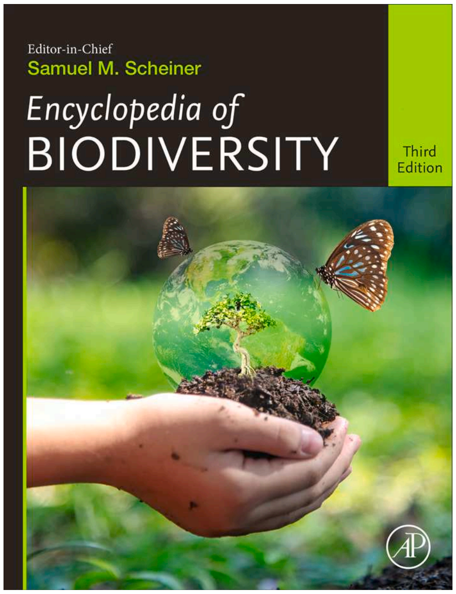

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Large-Scale Biodiversity Experiments

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Abstract

Biodiversity experiments are designed to identify how ecosystem functioning is affected by changes in diversity that can occur due to the decline, extinction, or invasion of species. Biodiversity experiments are a complement to theoretical and observational studies. There are two main ways to manipulate biodiversity: through the removal of species or by the assembly of experimental ecosystems of varying diversities. The latter allows comprehensive exploration of many possible combinations of species, some of which may be realized in the future. After three decades, more than 370 biodiversity experiments have been conducted. Results have been surprisingly consistent and indicate that biodiversity loss is causing substantial losses of ecosystem functioning, stability, and services.

Glossary

Biodiversity A contraction of biological diversity that encompasses all biological variation from the level of genes, through populations, species, functional groups, and ecosystems.
Ecosystem functioning All the pools and fluxes of matter and energy in an ecosystem.
Ecosystem services The benefits people receive from nature, for example, the provision of foods and materials, sequestration of carbon dioxide, and stabilization of soils.
Ecosystem stability Reduced variability of an aggregate ecosystem function, such as productivity, usually through time.
Factorial design Involves all possible combinations of the levels of the crossed treatments (fully factorial) or an incomplete but informative combination of levels (fractional factorial).
Functional group A group of species thought to have similar impacts on ecosystem functioning (functional effect groups).

Key Points

- Biodiversity experiments test whether changes in biodiversity cause changes in ecosystem functioning, stability, or services.
- Biodiversity experiments have helped disentangle the effects of changes in biodiversity (e.g., how many species are present) from the effects of changes in composition (i.e., which species are present).
- After three decades of research, more than 370 biodiversity experiments have been conducted in grasslands, forests, freshwater ecosystems, and marine ecosystems.
- Results from biodiversity experiments indicate that biodiversity loss is causing substantial losses of ecosystem functioning, stability, and services.

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Introduction

Biodiversity experiments aim to identify the consequences of changes in diversity for ecosystem functioning and services (as opposed to looking at the covariation of these two factors in comparative analyses of observational datasets). In the early 1990s, ecologists began formulating a set of hypothetical relationships between biodiversity and ecosystem functioning. These hypotheses ranged from those that implied a strong relationship in which the species are complementary and all play an important role, to those that include differing degrees of functional redundancy (species that overlap in functional role) to a null hypothesis of no causal relationship. In the early 1990s, these hypotheses were untested and the relationship between biodiversity and ecosystem functioning unknown. The subsequent years have seen a succession of experiments designed to test the effect of biodiversity on ecosystem functioning (Hooper *et al.*, 2005; Kinzig *et al.*, 2002; Loreau *et al.*, 2001, 2002; Balvanera *et al.*, 2006; Cardinale *et al.*, 2011; O'Connor *et al.*, 2017; Loreau *et al.*, 2022). These experiments have revealed significant effects of biodiversity on ecosystem functioning that are affecting the supply of ecosystem services to human societies.

