Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments

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Recent experiments, mainly in terrestrial environments, have provided evidence of the functional importance of biodiversity to ecosystem processes and properties. Compared to terrestrial systems, aquatic ecosystems are characterised by greater propagule and material exchange, often steeper physical and chemical gradients, more rapid biological processes and, in marine systems, higher metazoan phylogenetic diversity. These characteristics limit the potential to transfer conclusions derived from terrestrial experiments to aquatic ecosystems whilst at the same time provide opportunities for testing the general validity of hypotheses about effects of biodiversity on ecosystem functioning. Here, we focus on a number of unique features of aquatic experimental systems, propose an expansion to the scope of diversity facets to be considered when assessing the functional consequences of changes in biodiversity and outline a hierarchical classification scheme of ecosystem functions and their corresponding response variables. We then briefly highlight some recent controversial and newly emerging issues relating to biodiversity-ecosystem functioning relationships. Based on lessons learnt from previous experimental and theoretical work, we finally present four novel experimental designs to address largely unresolved questions about biodiversity-ecosystem functioning relationships. These include (1) investigating the effects of non-random species loss through the manipulation of the order and magnitude of such loss using dilution experiments; (2) combining factorial manipulation of diversity in interconnected habitat patches to test the additivity of ecosystem functioning between habitats; (3) disentangling the impact of local processes from the effect of ecosystem openness via factorial manipulation of the rate of recruitment and biodiversity within patches and within an available propagule pool; and (4) addressing how non-random species extinction following sequential exposure to different stressors may affect ecosystem functioning. Implementing these kinds of experimental designs in a variety of systems will, we believe, shift the focus of investigations from a species richness-centred approach to a broader consideration of the multifarious aspects of biodiversity that may well be critical to understanding effects of biodiversity changes on overall ecosystem functioning and to identifying some of the potential underlying mechanisms involved.

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Understanding the consequences of biodiversity changes (species loss and invasions) on ecosystem functioning is becoming increasingly critical in view of the profound influence of human activity on natural ecosystems (Vitousek et al. 1997) and the goods and services humans receive from them (Daily et al. 2000). The ground-breaking initiative by Schulze and Mooney (1993) highlighted our lack of knowledge concerning the effect of biodiversity loss on ecosystem functioning at that time. Subsequently, the body of pertinent experimental data and underlying theory has grown rapidly. The central research question has been whether ecosystems with increasingly fewer species would be able to maintain functional properties and process rates comparable with unaffected systems (reviewed by Kinzig et al. 2002, Loreau et al. 2002).

Most experimental evidence for biodiversity effects on ecosystem functioning has come from terrestrial ecosystems, particularly grasslands (Naeem et al. 1994, Tilman et al. 1997a, Hector et al. 1999, Schmid et al. 2001). These investigations have yielded valuable information yet a number of questions remain including: (1) to what extent can conclusions drawn from grassland studies be generalized to other biomes and ecosystems?; (2) how relevant are results from experiments with randomly assembled species for observed and predicted biodiversity loss in the real world (Jonsson et al. 2002)?; and (3) what are the mechanisms underlying observed biodiversity effects? Moreover, the design of experiments testing effects of biodiversity on ecosystem processes has led to a vigorous debate about hidden treatments and statistical versus biological effects (Huston 1997, Doak et al. 1998). Some major discrepancies in the statistical evaluation and interpretation of data have recently been settled and a common framework has emerged (Loreau et al. 2001, 2002). However, the statistical issues are highly complex (Fukami et al. 2001, Schmid et al. 2002, Morin and McGrady-Steed 2004), suggesting that exceptional care is needed in the design and analysis of experiments addressing biodiversity effects on ecosystem functioning.

This paper focuses on key features of aquatic systems that influence experimental approaches to understanding biodiversity effects on ecosystem functioning. We broaden the scope of diversity facets (from genotypes to phyla and to habitats) to be considered in experiments and present a classification of ecosystem functions (processes and properties) with a range of associated response variables that can be measured experimentally. We then briefly highlight some recent controversial and newly emerging issues relating to biodiversity-ecosystem functioning relationships. From a discussion of the lessons learnt from previous work, we develop designs of four novel types of experiments that have potential to significantly advance understanding of the importance of biodiversity for the functioning of ecosystems and that are particularly amenable to being carried out in aquatic environments.

Unique features of aquatic ecosystems

A number of features distinguish aquatic from terrestrial ecosystems in general and specifically in relation to biodiversity-ecosystem functioning relationships. Barriers to dispersal typically are weak and flow of energy and materials relatively rapid within and between habitats in aquatic ecosystems. Local patches can readily be recolonised by new recruits after disturbance or extinction events and the physical nature of water and hydrological processes ensures strong interconnection among habitat types, such as benthic and pelagic habitats. Thus there are likely to be stronger within-system boundaries in terrestrial systems (e.g. between soil and air) than in aquatic ones (e.g. between substrate/ sediment and water). Because each aquatic ecosystem is composed of multiple habitat types, assessing the effects of biodiversity changes on the functioning of aquatic ecosystems requires experimental designs that allow a scaling up from individual homogenous patches to large-scale, often highly heterogeneous, areas.

The mosaic of habitat patches in aquatic systems often is more spatially compact than in terrestrial environments, presenting more tractable experimental systems at the landscape scale (Schindler and Scheuerell 2002). In addition, some inland and coastal water bodies can be physically delineated, facilitating whole-system manipulations. Examples include whole-lake and whole-stream nutrient enrichment (Peterson et al. 1993, Carpenter et al. 2001), exclusion of leaf litter from streams (Wallace et al. 1997), and drastic food-web modification induced by the complete experimental removal of fish (Mehner et al. 2002) or benthic invertebrates (Monaghan et al. 2001).

