4.1). This is mostly due to the time intensive pre-sorting that is needed for macroinvertebrate analysis (Haase et al. 2004) and the large teams and expensive field equipment needed for electrofishing, which is not necessary in the case of periphyton and macrophytes. Surprisingly, a cost calculation for the investigation of bacteria applying T-RFLP fingerprinting revealed that the inclusion of this up to now widely neglected group into ecosystem assessments can be realized with similar financial resources as for periphyton or macrophytes at a comparable taxonomic resolution (75 € per substratum sample; including field sampling, T-RFLP fingerprinting by an industrial laboratory: nadicom GmbH, Karlsruhe, Germany; and data analysis). The cost-efficiency of the assessment of periphyton, macrophytes and macroinvertebrates can be strongly improved through the application of taxonomic sufficiency. Thereby, costs per sample can be reduced up to 53% for periphyton (order-level) and up to 16% for macroinvertebrates (family-level) as long as the statistical threshold levels proposed in Mueller et al. (2013*a*) are applied (Fig. 4.1). Application of the next coarsest level (TS group II, Fig. 4.1) would reduce costs of macroinvertebrate assessments to 45% (order-level) and costs of macrophyte assessments to 78% (genus- and family-level), involving some loss of information detail but still having high correlations with the species-level (≥ 0.9 in two of three data sets tested in Mueller et al. 2013*a*). However, it has to be considered that the applicability of taxonomic levels beyond the statistically approved thresholds can be data set-specific and has to be tested in a pre-assessment of species lists (see subsection 4.1.2), which also consumes part of the financial resources. However,

this can be very effective especially in repeated longterm investigations of the same sites. For effective and successful environmental monitoring programs, the optimal balance between cost-effectiveness and level of scienctific accuracy has to be found. For example, the sole assessment of macroinvertebrates at the species-level, as commonly practiced in freshwater biomonitoring (e.g. Menezes et al. 2010, Hajibabaei et al. 2012, Lunde & Resh 2012), makes up to 82% of the financial resouces that would be needed for the simultaneous assessment of periphyton, macrophytes, macroinvertebrates and fishes at group II taxonomic levels (60% of the costs for group I taxonomic levels, Fig. 4.1), while the representativeness for the entire ecosystem is limited. As Fig. 4.1 indicates, the inclusion of several ecosystem components at a rather coarse taxonomic resolution can show an ideal compromise that strongly increases the level of accuracy but does not increase costs manifold. Ideally, the money saved by the reduction of taxonomic resolution should be invested into additional ecosystem components, such as destruents, life stage-specific tests of target species and increased spatial and temporal replication.