Biodiversity Experiments

There are two main ways to manipulate biodiversity. One is by the assembly of synthesized model communities, either in laboratory cultures or in the field using artificial ponds or streams, forest plantations, plots in grasslands and similar experimental systems. An alternative approach is to remove species from natural communities, through weeding and herbiciding of plant species for example (Wardle *et al.*, 1999; Wardle and Zackrisson, 2005). Both approaches have strengths and weaknesses but both approaches attempt to hold other factors as constant as possible while manipulating the diversity of the experimental systems. Here we focus mostly on assembled biodiversity experiments, given that they are numerous and they allow exploration of combinations of species that are not yet present, but which may become increasingly relevant in the future (Jochum *et al.*, 2020).

A third nonmanipulative approach is to infer the relationship between biodiversity and ecosystem functioning by seeing how they are correlated across habitats or how they covary over time. The major advantage of this observational approach is to allow studying the relationship between biodiversity and ecosystem functioning under natural conditions (e.g., with dispersal) and realistic scenarios of biodiversity changes. Its major inconvenience is that it does not fully allow inferring causality in this relationship as both biodiversity and ecosystem functioning can be generally driven by confounding environmental factors (Loreau, 1998; Isbell *et al.*, 2013). Structural equation models are then often used to infer causality from correlations between factors (Isbell *et al.*, 2013; Grace *et al.*, 2016), but their inference power depends strongly on the quantity and quality of covariate data and prior knowledge of the ecosystem considered (Grace *et al.*, 2016).

Removal experiments are more familiar as they have a history of use in community ecology to examine interactions between species (principally competition). They are more realistic than assembly experiments in that they are based in real ecosystems. However, increased realism comes at the price of a loss of control through potential known and unknown confounding factors, including species composition. The approach also has its own potential limitations through the disturbance involved in species removal and other effects like fertilization through the decay of roots left behind after species removal.

The design and analysis of biodiversity experiments is not straightforward (Schmid *et al.*, 2002). One problem is the substantial levels of diversity present in most systems, which means that the number of possible experimental combinations of species soon exceeds what is logistically possible and fully factorial designs are only feasible when dealing with very small numbers of species (Kirwan *et al.*, 2009). One way around this is to combine species into a small number of functional groups—species that are expected to have similar effects on ecosystem functioning. However, functional diversity may not fall into discrete groups, for instance there can be strong effects of increasing diversity within functional groups (Reich *et al.*, 2004), and objectively identifying these groups a priori is not straightforward (Roscher *et al.*, 2004). A promising way to resolve this problem is a method that clusters species a posteriori based on their effects on ecosystem functioning. This approach has high explanatory and predictive powers, and turns out to be more accurate, more efficient and more parsimonious than traditional a priori functional groups (Jaillard *et al.*, 2018; 2021). A second problem is that different aspects of diversity, such as numbers of species and functional groups, are often confounded (but see Reich *et al.*, 2004). The combination of confounded explanatory variables and nonorthogonal designs has made it hard to definitively identify the importance of different aspects of diversity and the mechanisms by which they affect ecosystem functioning.

A further issue for community assembly experiments is how communities should be put together since the diversity gradient formed by the synthesized communities effectively simulates the assumed order of species loss. One approach is to assemble a full community and depauperate versions that simulate a single order of species loss (Naeem *et al.*, 1994). An alternative approach that provides a more general result by disentangling the number of species from the identities of the species is to replicate multiple communities of a given diversity by random selection of species from the pool available (Hector *et al.*, 1999; Tilman *et al.*, 2001). However, species are not usually lost from ecosystems in random order (although random loss may approximate some real-world cases). Instead, when a given extinction scenario can be predicted, communities can be assembled to simulate it (Zavaleta and Hulvey, 2004; Bracken *et al.*, 2008; Isbell *et al.*, 2008). Alternatively, a fully factorial approach simulates all possible combinations of species but is limited by logistics to small numbers of species (or more often functional groups). Community assembly experiments should give a high level of experimental control but with the usual trade-off with realism. For example, establishment of the assembled communities usually involves removing any existing vegetation and surviving propagules and the associated disturbance. Homogenization of the underlying substrate should reduce the variation among replicates but may also remove environmental heterogeneity that affects the relationship between diversity and functioning (Loreau *et al.*, 2003).