Differences between the terrestrial and aquatic environments influence the morphology, physiology, beha-

viour and ecology of organisms, the community structure, biotic interactions and abiotic constraints. The aqueous medium of aquatic systems, greatly influences uptake and transformation of materials by organisms. Accordingly, the faster rate of transfer across membranes and transformation of energy and materials in aquatic ecosystems leads to greater biogeochemical dynamics (e.g. nutrient cycling) and the rapid turnover of primary producers compared to terrestrial ecosystems (Warwick and Clarke 2001). Many primary producers in aquatic environments are small, and the high viscosity of water (compared to air) means that primary producers and consumers are often highly mobile and at the same time constrained and entrained by the medium as a result of water movements. The frequency and intensity of physical disturbance is likely to play a more fundamental role in structuring aquatic communities than in terrestrial systems. Steep environmental gradients are quite prevalent (especially in coastal marine systems, Underwood 2000), and such gradients and the abundance and variety of (often sedentary) organisms have made marine intertidal habitats one of the most important model systems for field-based ecological experimentation at the community level (Underwood 2000, Menge and Branch 2001).

Thirty-two of the 33 phyla of the plant and animal kingdoms are found in marine habitats, and 15 of these are endemic to them (Ray and Grassle 1991), offering a great opportunity to explore the influence of biodiversity across a range of taxonomic scales. Unique functional groups such as filter feeders have evolved and there is also a preponderance of size-dependent predation in aquatic systems. Finally, the microorganisms, which drive ecosystem functioning to a considerable extent, are more amenable to manipulation in aquatic than in terrestrial systems.

The unique features of aquatic ecosystems provide a compelling argument against directly extrapolating insights into biodiversity-ecosystem functioning relationships gained from terrestrial systems to lakes and open oceans, streams and coastal habitats. Further, these features provide abundant opportunities for challenging current hypotheses and for testing the generality of observed effects of biodiversity patterns on ecosystem processes and properties, as we illustrate in the following sections.

Expanding the scope of biodiversity facets and ecosystem responses

Experiments of biodiversity effects on ecosystem functioning have largely been confined to varying numbers of species and functional groups, with species being explicitly or implicitly used as proxies of functional entities (Díaz and Cabido 2001, Hooper et al. 2002). The relative importance of species versus functional group richness has been subject to considerable debate (Hooper and Vitousek 1997, Tilman et al. 1997a, Díaz and Cabido 2001), whereas both the scope and scale of approach and the range of diversity facets that may affect ecosystem functioning has been rather limited. We therefore propose expanding the range of diversity facets that should be considered for manipulation in experimental studies (Table 1).

Species richness is an important and readily quantified component of biodiversity. However, mechanisms by which diversity may influence ecosystem functioning are related more to some functional attributes of species, rather than to species richness per se. Although not invariably true, functional attributes tend to converge as phylogenetic relatedness of organisms increases. A rigorous methodology to separate groups of organisms based on their taxonomic relatedness has been developed (Warwick and Clarke 2001). Manipulating diversity directly at the functional group level is a most valuable approach, particularly in relation to more fully exploring the concept of species redundancy. However, the assignment of species to functional groups requires care (Díaz and Cabido 2001, Hooper et al. 2002) and several objective approaches have now been developed (Hooper et al. 2002, Petchey and Gaston 2002). It is possible that a more reliable representation of functional roles may be gained by allocating species into groups based on several relevant traits, rather than an individual trait.

Patch or habitat diversity is a critical component of biodiversity, and ways must be sought to examine the potential ecosystem-level effects of habitat diversity experimentally. Addressing the effects of habitat diversity on ecosystem functioning is particularly important for ecosystem management and conservation, which increasingly operates within the heterogeneous environments of whole complex ecosystems, catchments or at even larger scales (Cardinale et al. 2004).

Table 1. An expanded range of diversity facets. Diversity can be manipulated within or across habitat patches, functional groups or taxonomic levels. Evenness can be manipulated within or across any level of the hierarchy.

Aspect of diversity	Level of manipulation
Taxonomic	Genotype Species Any higher taxonomic level
Functional	Functional group Taxonomic richness within functional groups
Habitat (β diversity)	Habitat piatch Functional group within habitat patch Taxonomic richness within habitat patch
Evenness	Taxonomic group Functional group Habitat patch

Anthropogenic disturbances can bring about significant changes in evenness without associated changes in richness (Gray et al. 1990, Dawson Shepard et al. 1992). Consequently, evenness is an additional critical aspect of biodiversity that has been neglected when evaluating the functional consequences of species loss (Connolly et al. 2001). In marine systems, quantitative changes in community structure (i.e. changes in the relative and absolute abundance of different taxa) have been demonstrated for a range of taxonomic levels (Warwick and Clarke 2001), pointing to the importance of manipulating evenness at different taxonomic levels, just as is true for richness.

Turning from the biodiversity components to the ecosystem responses, it is important to understand that there is no single index that can capture the entire scope of processes and properties characterizing the overall functioning of a given ecosystem. Most studies to date have used a restricted set of ecosystem-level response variables, primarily centred on plant productivity. This approach can be misleading if the indicators are not appropriately chosen and the risk is especially pronounced when the number of indicators is small and strong interactions exist among key processes or properties. It is this recognition that provides the basic rationale for the hierarchical classification of functions proposed in Table 2. The hierarchy facilitates selection of a relevant sub-set of functions, and corresponding response variables, to represent ecosystem functioning as a whole. Choosing the proper process and property (and therefore response variable) for study requires consideration of a number of features. These include (1) the average rate or value; (2) the temporal variation; (3) the return rate to 'pre-perturbation values' (elapsed time after disturbance; resilience sensu Pimm 1984); and (4) the extreme values. The relative importance of these features will vary with the particular ecosystem process and habitat studied.