4.3 Research perspectives

4.3.1 Universal guideline for multivariate data integration

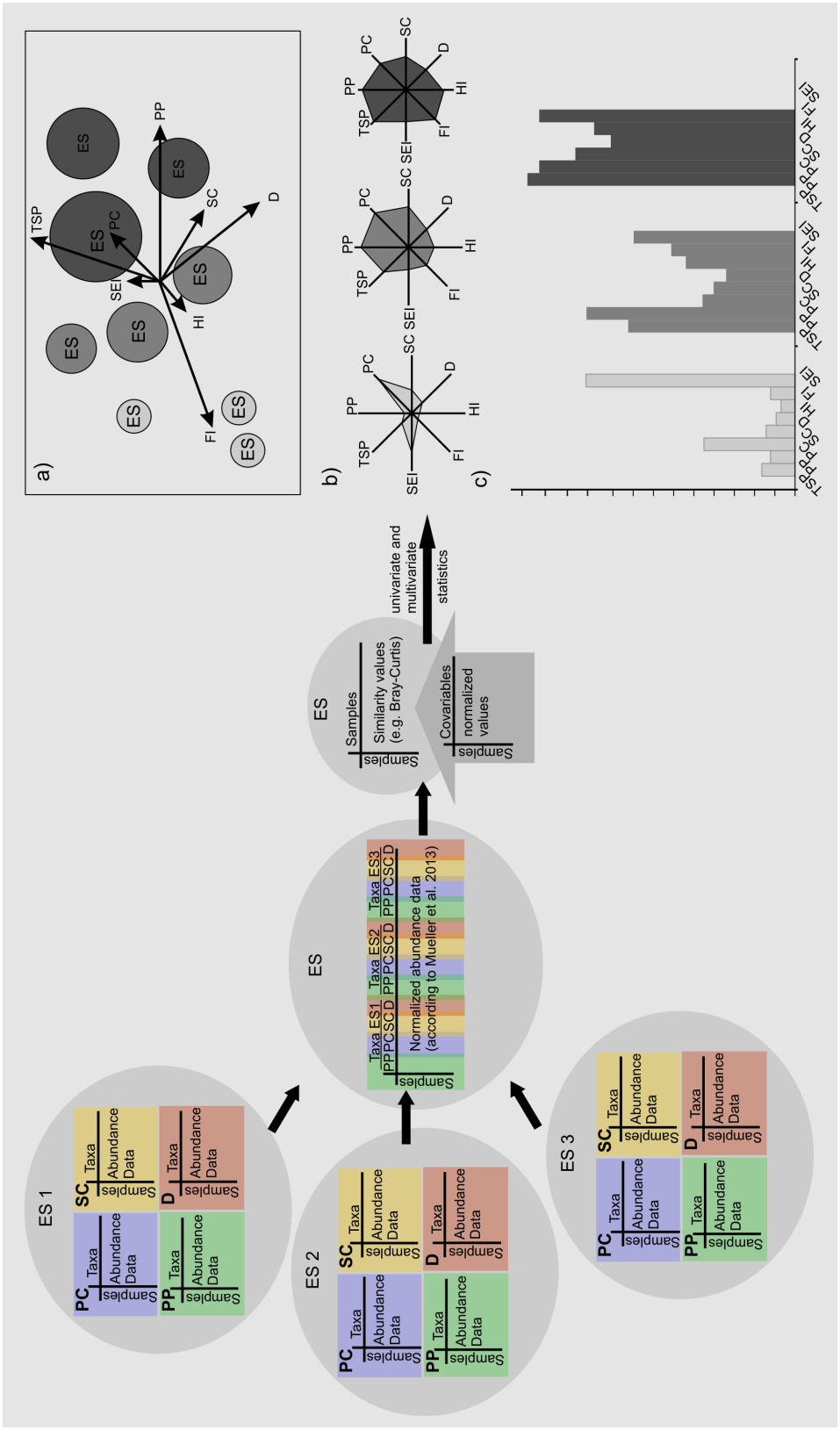
By merging the results from the five scientific publications discussed in this chapter, a step-by-step guideline for the integration of data from different taxonomic groups across ecosystems can be established (Fig. 4.2).

Essential prerequesites for following this guideline include the definition of a common spatial sampling unit for all components to be included beforehand (compare subsection 4.1.1). This common sampling units can be applied to different treatments (e.g. impacted and unimpacted parts of the studied system) and constitute the "samples" in Fig. 4.2. The basis for data integration and following multivariate analyses are separate matrices containing abundance data for each investigated ecosystem component. In these matrices each cell contains individual counts for a specific taxon in a specific sample. In this context, the taxa can either be species, genus, family, order, or OTU in the case of genetic identification methods. The presented integration and analysis methods allow the combination of different levels of taxonomic resolution, so that resolution can be individually adapted for each taxonomic group according to the group-specific statistical reliablity, the purpose of the study and the available financial resources (e.g. for freshwater taxonomic groups see Mueller et al. 2013*a*). Since many scientific studies from diverse ecosystems concluded that single taxonomic groups

are not suitable as indicators for ecosystem health (Niemelä & Baur 1998, Maes & Dyck 2005, Mueller et al. 2011, de Lorenzo & Fulton 2012, Larsen et al. 2012, Sutcliffe et al. 2012) , and in respect of the strong interactions among trophic levels (Campbell et al. 2009), ideally the selected set of ecosystem components should include at least one representative community for each trophic level (e.g. algae or vascular plants for primary producers, invertebrates for primary consumers, vertebrates for secondary consumers and bacteria for destruents).

The abundance data matrices can exhibit discipline-specific differences in quantity (e.g. full quantitative data, relative abundance or presence-absence data) and numerical magnitude (i.e. natural differences in numbers of individuals of different groups), which are accounted for in the following "normalizing" procedure.