To date, no one had conducted the same biodiversity manipulation in a given system using both species removal and the assembly of experimental communities and compared the results of the two alternative approaches. There are, however, side-by-side comparisons between assembly experiments and natural systems in forests (e.g., Huang *et al.*, 2018; Liu *et al.*, 2018) and grasslands (Jochum *et al.*, 2020). Similar results in these studies provide strong support for general effects regardless of experimental methodology. Differences in results could be due to artifacts of the methodology but could also reflect important biological processes. For example, in removal experiments species compensation depends in part on natural levels of colonization of the newly free space, whereas community assembly experiments usually introduce equal numbers of propagules of all species as part of the method.

Ecological experiments can be large scale in different ways as this article illustrates specifically for biodiversity manipulations. Natural experiments may use only a single experimental unit but of a very large size: whole lakes or forest catchments for example. Alternatively, experiments may use a level of replication which is not especially large (a sample size of 30 for example) but where individual experimental units are of a size large for the study system (e.g., fields-scale trials with genetically modified crop species). Other large-scale projects use experimental units of conventional size but in complex and highly replicated designs. Multisite studies can be large if they combine experiments from several locations into a single coordinated project (Hector *et al.*, 1999; Finn *et al.*, 2013). Long-term studies are increasingly available (e.g., Reich *et al.*, 2012) and can be of great value and are large scale in a temporal context.

Mechanisms

Ecological theory identifies two classes of underlying mechanisms that can generate impacts on ecosystem processes when diversity changes. Species influence ecosystem processes partly through their intrinsic properties. Whether a species with relatively extreme traits is dominant or absent from a system is one simple mechanism for impacts of biodiversity change. When species have similar traits affecting a specific ecosystem function, they are said to be functionally redundant for that function. They then compensate for each other, such that there is little if any change in functioning when one of them is lost. Redundancy, however, is a function- and scale-dependent concept as different species or combinations of species are generally needed to ensure different functions at different places and different times (Isbell *et al.*, 2011). Thus, redundancy for a single function at one particular place and time has value as biological insurance across space and time (Loreau *et al.*, 2021). Conversely, when species occupy clearly distinct and complementary niches it will be impossible for full compensation to occur. The degree of niche overlap should control the shape of the relationship between increasing diversity and ecosystem functions; low niche overlap will produce linear patterns while high overlap will lead to saturating curves as the addition of new species increases redundancy (Loreau, 1998).

The interpretation of biodiversity experiments is complicated by these alternative classes of mechanism. Selection effects (Loreau and Hector, 2001) occur when communities become dominated by particular species and if dominant species occur more frequently in high-diversity communities then the traits of these species may drive ecosystem functioning. Alternatively, complementary or positive interactions between species may allow diverse systems to utilize more resources or to suffer lower levels of pests and diseases. An additive partitioning method (Loreau and Hector, 2001) was developed as part of the BIODEPTH project (see below) to distinguish between these alternative mechanisms. The additive partitioning method (Loreau and Hector, 2001) defines a net effect of biodiversity which is the difference between the observed yield of a mixture and that of the average monoculture yield. This net effect is then partitioned into two additive components: the selection and complementarity effects. The selection effect is the standard statistical measure of covariance applied to the relationship between yield in monoculture and the change in relative yield in mixture. Selection effects will be positive when species with higher-than-average monoculture yield dominate communities and negative when species with lower-than-average monoculture yield dominate. The complementarity effect uses relative yields to ask whether increases in the abundance of some species exactly cancel with declines in others. When this is the case, resource partitioning is a zero-sum game with some species taking more of a fixed total pool of resources and others taking less, leading to no effects of diversity. In contrast, positive complementarity effects occur when decreases in the abundances of some species do not compensate for the increases in the abundance of other species, which could result from interspecific facilitation, resource partitioning, or decreased impact of natural enemies in more diverse communities. The additive partitioning method has revealed widespread complementarity contributing to most published results (Cardinale *et al.*, 2011), especially in long-term experiments (Reich *et al.*, 2012). However, unanticipated negative selection effects have also emerged as a widespread result (Cardinale *et al.*, 2011; Reich *et al.*, 2012). One consequence is that complementarity effects are often partly masked by the negative selection.