Lessons learned and emerging issues

Considerable discussion of biodiversity-ecosystem functioning experiments has centred on appropriate experimental design and the proper statistical analysis and interpretation of results. These issues have been discussed elsewhere in detail (Huston 1997, Allison 1999, Fukami et al. 2001, Schmid et al. 2002, Morin and McGrady-Steed 2004), so we only summarise the main points and raise some additional issues to provide the background explanations to the hypotheses and experimental designs that we propose in the following section.

Manipulating diversity directly

From the early experiments, it has become clear that diversity must be manipulated directly in order to avoid the confounding effects of hidden treatments (Huston 1997). Direct manipulation of diversity also underlines the nature of diversity as an independent factor eliciting effects on a response variable that reflects ecosystem functioning. Separating cause and effect experimentally has the evident benefit of minimising the range of possible interpretations that can be put on the results, a problem that has fuelled much of recent debate on biodiversity-ecosystem functioning relationships (Loreau et al. 2001).

Replicating both species combinations and diversity levels

Another of the lessons learnt from the first generation of biodiversity experiments is that, if possible, designs should replicate both richness and composition (Schmid et al. 2002). The high number of experimental units required to test all possible combinations when drawing from a common pool of diversity elements (e.g. species) can be a severe logistical constraint (Allison 1999, Morin and McGrady-Steed 2004). To illustrate the point, 16 species at richness levels of 1, 2, 4, 8 and 16 would lead to an unreplicated, and clearly impractical, 14 827 combinations. To circumvent this problem, previous experiments have replicated diversity levels either by (1) generating multiple combinations within each diversity level from a common species pool or (2) by selecting one particular replicated combination within each diversity level (Naeem et al. 1994). Both approaches have major drawbacks. In the former (1), assemblages at higher diversity levels become more similar (i.e. species composition overlaps more), which becomes a problem when variation of a state or process is the dependent variable (Fukami et al. 2001). One solution (Morin and McGrady-Steed 2004) minimally requires at least two, well-replicated, alternate species compositions, so that variation is measured only among replicates of initially identical composition. Then the different compositions can be statistically re-sampled, using one composition at a time per diversity level, to describe relations between diversity and variation. In the latter case (2), the effects of diversity level and the particular combination of species used are inseparable (Huston 1997). In the trade-off between manageability of experiments and desired breadth and strength of data interpretations, we generally advocate testing fewer diversity levels in favour of replicating more than one replicated species combination. A positive consequence of replicating both multiple combinations and diversity levels is that species identity effects can be identified in addition to diversity effects.

Incorporating non-random species loss

Species loss from ecosystems is typically not random because of factors such as species-specific responses

Table 2. A hierarchical classification of ecosystem functions that can be used to characterize aquatic ecosystems. *Resistance and resilience sensu Pimm 1984.

Basic type of ecosystem function	Class of process, property or value	Ecosystem process, property or value	Example of response variables
Ecosystem processes (defined as rates: dx/dt = f(x,...z))	Biomass production	Primary production	[14C]bicarbonate incorporation into seston, O_2 release; biomass present at the end of growing season
		Secondary production	Change in weight and number of individuals; [3H]leucine incorporation into bacterial protein
	Organic matter transformation	Organic matter decomposition	Decay coefficient of leaves in litter bags; wood density; profiles of organic matter content in sediment cores
		Import/export of organic matter	Amount of litter that falls in traps; seston concentration and discharge
		Organic matter removal	Ingestion rate of animals; organic matter standing stocks; enzyme activities
	Ecosystem metabolism	P:R ratio	Diel changes in oxygen concentration
		Carbon mineralisation	Release of CO_2; decline in DOC concentration; release of CH_4
		Oxygen consumption/production	Change in O_2 concentration over time
	Elemental cycling	Denitrification, nitrification, nitrogen fixation	Rate of acetylene reduction; rate of $^{15}NO_3^-$ rate of $^{15}N_2$ incorporation into biomass; activity of genes involved in N transformations
		Exchange of limiting nutrients	Movement of P, N, Si or Fe
	Physical structuring	Bioturbation	Depth of unstratified sediment; P release; NH_4^+ release
		Reef-building	Change in reef perimeter; calcite precipitation rate
		Sedimentation	Accumulation of matter in sediment traps
		Microbial biofilm development	Biofilm thickness; accumulation of exopolymeric substances (EPS)
		Island formation in river floodplains	Age structure of set of islands as inferred from tree height and dendrochronology
		Particle flow	Seston concentration and water velocity
Ecosystem properties (presence/absence and levels of states)	Stability of ecosystem processes	Resistance*	Change of productivity following disturbance; establishment of an invasive species
		Resilience*	Rate of return of litter decomposition rates following disturbance
		Temporal variability	Interannual coefficient of variation of primary production
	Trophic structure of community	Biomass of groups of species	Plant biomass
		Food web topology	Community connectedness; predator:prey ratio; proportion of omnivorous species
Ecosystem values (tangible benefits to humans)	Ecological goods	Food provision	Size of fish stocks, income from angling licences
		Provision of specific chemicals	
	Ecological services	Water quality	Biological oxygen demand (BOD), concentration of coliforms
		Nutrient and carbon sink	
		Wave breaking	

to stressors, differences in population size, propagule pool size, and the rate of immigration or rescue from connected populations (Vinebrooke et al. 2004). It is impractical to mimic all these factors in experimental systems, but it is clearly an oversimplification to accept random extinction as a representative scenario of species loss in nature. Nonetheless, random species loss is a common assumption in biodiversity-ecosystem functioning experiments (Naeem et al. 1994, McGrady-Steed et al. 1997, Downing and Leibold 2002). Because compositional changes can be just as important as changes in diversity (Hooper and Vitousek 1997, Huston 1997, Downing and Leibold, 2002), we recommend including the major factors likely to influence the order and magnitude of biodiversity loss in experimental systems, as Jonsson et al. (2002) have recently shown for a simple aquatic system.