Figure 4.2 ***(on the next page)*****:** Guideline for the multivariate integration of data from different taxonomic groups and ecosystems. ES 1-3 = different ecosystems, compare Fig. 1.2). Ecosystem components: PP = primary producers, PC = primary consumers, SC = secondary consumers, D = destruents. Samples = single replicates for different treatments at highest common spatial and temporal sampling resolution for all taxonomic groups and ecosystems. Abundance data = individual counts for each taxonomic group. Similarity values = any measure of similarity that is appropriate for the used data type. Covariables = parameters that are associated with changes in community composition (normalized values; e.g. HI: physicochemical habitat characteristics, FI: functional traits, TSP: target species and life stage-specific variables or SEI: socioeconomic values). a), b) and c) show different possibilities of data presentation, adaptable to the purpose of the study and the end user: a) comprehensive scientific presentation of overall community composition as ordination plot (e.g. NMDS). Covariables are correlated onto the ordination plot and displayed as vectors of different size and direction, indicating the strength of the correlation and the direction of gradient, b) sunray plots including selected criteria of special management interest, c) barplots including selected criteria of special management interest. Different shades of grey indicate different sampling periods or treatments


a)
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PP Taxa Abundance Data Samples
SC Taxa Abundance Data Samples
D Taxa Abundance Data Samples
Taxa ES1 PPPCSCD
Taxa ES2 PPPCSCD
Taxa ES3 PPPCSCD
Normalized abundance data (according to Mueller et al. 2013)
Samples
Samples Similarity values (e.g. Bray-Curtis)
Covariables normalized values
univariate and multivariate statistics

According to Mueller et al. (2013*b*), this is performed by dividing the value of each cell by the sum of the entire matrix and multiplying by a factor of 1,000 to increase readability of the values. The multiplication factor can be specifically adapted, if a distinct weighting of specific ecosystem components is desired. After normalizing, single abundance data matrices are combined to one large matrix. At this step it is important that samples are in the same order and have the same spatial and temporal resolution in each single matrix.

The normalized overall abundance data matrix can then be used for the calculation of multivariate similarity indices (e.g. Bray-Curtis Similarity as applied in the presented case studies) that are further used in ordination techniques or for other data analyses purposes (e.g. calculation of species richness and diversity indices). At this stage of analysis it is also possible to integrate covariables from different scientific disciplines, such as physicochemical habitat characteristics (e.g. structural habitat diversity, water or soil chemistry, elevation, inclination, Pander et al. 2013), target species and life stage-specific factors (e.g. abundance of threatened species, survival rates of critical life stages attained by active bioindication, Pander & Geist 2013, functional traits and ecosystem processes, or socioeconomic variables such as fish landings per year, number of visitors for recreation). Since the assessment of the degree of uncertainty is a critical process in any study, especially in monitoring programs where results could influence future management decisions that involve the investment of huge financial resources, it is highly recommended to apply multivariate and univariate statistics at this stage. Examples for appropriate testing for changes in community composition and the consideration of covariables are given in subsection 4.1.3.

Despite the high complexity inherent to such comprehensive ecosystem investigations, the most management relevant variables should be selected according to the case-specific conservation targets, and presented in a way that is easily accessible for conservation practitioners. For instance, this can be simple bar plots or sunray plots (Fig. 4.2) that are accompanied by the results from multivariate statistics in order to provide an estimation of uncertainty.

The presented multivariate techniques produce an outcome of the monitoring that is no more restricted to coarse categories of impact (e.g. Ecological Quality Classes according to the European WFD) and effects on single taxonomic groups, but provides a diagnostic assessment of the quantity and gradient directions of effects (Mueller et al. 2011, 2013*b*, 2014) considering the entire ecosystem. The possibility of the simultaneous and multivariate consideration of multiple taxonomic groups and covariables provides a powerful tool that can give important insights into the causes and effects of impact in future research, which was limited

to conjectural expert judgement in traditional monitoring approaches (Baird & Hajibabaei 2012).

4.3.2 Integration of different data types

In general, each of the ecological community analysis methods applied in this thesis (e.g. NMDS, DCA, PERMANOVA, ANOSIM, SIMPER) depends on some kind of nomenclature for the single elements of the community under study. However, it is not essential for the names to follow Linnean taxonomy. The names of the taxa in the abundance data matrices can be equally be substituted by numbers that individually mark and distinguish each taxon, labels of DNA fragments (as commonly applied by microbiologists), or any other identifiers for the biological units investigated. The variables studied do not necessarily have to be species, but can also be measures of functional processess, gene activity, or metabolites. Similarly, the covariables included can be extended from traditional measurements of physicochemical habitat characteristics to socioeconomic measures of ecosystem services. Furthermore, the number of variables and covariables included is not restricted to a maximum as long as the available statistical software can handle the size of the data set.