Historical Precedents

Many effects of biodiversity on ecosystem functioning and services have, for millennia, been understood and leveraged by indigenous peoples. For example, in North America, it has been common to plant a mixture known as The Three Sisters: corn (maize), beans, and squash (Kimmerer, 2013). The corn provides a tall structure upon which the beans climb, helping fill

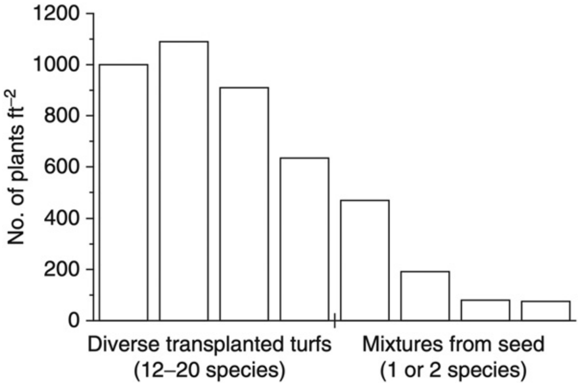

Fig. 1 Rediscovered data dating to the early nineteenth century which was collected from a large-scale experimental garden at Woburn Abbey, UK has many similarities to modern biodiversity experiments and is arguably some of the earliest experimental work conducted in ecology. Graph by A. Hector. Data from Hector, A., Hooper, R. E. (2002) Darwin and the first ecological experiment, Science 295, 639–640.

aboveground space and more completely utilize light resources. The beans are legumes that have symbiotic relationships with N-fixing bacteria, which enhances soil fertility. The squash sprawls as it grows, covering the ground, reducing weeds, and helping maintain soil moisture. Together, these three crops sustain fertile soil, enhance yields, and provide a healthy diet (Kimmerer, 2013). A fourth sister is sometimes added in the form of a plant attractive to pollinators that increase the fertilization effects of the beans.

The intellectual linkage of biodiversity with ecosystem functioning can also be traced back to the *Origin of Species* where Darwin describes what is arguably one of the first ecological experiments: a large-scale experimental garden comparing the properties of plant monocultures and mixtures (Hector and Hooper, 2002). Interestingly, the results prefigure those of recent experiments in associating higher levels of diversity with higher levels of biomass production (**Fig. 1**). However, this early work inevitably lacks features of modern experimental design such as replication (in this experiment diversity was confounded with the origin of the experimental communities; that is whether they were transplanted natural turfs or established from seed).

The Ecotron Large-Scale Controlled Environment Facility

The first modern biodiversity experiment was carried out with model communities based on annual plant species grown in the Ecotron large-scale controlled environment facility (Naeem *et al.*, 1994). Most first-generation biodiversity experiments concentrated on a single trophic level. The Ecotron experiment was unusual in taking a single intact community and simultaneously reducing diversity at four trophic levels to produce two increasingly depauperate versions from which species had been omitted at random. The key result of this experiment was that the impoverished communities were progressively less productive. However, because all replicates at each diversity level were identical in composition, the effects of numbers and types of species were confounded and the results may be specific to the particular order of species extinction examined in this experiment and not general across a wider range of possible extinction scenarios.

Large-Scale Field Experiments

The first large-scale biodiversity experiments performed under field conditions were a series of studies by Tilman and colleagues working at Cedar Creek, Minnesota in a grassland. A pair of biodiversity experiments concentrated on species (Tilman *et al.*, 1996) and functional group effects (Tilman *et al.*, 1997, 2001, 2006), respectively while the BioCON experiment (Reich *et al.*, 2001, 2004, 2012) looks at the interactions between biodiversity loss, elevated CO_2, and nitrogen enrichment. In contrast to the Ecotron experiment, more extensive diversity gradients were established where each level of diversity (a given number of species) was replicated with different mixtures of species selected at random from the species pool. Biodiversity effects could then be quantified with linear regression and tested against the residual differences between different composition communities within diversity levels. Increasing the numbers of species and functional groups caused a substantial increase in productivity, with relationships growing stronger over time (Tilman *et al.*, 2001, 2012; Reich *et al.*, 2012). Levels of unconsumed soil nitrate and (potentially leachable) nitrate below the rooting zone were both reduced at higher diversity levels. Detecting how many species contribute to biodiversity relationships has proved one of the most contentious issues in interpreting biodiversity experiments. Tilman *et al.* (2001) used a diversity index approach to see how many of the most productive species in a plot had to be present to best explain their aboveground and total biomass production. For these two ecosystem processes between one quarter and three quarters, respectively, of the species in the high-diversity treatment were needed to best explain the productivity of a plot. Additional analyses of species-specific contributions suggest that much of the biodiversity effects in this experiment can be explained by