Incorporating greater temporal and spatial scales

To date, most experiments addressing biodiversity effects on ecosystem functioning have been conducted at fine spatial (patch or local level) and temporal scales (Bengtsson et al. 2002). This is not surprising as experimental control of particular factors, including biodiversity, becomes increasingly difficult at broader scales. However, organisms both on land and in water can operate over large scales. In addition, dispersal and immigration may be among the most important processes affecting the biodiversity of local assemblages, whereas experimental systems thus far have mainly been demographically closed (Cardinale et al. 2004). Thus to understand the effects of biodiversity on the functioning of ecosystems and to make effective management decisions, either results from these small-scale experiments must be extrapolated to large scales or the experiments must be directly conducted at the larger landscape level by explicitly incorporating both the grain (the individual sampling unit) and the extent (or overall area) of the ecosystems. Both aspects are important because broad-scale processes like dispersal constrain local phenomena such as species loss and vice versa.

Investigating mechanisms underlying observed patterns

The discussion of the relative importance of the sampling effect (= selection effect or selection probability effect) vs the 'complementarity' effect sensu lato as used by Loreau (2000) and of the significance of species versus functional diversity (Hooper and Vitousek 1997, Tilman et al. 1997a) indicates that most experiments to date have not adequately addressed the mechanisms underlying observed effects of biodiversity on ecosystem functioning. As the mechanistic understanding of biodiversity effects is of considerable theoretical and practical relevance, we discuss some of the possible mechanisms in the following sub-sections.

Diversity of traits

The number of traits, represented either by species or functional groups, forms the basis of two early hypotheses explaining biodiversity effects on ecosystem functioning. The complementarity hypothesis states that the exploitation of an n-dimensional resource space should increase with a higher number of traits present (Tilman et al. 1997b). The insurance hypothesis states that a higher number of traits increases the probability that the functional role of a species that goes extinct is assumed by other surviving species, thus maintaining ecosystem functioning at the previous level (Lawton and Brown 1993, Yachi and Loreau 1999). The insurance hypothesis

involves an explicit temporal aspect, thereby shifting the emphasis from short-term effects on the average rate or value of ecosystem functioning to longer-term effects relating to the stability of ecosystem functioning, including the degree of temporal variability and the resistance and resilience of ecosystem functioning in response to perturbations. Despite their appealing logic, central predictions from these hypotheses lack rigorous experimental testing. Observational and experimental data have, however, shown a negative relationship between species numbers and their densities, indicating that the activities of lost species can be compensated to some extent by an increase in the population sizes of the remaining species (McGrady-Steed and Morin 2000).

Facilitation

A diversity effect on ecosystem functioning would also arise if one species facilitates another (Mulder et al. 2001). There are many examples of synergisms in nature but examples in the context of a diversity–ecological functioning perspective are rare, although they do exist in aquatic ecosystems (Jonsson and Malmqvist 2000, Cardinale et al. 2002). Addressing the possible importance of positive interactions for ecosystem functioning appears to be important.

Sampling effect

The lively debate on the first generation of experiments testing biodiversity effects on ecosystem functioning partly originated around the so-called sampling or selection effect (Huston 1997, Loreau 2000, Loreau et al. 2001). A sampling effect occurs when ecosystem functioning increases in higher-diversity assemblages solely because the probability is increased that the dominant species are present. This sampling effect may either represent an experimental artefact (Huston 1997) or a valid ecological mechanism by which higher diversity translates into enhanced ecosystem functioning (Tilman et al. 1997b, Loreau 2000). In an attempt to reconcile these opposing views, Loreau et al. (2001) suggested incorporating tests of sampling effects explicitly in experimental designs. This call is important, because if the critical ecosystem processes and properties were indeed supported mainly by dominant species and only rare species went extinct, ecosystem functioning would be largely unaltered following species losses from ecosystems. An implicit assumption behind the idea of a sampling effect is, however, that the dominant species are also those that contribute most to the measured process or property even on a per capita or per biomass basis. This condition may not always be met, suggesting that teasing apart putative sampling effects from straightforward diversity effects is crucial.

Species identity

The efficiency of ecosystem processes often depends exactly on which species are present (Hooper and Vitousek 1997, Mikola and Setälä 1998). In the presence of a species that is very effective in driving a particular process, the performance of the system with respect to this process will be high. Examples of strong effects of species identity in aquatic ecosystems include nutrient recycling by fish (Vanni et al. 2002), and the ecological engineering of beavers (Naiman et al. 1988). Thus, when a species is lost from a community, predictions of the effect on ecosystem functioning will depend on the species' specific influence on those responses. Species identity effects are thus related to, but not the same as, the sampling effect discussed above. The individuality and complexity of the roles of different species clearly upset attempts to predict process rates (and other measures of ecosystem functioning) at varying species richness. The issue becomes even more intricate when the contribution made by an individual species varies with the environmental conditions (Cardinale et al. 2000, Wellnitz and Poff 2001). This suggests that the results of simple experiments with few species may be a weak foundation for predicting effects in complex natural systems.

Analytically, it may be possible to separate identity effects from richness effects originating from other factors (e.g. from complementarity and facilitation, Schmid et al. 2002). If the number of diversity levels is small, a nested ANOVA can be used to assess contributions from species identity and richness by partitioning the variance between identity nested under richness (Hector et al. 1999, Jonsson and Malmqvist 2000, Downing and Leibold 2002). Alternatively, Loreau and Hector (2001) advised an additive partitioning method, which requires the effect (e.g. biomass production) of the individual species to be separable. However, in cases where only the overall process rate is recorded (e.g. the rate of litter decomposition), this method does not work. Moreover, the additive partitioning approach may allow partitioning of species effects based on competition, mutualism or commensalism, but not based on predation (Loreau and Hector 2001), which limits the usefulness of the method in experiments involving more than one trophic level.