This is of particular relevance for the future integration of data gained from modern approaches in the field of molecular biology, such as the currently arising environmental DNA (e-DNA) metabarcoding (Ficetola et al. 2008) or non-targeted metabolomics (Suhre & Schmitt-Kopplin 2008). Often scientists in these fields use similar data analysis methods as applied in taxonomy based community ecology (e.g. Nylund et al. 2011, Gonzalez & Knight 2012, Lefévre et al. 2013, Rocha et al. 2013), but currently information from traditional and molecular approaches are hardly integrated in multivarite statistics. Following the universal guideline for multivariate data integration proposed herein, classical community data and information gained from molecular approaches can be normalized independently of data structure and analysed integratively in future ecosystem assessments.

4.3.3 Transferability to other ecosystems and geographic regions

Since ecological community data usually are structured similarly throughout ecosystem types ("taxa" x "sites" matrices), the presented methods for data combination may be universally applied to integratively assess community change in multiple taxonomic groups from all types of habitats. The matrix normalization (Mueller et al. 2013*b*) allows the integration of an unlimited and flexible number

of taxonomic groups (as long as there is a minimum common sampling resolution) that can also comprise different ecosystems, such as a stream system and the surrounding alluvial forests and wetlands, or the entire landscape (Fig. 1.2). The multivariate consideration of taxa abundance data based on the similarity measure for all ecosystem types brings along the advantage that the results allow the comparative quantification of effect size across ecosystems (e.g. comparison of the effect size of floodplain restoration measures on the alluvial forest and aquatic habitats) and geographic regions. Furthermore, this approach is independent of minimum abundances of certain indicator taxa and can also include neobiota, which can both be critical constraints that limit the applicability of traditional monitoring approaches in heavily altered ecosystems (e.g. weir-influenced river sections, Mueller et al. 2011; Arndt et al. 2009, Mueller et al. 2014) and their transferability to other geographic regions. Unfortunately, systematic scientific evidence supporting taxonomic sufficiency methods in other ecosystems is still restricted to single taxonomic groups (marine macroinvertberates, terrestrial invertebrates), making the application of the ecosystem approach very costly in terms of labour.

The applicability to a broad range of data types, together with the high transferability between ecosystem types and geographic regions makes the new data integration and analysis approach presented herein a highly flexible and promising tool for the future development of ecological monitoring.

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7 Appendix

7.1 List of frequently used abbreviations

ANOSIM	ANalysis Of SIMilarities
ANOVA	ANalysis Of VAriance
AQUEM	Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates
AR	Argentina
ARISA	Automated Ribosomal Intergenic Spacer Analysis
ASTERICS	Software for the German macroinvertebrate evaluation system PERLODES
AT	Analysis Type
AUS	Australia
AUSRIVAS	AUstralian RIVer Assessent System
β_t	Beta-Diversity
BACI	Before After Control Impact design
BDA	Biological Diversity Act
BEST	Biota Environment STepwise matching
BestAgg	Best practicable Aggregation of species
BEAST	BEnthic Assessment of SedimenT
BSA	Bovine Serum Albumin
CBD	Convention on Biological Diversity
CCA	Canonical Correspondence Analysis
CDN	Canada
CHI	Chile
CHN	China
CPOM	Coarse Particular Organic Matter
CPUE	Catch Per Unit Effort
CME	Cellulose Mixed Ester
CWA	Clean Water Act
Δ^+	Average taxonomic breadth according to Clarke et al. (2001)