Fig. 2 The Jena Biodiversity Experiment manipulates numbers of grassland plant species and functional groups in small (3.5 × 3.5 m) and large (20 × 20 m) plots. Photo by The Jena Experiment.

legumes and C_4 grasses but with additional species coexisting alongside them and contributing to total productivity (Lambers *et al.*, 2004). However, strong effects of biodiversity on productivity have also been found in other grassland experiments that did not include legumes (Van Ruijven and Berendse, 2003; Wilsey and Polley, 2004).

Furthermore, in the BioCON experiment, which examined a biodiversity gradient under different conditions of elevated CO_2 and N enrichment, species and functional group richness had largely independent effects across the whole range of conditions such that species within groups were not functionally redundant but made separate contributions (Reich *et al.*, 2004). Although species may appear functionally redundant under one set of environmental conditions, different sets of species support different ecosystem functions (Hector and Bagchi, 2007) and different sets of species provide any given ecosystem function during different years, at different places, and under different global change scenarios for the future (Isbell *et al.*, 2011).

The Jena Biodiversity Experiment (Roscher *et al.*, 2004; Weisser *et al.*, 2017) in Germany simultaneously manipulates numbers of both species and functional groups in a randomized design but in a more balanced way than earlier experiments and in small (3.5 × 3.5 m) and large (20 × 20 m) grassland plots (**Fig. 2**). The project also contains a dominance experiment comprising plots composed only from a reduced pool of nine grassland plant species predicted to be dominant (high relative abundance) in the full community.

Initial results from the Jena main experiment showed that relationships from small plots can be scaled up to the large plots: results from the small and large plots were statistically indistinguishable (Roscher *et al.*, 2005). Another key strength of the Jena Experiment is its comprehensive measurements of animals both aboveground and belowground, which provides a multitrophic food web perspective (Weisser *et al.*, 2017; Eisenhauer *et al.*, 2019). For example, this has shown that plant diversity effects on animals dampen with increasing trophic level (Scherber *et al.*, 2010). That is, both aboveground and belowground, herbivores responded more (in terms of their abundance and diversity) to changes in plant diversity than predators or omnivores (**Fig. 3**).

The BEF China Experiment manipulates tree species richness by planting more than 150,000 trees in plots ranging from single-species monocultures to 16-species mixtures (Huang *et al.*, 2018). By eight years into the study, 16-species mixtures had accumulated over twice the amount of carbon found in monocultures, on average, and as much carbon as in two commercial monocultures. These results suggest that diverse afforestation could help restore biodiversity and mitigate climate change.

Multisite Biodiversity Experiments

The European BIODEPTH project (Hector *et al.*, 1999; Spehn *et al.*, 2005) added two new features to the single-site experiments described above. First, in addition to replicating diversity levels with different species compositions, each composition was itself replicated which allowed the effects of both diversity and composition to be quantified. Second, the same biodiversity experiment was replicated at eight different grassland sites using standardized methodologies. Individual site analyses illustrate the broader