Novel experimental designs

The experimental designs described below address important aspects of biodiversity effects on ecosystem functioning that could be readily established across a range of different habitats. Besides their novelty with respect to non-random species loss and the role of habitat patchiness and heterogeneity and hence the issue of scaling up, they also take into account the significance of propagule supply and the influence of multiple stressors on the sequence of species loss and ecosystem functioning. Schmid et al. (2002) have identified some of these issues in relation to the next generation of biodiversity experiments on terrestrial plant communities and Raffaelli et al. (2002) and Petchey et al. (2004) consider experimental and theoretical approaches to multitrophic systems.

The experiments we propose also go beyond the previous tests by addressing specifically the impact of the diversity of traits, as suggested by Schmid et al. (2002). We use different organisational levels, testing for the additivity of species effects on the community level. We include propagule supply to test whether species diversity is more important within a community or within a pool of possible colonisers, thus addressing the transient nature of trait loss and the role of dispersal. We include a design explicitly testing the insurance hypothesis, but we use multiple stressors to ask whether increased trait-diversity decreases the impact of naturally co-occurring disturbances. We also propose having more dominant and more tolerant species in the low-species treatments, under the assumption that species loss is non-random.

The important species identity effects are considered explicitly in several of the experimental designs; where not, we propose the inclusion of more than one species combination to test for compositional effects. In two of the designs, we use a small number of species combinations, which, whilst allowing us to assign species composition as a factor, strongly restricts the possibility of testing different community compositions ('C' in Fig. 1). Realistically, this could be done only for C = 2 or 3. In contrast, the multiple stressor experiment itself should be repeated using different species combinations to test the robustness of the results. Here and in the two-habitat experiment, idiosyncratic results would then indicate strong identity effects (Emmerson et al. 2001).

Non-random species loss and the design of dilution experiments

A dominant factor affecting the order of species loss from a community is the relative size of the constituent populations. Species with fewer individuals may be more vulnerable to extinction from stressors (Vinebrooke et al. 2004), habitat fragmentation, and stochastic effects (Caughley 1994). Therefore, biodiversity-ecosystem functioning experiments with assembled communities would be more realistic if the order of simulated species loss followed a species accumulation curve rather than simply being random. This would simulate rare species being lost first (and in low-loss treatments) and abundant species last (in high loss treatments).

Species richness and abundance often correlate with trophic level (Petchey et al. 2004) and productivity (Kautzinger and Morin 1998). Under conditions of stress, fewer individuals and therefore fewer species would be expected to persist in higher trophic positions as demonstrated in experimental systems (Petchey et al. 1999). Thus instead of creating species rank curves inclusive of all trophic levels, the loss of species from each trophic level could be examined independently, and the magnitude of loss, from rare to abundant, could be separately controlled.

The challenge is to devise treatments controlling the order, magnitude, and trophic position of species loss in experimental systems. One approach could be to construct invertebrate assemblages in aquatic mesocosms, based upon species accumulation curves from the natural environment (Emmerson and Raffaelli, 2000). Treatments could then involve the physical removal of differing numbers of species within different functional groups/trophic levels (corresponding to different magnitudes of species loss) along the accumulation curves moving from the least to most abundant species.

For microbial systems mimicking simple plankton communities, a similar experiment could be performed where the initial proportion of species in the mixture is controlled by the researcher or determined by growth rate prior to trophic fractionation. Filters of different pore sizes could separate decomposers ($< 2 \mu m$; essentially bacteria), primary producers ($5-50 \mu m$; provided autotrophic picoplankton is unimportant), and metazoan consumers ($> 50 \mu m$), representing the majority of the broad trophic levels of many published biodiversity-ecosystem functioning studies (Naeem and Li 1997, Petchey et al. 1999). A constant volume dilution-extinction series (e.g. 1 ml inoculating 10 ml of sterile medium, then mixed and 1 ml removed to inoculate the next dilution tube) of these size fractions would create a gradient of species loss, and hence of species richness, within each trophic level from complete communities to communities consisting of only the most abundant species. These diversity treatments could be normalized to the desired initial number of individuals or biomass by either taking advantage of growth before the experiment or concentrating by filtration. This approach would allow richness to be manipulated along the species accumulation curve, while abundance could be controlled prior to community reconstruction. Using these methods, trophic levels could then be reassembled with diversity treatments based upon the loss of numerically rare species, and the magnitude of species loss could be experimentally controlled for each trophic level. The same dilution technique could be used to investigate the functional importance of diversity in both natural and artificial microbial communities (Franklin et al. 2001, Garland and Lehman 2001). For example, the microbes from a pelagic system could be sampled and the natural diversity used as the high diversity treatment in a microcosm. Two dilutions from the series could be used to construct the medium and low diversity treatment. Trophic fractionation followed by dilution series-diversity treatments and community reconstruction could be applied in microcosms such as those used by McGrady-Steed et al. (1997), with the exception of those few taxa that fall outside the size range for proper fractionation.

In both natural and artificial communities, we hypothesize that an increase in biodiversity leads to an enhanced or less temporally variable rate of a given ecosystem process. The response variable could be, for example, the rate of a biogeochemical transformation or the total produced biomass of heterotrophic organisms as a measure of secondary production. In the simplest form of the proposed approach, the order of species loss would not vary, that is there would only be one composition ($C = 1$) per richness level. The number of experimental units would then be the number of replicates, R, multiplied by the number of treatments, T.

Results obtained with this design can be confounded by compositional effects, so that the experiment is not a true test of the functional consequences of richness alone. However, if we are interested in the functional consequences of natural species loss and our assumption of the importance of numerical effects on the extinction process is correct, then this approach is preferable to random loss with multiple compositions per diversity treatment. Experimental approaches that manipulate additional factors contributing to the order of natural species loss should also be investigated, but care must be taken to ensure that the additional variable used to create the experimental biodiversity gradient does not itself induce a change in the aspect of ecosystem functioning being investigated thus leading to a spurious effect (Huston 1997).