D	Destruents
d	descriptive analysis
DCA	Detrended Correspondence Analysis
dg	geometric mean particle diameter
DI	Data Integration
DistLM	Distance based Linear Modelling
DIVERSE	Diversity calculation function in Primer v6
DNA	DesoxyriboNucleicAcid
dNTP	desoxy-Nucleotide-Tri-Phosphate
D side	Downstream side of weir/dam
EA	Ecosystem Approach
ECA	Environmental Conservation Act
EFI	European Fish Index
Eh	Redox potential
EIA	Environmental Impact Assessment
EMA	Environmental Management Act
EPA	Environmental Protection Act
EPBCA	Environmental Protection and Biodiversity Conservation Act
EPT	Ephemeroptera, Plecoptera, Trichoptera
EQC	Ecological Quality Class
ES	Ecosystem
EU	European Union
EV	Environmental Variables
F	Fishes
FFG	Functional Feeding Group
FFH	Fauna Flora Habitat, related to European Parliament (1992)
FI	Functional Index
FiBS	German evaluation system for fishes
FPOM	Fine Particular Organic Matter
FRI	Index of Fish Region
FW	Freshwater ecosystems
GER	Germany
Global *R*	Test statistics from ANOSIM
H	Shannon Index
HD	Habitats Directive

HEDW	official author citation of Johannes Hedwig, German botanist
HI	Habitat Index
H_{max}	maximum Diversity
IBI	Index of Biotic Integrity
IND	India
J	Evenness
KEN	Kenya
KOR	Korea
L.	official author citation of Carl von Linné
LGA	Ley General del Ambiente
M	Marine ecosystems
MANOVA	Multivariate ANalysis of VAriance
MIV	Macroinvertebrates
MM	Multi-Metric index
MP	Macrophytes
MV	Multivariate analysis
NAM	Namibia
NMDS	Non-metric MultiDimensional Scaling
nsp.	not specified
O	OTU Richness
OTU	Operational Taxonomic Unit
ϕ	higher taxa/species ratio
P	Periphyton
PAN	Panama
PC	Primary Consumers/Consumption
PCR	Polymerase-Chain-Reaction
PERLODES	German macroinvertebrate monitoring system according to WFD
PERMANOVA	PERmutational Multivariate ANalysis Of VAriance
PhD	Doctor of Philosophy
PhyLib	German evaluation system for macrophytes and phytobenthos
PP	Primary Producers/Production
PRIMER	Software for statistical analysis of multivariate data
Pseudo-*F*	Test statistics from PERMANOVA
PU	Political Unit
ρ	Multivariate Spearman rank correlation coefficient

r^2	Univariate Spearman rank correlation coefficient
R	free software programming language and software environment for statistical computing and graphics
RC	Reference Condition
RCC	River Continuum Concept
RDA	ReDundancy Analysis
REL	Related Environmental Legislation
RF	ReFerence type
RGA	Regimen de Gestion Ambiental de Aguas
RIVPACS	River Invertebrate Prediction and Classification System
rRNA	ribosomal RiboNucleicAcid
RU	Russia
S	Species Richness
SA	Statistical Analysis
SC	Secondary Consumers/Consumption
SD	Standard Deviation
SE	Socio-Economic factors
SI	Saprobic Index
SIMPER	SIMilarity PERcentages
SSI	Single Score Index
SRP	SunRay Plot
T	Terrestrial ecosystems
TG	Taxonomic Group
TITAN	Threshold Indicator Taxa ANalysis
T-REX	Online tool for the analysis of T-RFLP data
T-RFLP	Terminal Restriction Fragment Length Polymorphism
T-RF	Terminal Restriction Fragment
TS	Taxonomic Sufficiency
TSP	Target SPecies related endpoints
USA	United States of America
U side	Upstream side of weir/dam
UV	Univariate analysis
WA	Water Act
WFD	Water Framework Directive
WQECA	Water Quality and Environmental Conservation Act
ZA	South Africa

7.2 Full list of references contributing to Fig. 1.2

The following studies were part of the literature review presented in Fig. 1.2 (listed alphabetically). Full references can be found in the Bibliography.