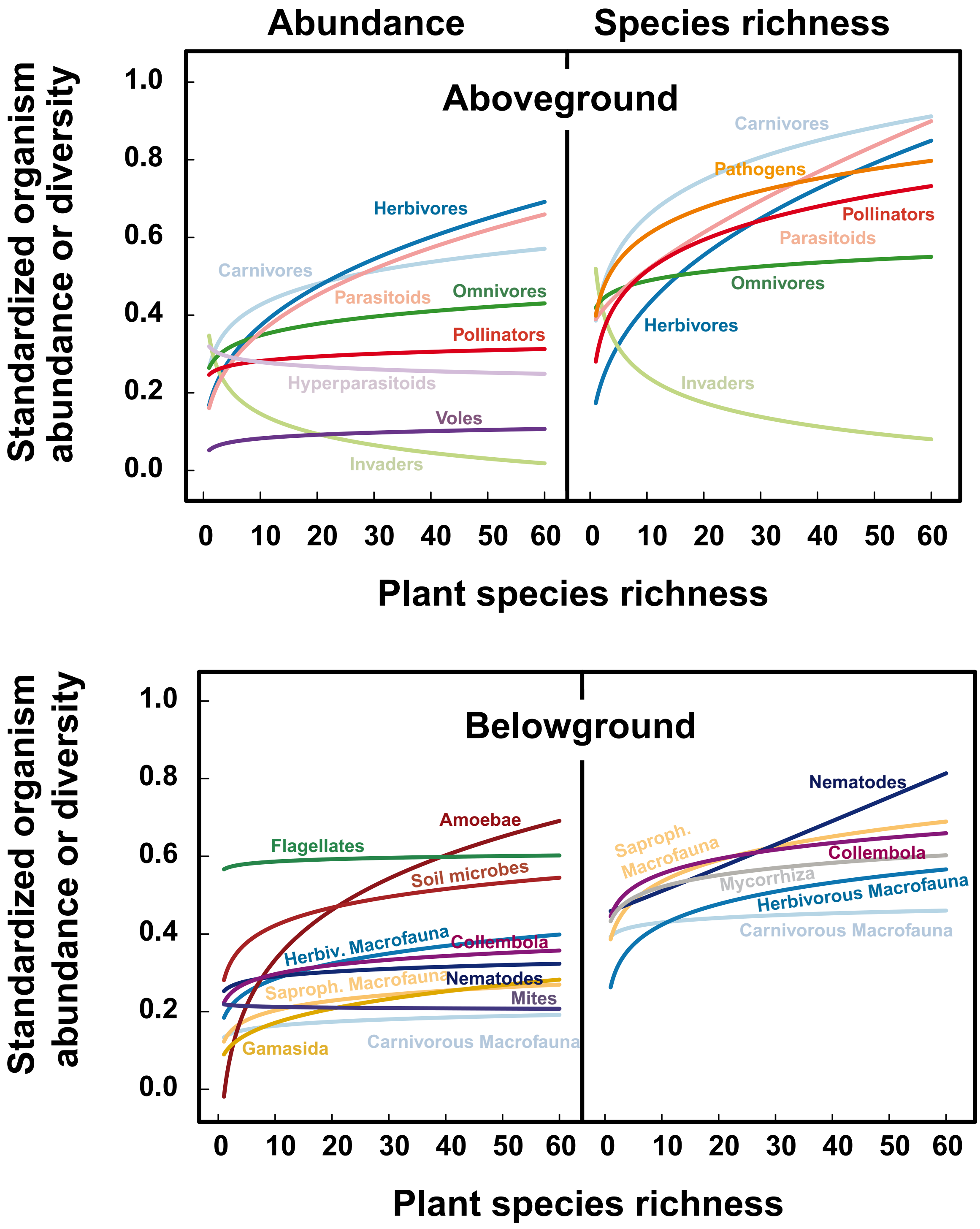

Fig. 3 The effect of grassland plant diversity on the abundance (a, c) and diversity (b, d) of many groups of species both aboveground (a, b) and belowground (c, d) in the Jena Biodiversity Experiment. Changes in plant diversity tended to impact herbivores, which directly and exclusively feed on plants, more than predators or omnivores. Graph by C. Scherber. Data from Scherber, C., Eisenhauer, N., Weisser, W. W. *et al.* (2010) Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. Nature 468, 553–556.