Scale, patchiness and biodiversity effects on ecosystem functioning

As indicated earlier, one of the major challenges to understanding effects of biodiversity on ecosystem functioning is whether patterns observed at small scales also occur at larger ones. Natural systems can be viewed as heterogeneous landscapes composed of numerous patches that differ in both their biotic and abiotic attributes. These patches taken together represent a fundamentally different type of biodiversity than the diversity of species, other taxonomic units or functional groups (Table 1).

As a starting point, it is useful to consider systems that fit into a two-patch or two-compartment conceptual framework. Examples from aquatic environments include the benthic and pelagic zones of lakes, pools and riffles in streams, rocky benches and tide pools in marine

intertidal zones, and vegetated and non-vegetated areas in marine soft-bottom systems. In all of these systems, patches or compartments are connected by the flow of water, dissolved and suspended materials, and transported or actively moving organisms. This high connectivity makes it likely that ecosystem processes in different kinds of patches will mutually influence each other. It is the extent of those interactions that needs to be understood when scaling up from processes observed within small homogeneous patches to heterogeneous landscapes (Stoll and Prati 2001 for a terrestrial perspective). Cardinale et al. (2004), using a simple patch-dynamics modelling approach to examine the diversity-productivity relationship, suggest that even if the relationship is not affected by spatial scale per se, the underlying mechanism producing the relationship will change with scale.

In developing an experimental design that addresses the significance of patch interactions, we begin with the simple and probably unrealistic hypothesis that observed effects of biodiversity on ecosystem functioning are independent at the spatial scale of individual patches (i.e. one patch type operates independently of all others), thus the overall effects of biodiversity in a patchy landscape will simply be the sum of processes operating in all the patches. We will refer to this as the additivity hypothesis. The alternative is that the impact of biodiversity on ecosystem functioning in one kind of patch depends critically on biodiversity effects in another patch type. To develop this scenario further, consider, a small pond with two patches or compartments: bottom sediments and open water. In an experimental container used as a pond surrogate, these patches would be the hard surface compartment colonized by benthic organisms and the open water with its pelagic organisms. By experimentally varying the diversity of organisms in both habitats, it would be possible to determine whether biodiversity effects in one compartment influenced effects in the other.

Table 3 outlines a simple design to test for such effects, and suggests some of the logistical limitations in such designs. Three levels of species richness are used for each patch type, benthic or pelagic: low, determined by the minimum value observed across a range of ponds; medium, corresponding to 1/2 of the maximum value observed in the same survey; and high, which is the maximum value observed. Note that these absolute values might be quite different for the two different habitat types and different combinations of species with the same total species richness could be used and replicated to ensure that any effects of diversity were not confounded with species composition (see above) and to assess the effects of composition on measured responses. Let C = the number of different species compositions used in each level of patch diversity, and let R = the number of replicates of each composition within each patch. Then the minimal design shown in Table 3 requires $3 \times 3 \times C \times R$ experimental units, with a total of 81 experimental units needed even for values of C and R as low as 3. Larger designs could involve more levels of diversity within each patch type (Table 3B), where n_b and n_p refer to the numbers of diversity levels in benthic and pelagic patches, respectively, but such designs rapidly become unmanageable. Similar caveats apply to factorial designs including more than two patch types. These constraints on the number of levels for the factors being experimentally manipulated may be alleviated if the experiment is carried out under controlled conditions, which may allow the splitting of the experiment into smaller sets that can be carried out sequentially (rather than simultaneously) and then reassembled for the analysis.

Fig. 1 outlines what a null hypothesis of additive effects of diversity on functioning may look like, along with one of many alternative ways in which observed results might depart from additivity. For simplicity, consider the biomass of organisms in each patch type as the ecosystem response of interest. In the parlance of analysis of variance, if significant effects of benthic and pelagic diversity on biomass were detected, but no significant interaction effects occurred, then the total biomass in both patch types can be readily predicted

Table 3. A simple (Scenario A) and more comprehensive (Scenario B) experimental design to evaluate the additivity of biodiversity effects on ecosystem functioning in different patch types or habitat compartments. S_{max} and S_{min} refer to the maximum and minimum species richness in a particular habitat patch, respectively. C refers to the number of different combinations of species used to create a given level of richness in patch types A and B. C must be ≥ 2 to assess effects of species identity.

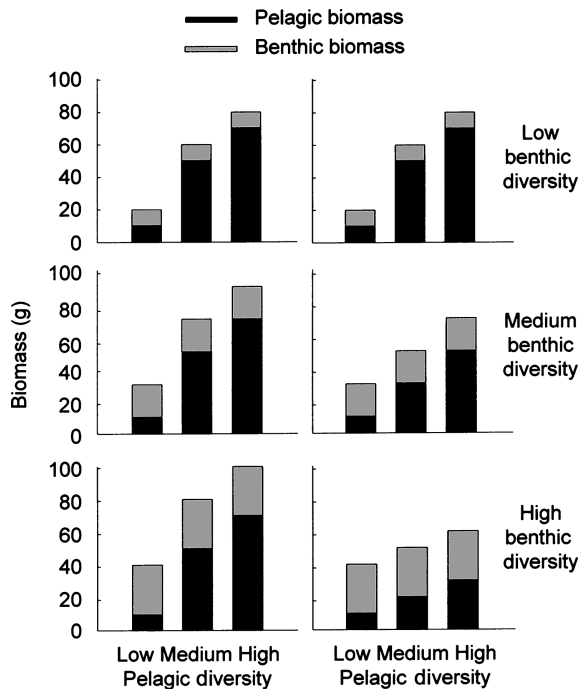| Scenario	Species richness in patch type B	Species richness in patch type A				
		Low (S_{min})	Medium (1/2 S_{max})	High (S_{max})		
A	Low (S_{min})	C	C	C		
	Medium (1/2 S_{max})	C	C	C		
	High (S_{max})	C	C	C		
		1	2	4	...	High (S_{max})
	1	C	C	C	...	C
B	2	C	C	C	...	C
	4	C	C	C	...	C
	...	...	...	...	...	C
	High (S_{max})	C	C	C	...	C