Allen et al. (1999), Bailey et al. (2007), Barrows & Allen (2007), Baur et al. (2006), Becker et al. (2011), Betrus et al. (2005), Billeter et al. (2008), Blaise et al. (2008), Blasi et al. (2010), Brazner et al. (2007), Brix et al. (2001), Brook & Bradshaw (n.d.), Burel et al. (1998), Carlisle et al. (2009), Crosswhite et al. (1999), Debenest et al. (2010), Della Bella & Mancini (2010), de Lorenzo & Fulton (2012), Doka et al. (2003), Fabricius et al. (2003), Ficetola et al. (2007), Fleishman et al. (2002), Flinders et al. (n.d.), Flynn et al. (2009), Gao et al. (2004), Golet et al. (2011), Grenouillet et al. (2008), Guerra-García et al. (2006), Heegaard et al. (2006), Heino (2002, 2010), Heino et al. (2005), Hsu et al. (2011), Hughes et al. (2009), Infante et al. (2009), Jackson & Harvey (1993), Johnson & Hering (2009), Kadoya et al. (n.d.), Kavanagh & Stanton (2005), Kruk et al. (2009), Kunte et al. (1999), Lammert & Allan (1999), Landeiro et al. (2012), Larsen et al. (2012), Lawton et al. (1998), Lougheed et al. (2007), Maes & Dyck (2005), Mac Nally et al. (2002), Manley et al. (2004), Martínez-Crego et al. (2010), Mazor et al. (2006), Mueller et al. (2011), Mykrä et al. (2008), Nandakumar (1995), Niemelä & Baur (1998), O'Connor et al. (2000), Oliver et al. (1998), Paavola et al. (2006), Padial et al. (2012), Patrick (1949), Peintinger et al. (2003), Perfecto et al. (2003), Postma-Blaauw et al. (2012), Rooney & Bayley (2012), Roth & Weber (2008), Santos et al. (2011), Schouten et al. (2009), Schulze et al. (2004), Soininen & Könönen (2004), Spitale et al. (2012), Stoler & Relyea (2011), Stendera & Johnson (2006), Sutcliffe et al. (2012), Thompson & Townsend (2000), Tolonen et al. (2005), Tsui & Chu (2003), Vanderklift et al. (1998), Vera et al. (2011), Wang et al. (2011), Weibull et al. (2003), Woinarski et al. (2005), Yates & Bailey (2011)

7.3 Publication list

The following papers were included in this thesis

Mueller, M., Pander, J. & Geist, J. (2011) The effects of weirs on structural stream habitat and biological communities. *Journal of Applied Ecology* **48**(6), 1450-1461.

Mueller, M., Pander, J., Wild, R., Lueders, T. & Geist, J. (2013) The effects of stream substratum texture on interstitial conditions and bacterial biofilms: Methodological strategies. *Limnologica-Ecology and Management of Inland Waters* **43**(2), 106-113.

Mueller, M., Pander, J. & Geist, J. (2013) Taxonomic sufficiency in freshwater ecosystems: Effects of taxonomic resolution, functional traits and data transformation. *Freshwater Science* **32**(3), 762-778.

Mueller, M., Pander, J. & Geist, J. (2013) Simultaneous consideration of multiple taxonomic groups in ecological monitoring: From single indices to multivariate indication of ecosystem change. *in prep*.

Mueller, M., Pander, J. & Geist, J. (2014) The ecological value of stream restoration measures: An evaluation on ecosystem and target species scales *Ecological Engineering* **62**, 129-139.

Co-authorships (not included in this Thesis)

Pander, J., **Mueller, M.**, & Geist, J. (2013). Ecological functions of fish bypass channels in streams: Migration corridor and habitat for rheophilic species. *River Research and Applications*, **29**(4), 441-450.

Stammel, B., Cyffka, B., Geist, J., **Mueller, M.**, Pander, J., Blasch, G., Fischer, P., Gruppe, A., Haas, F., Kilg, M., Lang, P., Schopf, R., Schwab, A., Utschick, H. & Weißbrod, M. (2012). Floodplain restoration on the Upper Danube (Germany) by re-establishing water and sediment dynamics: a scientific monitoring as part of the implementation. *River Systems*, **20**(1-2), 1-2.

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Pander, J., **Mueller, M.** & Geist, J. (2013) A comparison of four stream substratum restoration techniques concerning interstitial conditions and downstream effects. *River Research and Applications*, minor revisions.

Oral contributions related to the PhD thesis

Mueller, M., Pander, J. & Geist, J. (2011) The effects of weirs on structural stream habitat and biological communities. *Jahrestagung der Deutschen Gesellschaft für Limnologie*, Freising, Germany, September 2011.

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Mueller, M., Pander, J. & Geist, J. (2013) Weir-induced serial discontinuity and mitigation strategies. *Annual Meeting of the North American Fisheries Society*, Little Rock, USA, September 2013.

Mueller, M., Pander, J. & Geist, J. (2013) How much taxonomic detail is required?

The applicability of taxonomic sufficiency in freshwater biodiversity conservation. *Leichhardt Symposium*, Brisbane, Australia, October 2013.