range of responses seen in the literature—from null to positive—and the combined multisite analysis revealed an overall log-linear (asymptotic with increasing diversity) relationship that was statistically common to all sites. Reconciling the results of the individual site analyses with the general outcome of the overall analysis has been a point of both confusion and contention. The results of the BIODEPTH project also revealed that productivity could be well explained in terms of the multiple influences of location (∼30% of the total sums of squares), the diversity of both species and functional groups (∼20%), and composition (∼40%). Later results revealed that the effects of biodiversity were not limited only to aboveground biomass production but extended to a wider suite of ecosystem processes and properties including root biomass, levels of intercepted light, nitrogen pools in aboveground vegetation, and available soil nitrogen as well as the decomposition of various substrates. However, the relationship of some of these variables to biodiversity was often more complex and less clear than that of aboveground biomass production (Spehn *et al.*, 2005). Furthermore, different sets of plant species provided high levels of different ecosystem functions (Hector and Bagchi, 2007). Other multisite experiments have subsequently been performed in terrestrial ecosystems (e.g., Van der Putten *et al.*, 2000; Kirwan *et al.*, 2007; Verheyen *et al.*, 2016). Below we elaborate on two of these more recent experimental networks, the Agrodiversity studies and TreeDivNet.

Experimental Tests of Biodiversity-Stability Relationships

A decade of monitoring of the Cedar Creek BioDIV experiment confirmed the insurance hypothesis (Loreau *et al.*, 2021), which predicts that biodiversity promotes greater temporal stability of ecosystem functioning (Tilman *et al.*, 2006). The highest diversity plots had an ecosystem stability measure (mean/standard deviation for a given time period) for biomass production that was 70% greater than the average of the monocultures. Temporal stability at the ecosystem level was positively correlated with high root biomass but, interestingly, negatively correlated with the presence of legumes (even though they promote overyielding in this system).

A later synthesis of results from 46 experiments found that increasing grassland plant diversity almost always increases ecosystem stability (Isbell *et al.*, 2015) (**Fig. 4**a). Often, it did so by increasing resistance of productivity to climate events (**Fig. 4**b). For example, diverse communities lost less productivity during moderate and extreme droughts of short or long duration. In contrast, grassland plant diversity did not increase resilience (*sensu* rapid recovery) after climate events because resilience was high in all plant communities, even in monocultures (**Fig. 4**c).

Highly Replicated Laboratory Experiments With Microbial Microcosms

In response to the complexities in the analysis and interpretation of biodiversity experiments, Bell *et al.* (2005) used a highly replicated laboratory experiment with a novel design to test whether the relationship between biodiversity and ecosystem functioning found for terrestrial plant communities extended to microbial microcosms. They took a pool of 72 laboratory culturable bacterial species isolated from the communities that develop in tree holes (the pools of water that collect between the exposed roots of trees at the base of their trunks). They took all the factors of 72 as their diversity levels (1, 2, 3, 4, 6, 8, 9, 12, 18, 24, 36, and 72) and replicated each level by drawing species from the pool without replacement. Five independent diversity gradients were formed in this way to give five alternative partitions of the species pool. Finally, each particular partition was repeated twice to provide compositional replication (a feature first introduced by the BIODEPTH experiment; see below). In total, the experiment