Fig. 1. Hypothetical examples of additive (left panels) and non-additive (right panels) effects of patch type on biodiversity effects on ecosystem functioning. In the non-additive case, increasing benthic biomass leads to a decrease in pelagic biomass. The panels should be read from top to bottom.

from the diversity–biomass relation observed in each patch type alone. In the counterexample, there is a positive effect of diversity on biomass in one patch type, but high diversity/biomass in that patch depresses biomass in the second patch, regardless of its own diversity. In the analysis of variance of these results, this pattern would generate a significant interaction term, and consequently effects of diversity in one patch type would depend on diversity in the other. Such effects might occur, for example, if shading by dense phytoplankton depressed the growth of benthic macrophytes, or if nutrients sequestered in large long-lived benthic organisms are unavailable to smaller short-lived pelagic organisms.

Although this discussion has focused on one diversity facet, species richness, the general design could be readily used with other taxonomic and functional diversity levels and is equally applicable to non-aquatic systems.

Significance of propagule supply

The exchange of organisms by dispersal and by emigration and immigration is among the most important processes affecting the diversity of local assemblages and is likely to be highly pertinent to the biodiversity-ecosystem functioning issue. Indeed, incorporating re-

gional processes of dispersal (and disturbance) into a simple patch-dynamics model showed that these processes can amplify the effect of species richness on productivity (Cardinale et al. 2004). Marine ecologists, for example, have long recognised the importance of recruitment fluctuations in regulating community dynamics (Underwood and Fairweather 1989) and recruitment limitation can be important in determining local species richness in terrestrial communities (Hubbell et al. 1999, Zobel et al. 2000). The implication is that the loss of species from a local habitat patch may only be transient and the effect on ecosystem functioning consequently small where the flow of propagules is high (Palmer et al. 1996). However, in some marine systems larvae may be retained nearer their natal sites more often than previously thought (Swearer et al. 1999); therefore, one would expect a continuum from very open to almost enclosed systems, determined by hydrographic factors, life history, and propagule behaviour.

Propagule supply, as used here, includes the arrival of propagules to the local community, settlement (e.g. the rate of new larvae settling and metamorphosing to the next life stage), and recruitment (i.e. the rate of juveniles surviving beyond the settlement stage, Fig. 2). Propagule density and diversity affect local species richness as well as biotic interactions within the communities (Palmer et al. 1996). Propagule supply may increase the number of colonising species, whereas fluctuations in recruitment can promote co-existence of species as equilibrium conditions necessary for competitive exclusion will not occur (Menge and Sutherland 1987). While there have been few investigations of the effects of propagule diversity on ecosystem processes, aquatic communities seem particularly suitable for experimental manipulations of propagule density at a range of spatial scales: from the laboratory (Long and Karel 2002) to mesocosms (Shurin 2001) and to field manipulations of local biological interactions at sites characterised by large-scale oceanographic differences (Menge et al. 1997).

From this previous work, several interactions between local community processes and propagule supply can be postulated and we propose to address two hypotheses: (1) increasing propagule diversity leads to higher (or lower) rates of ecosystem processes when resident species diversity is constant, and (2) increasing propagule diversity mediates the effects of reducing resident species richness. To test these hypotheses, a factorial design can be used where diversity is manipulated simultaneously in assembled communities and the propagule supply pool (Fig. 2B). Species are drawn from a common species pool and used in random assemblages. If three levels (low, mid, high) of propagule supply are combined with three levels of resident species richness, the total number of experimental units would be $3 \times 3 \times C \times R$. Thus, a minimal fourfold replication would result in 36 experimental units without replication of certain combinations
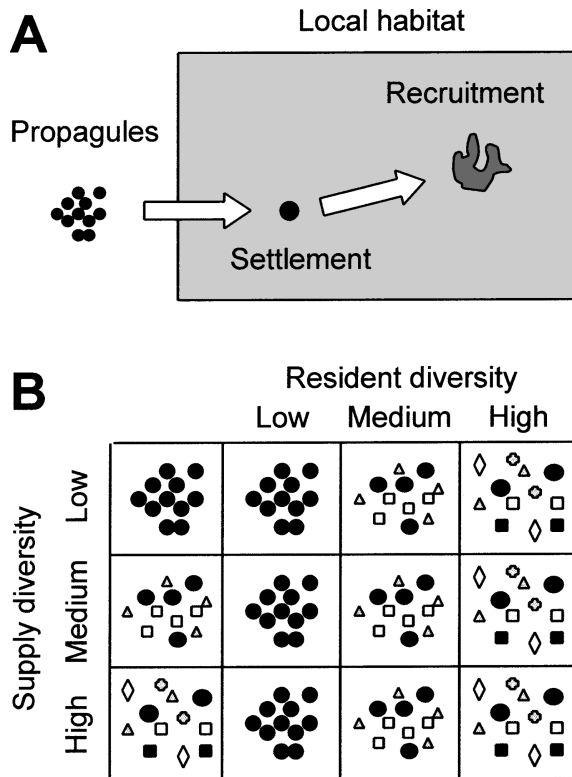
Fig. 2. Propagule supply and biodiversity effects on ecosystem functioning. (A) steps in the recruitment process, from external supply of propagules to settlement of larvae and recruitment into the local assemblage. (B) experimental design involving factorial manipulation of the diversity both of the resident community and the supplied propagules. Three diversity levels of the resident community are established in replicated plots, in this example with equal number of individuals, but 1, 4 and 8 species. Propagules are supplied to each plot by factorially crossing the three levels of resident species richness with the three levels of supply diversity. These levels consist of equal numbers of individuals, but differ in species richness.

(C = 1), and replication of three species combinations would result in 108 units. Such an experiment is still small enough to be conducted in the laboratory with independent combinations of species. Depending on the specific context of the experimental system, a range of ecosystem processes or properties can be used as response variables (Table 2).

Effects of multiple stressors

Implicit in the insurance hypothesis of biodiversity is the assumption that more diverse communities contain a wider set of physiological, morphological, behavioural and other traits. In the face of environmental perturbations, some species would thus always be likely to survive the negative impacts, increasing the probability that a species loss can be replaced by another functionally similar species, thus maintaining ecosystem functioning.

The random assembly, or disassembly, of species from a species pool to experimentally mimic the loss of biodiversity has desirable statistical properties; however, in natural ecosystems, local extinction will occur in a non-random manner with the highest adverse impact on the rarest species (see above) or on species which are least adapted to cope with a certain stressor (Vinebrooke et al. 2004). Thus, any given stressor will affect the species composition along a gradient of susceptibility, not randomly (Jonsson et al. 2002). A second simplification in previous experiments has been the use of a single stressor at a time, whereas most natural aquatic systems suffer from multiple stressors (Hughes and Connell 1999, Vinebrooke et al. 2004).

The experimental design proposed here incorporates different sequences of naturally occurring stressors and thus reflects consequences of natural extinction scenarios more realistically. The nature of these stressors is kept general in the example below (stressors A and B). The specific hypotheses that can be tested include: (1) more diverse assemblages will respond less to or recover faster from multiple disturbance events if the species-specific adaptations to the different disturbances are similar, and (2) more diverse assemblages will not increase resistance or resilience if the different disturbances antagonistically affect different species clusters. The experiment requires manipulation of three factors: (1) diversity, which is most easily represented by two levels of species richness (Table 1 for alternatives); (2) disturbance (Fig. 3), where in addition to the control, two sequences of disturbance which pose similar species-specific adaptations (AA, BB) are contrasted with two sequences of disturbance with different required adaptations (AB, BA); and (3) time, with several sampling dates), before application of the stressors (A, B or control), after the first application of
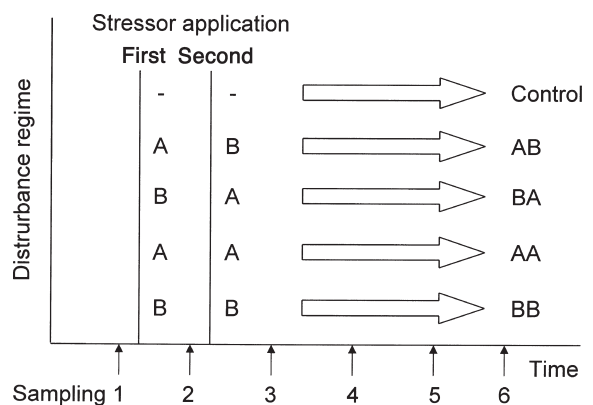

Fig. 3. Outline of an experimental set-up addressing the effect of multiple stressors on the effect of biodiversity on ecosystem functioning. The depicted experiment involves a high-diversity and a low-diversity treatment using an identical design, five different sequences of stressors and six sampling dates (see text for explanation).

stresses, after the second application of stresses, and on additional occasions after the second application to follow recovery of the system (Fig. 3). The exact sampling dates would depend on the generation times and growth rates of the species in the target community.

If replication is fourfold, an experiment with 2 diversity levels × 5 disturbance sequences × 6 sampling dates would require 240 experimental units. This number may be tractable for a microbial microcosm experimental system, but where larger units are required, repeated measurements within each unit would be needed. Species identity effects are not tested within this design, since only a sub-set of all possible species combinations is compared. Therefore, several experiments need to be run to test how different realistic species compositions respond to multiple stressors. As in the experiments proposed above, a variety of ecosystem processes and properties may be considered to assess the ecosystem responses (Table 2).

Concluding remarks

The unique characteristics of aquatic ecosystems limit our ability to directly transfer conclusions on biodiversity effects on ecosystem functioning from terrestrial experiments to aquatic habitats. In the light of the long history and past achievements in the study of aquatic ecosystems, we suggest that these systems should provide excellent testing grounds for challenging current hypotheses more fully and for testing the generality of the patterns and processes identified to date. We should build on the success of experimental work manipulating whole freshwater food webs (Carpenter et al. 2001, Persson et al. 2001), and merge the twin traditions of the systems approach of limnology and biological oceanography with the experimental community ecology approach adopted in a variety of marine (Archambault and Bourget 1999, Underwood 2000, Emmerson et al. 2001, Menge and Branch 2001) and freshwater habitats (Jonsson and Malmqvist 2000, Carpenter et al. 2001, Mehner et al. 2002).

We support the views of Schmid et al. (2002) that the next generation of biodiversity experiments should utilize a diversity of approaches. To help achieve this, we have identified the levels at which diversity should be manipulated and the properties and processes that can be measured to indicate levels of ecosystem functioning. We have suggested that there should be a greater emphasis on diversity traits, which will enable us to evaluate the effects of biodiversity on ecosystem functioning and the mechanisms underlying them, at a range of spatial and temporal scales. This shifting of the focus of investigations from a species richness-centred approach to a broader consideration of the multifarious aspects of biodiversity may well be critical to under-

standing the functional consequences of biodiversity loss on overall ecosystem functioning. To this end, the novel experimental designs presented here can be applied across a range of habitat types, both aquatic and terrestrial.

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