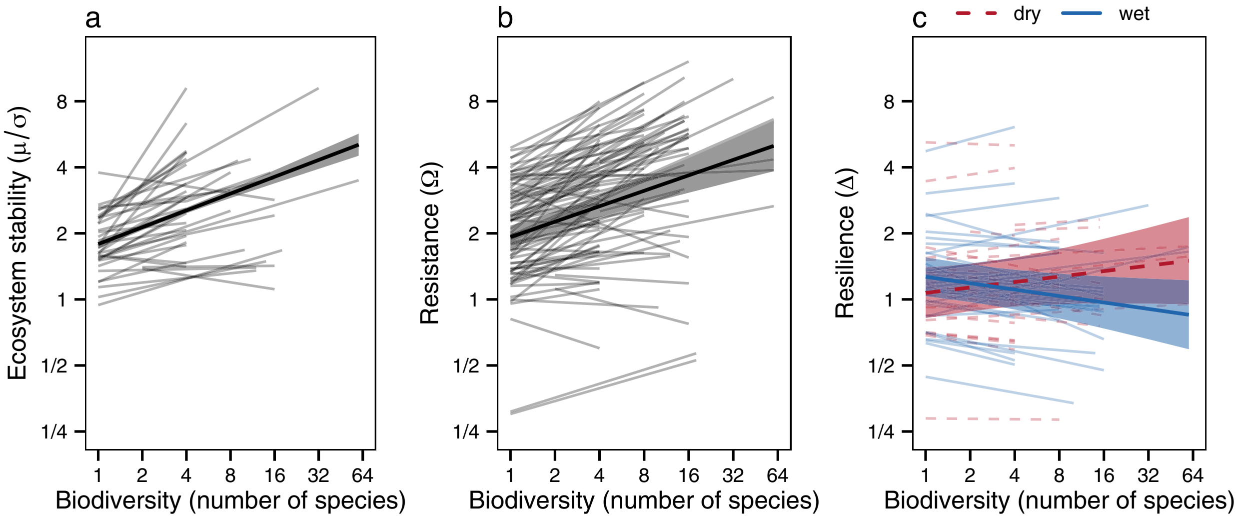

Fig. 4 Increasing grassland plant diversity increases ecosystem stability (a) and resistance (b), but inconsistently affects resilience (c). Results shown for 48 experiments that manipulated grassland plant diversity and observed how much ecosystem productivity varied over time, including during (b) and the year after (c) climate events. Graph by F. Isbell. Data from Isbell, F., Craven, D., Connolly, J. *et al.* (2015). Biodiversity increases the resistance of ecosystem productivity to climate extremes. Nature 526, 574–577.

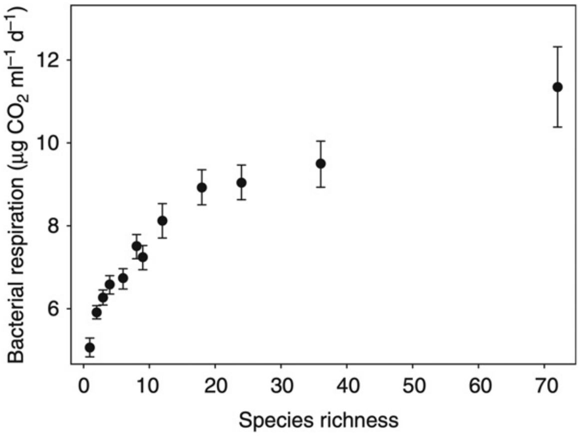

Fig. 5 The nonlinear relationship between the diversity of laboratory microbial microcosms and respiration rate (mean ± 1 S.E.M.). Graph by T. Bell and J. Newman. Data from Bell, T., Newman, J. A., Silverman, B. W., Turner, S. L., Lilley, A. K. (2005). The contribution of species richness and composition to bacterial services. Science 436, 1157–1160.

required 1374 microbial microcosms. This novel approach resulted in an analysis where the log-linear effect of species richness (numbers of species on a log-scale) was orthogonal to the effect of species composition (the collective effects of 72 variables coding for the presence or absence of each species in each microcosm). The main result was a log-linear relationship between diversity and community respiration (**Fig. 5**), which was similar to the relationship between diversity and biomass production in experiments with plants. The independence of the log-linear diversity effect from the composition effect, combined with only a small number of strong individual species effects suggests that the result was not produced by dominance of the microbial communities by species with high respiration rates but more likely resulted from complementary or facilitatory relationships or a combination of the two.

Utilizing Biodiversity in Applied Settings

The large-scale biodiversity experiments described above all come from pure ecology and aim to identify generalities in the relationship between biodiversity and ecosystem functioning in various types of ecosystems. However, in the real world, biodiversity is lost due to specific processes (e.g., eutrophication, loss and fragmentation, overharvesting) and in some cases pure studies may not give general predictions which apply to particular real-world cases. A small number of applied experiments have been established to see whether biodiversity can be used in the management of habitats in a way which is beneficial to the provision of ecosystem services to humans.

Forage Diversity Experiments

Bullock *et al.* (2001, 2007) performed a biodiversity experiment on the restoration of hay meadows at seven locations across southern Britain. At each site, plots were sown with "low-diversity" treatments (6–17 species depending on the site) which consisted of the seed mixtures recommended by the (then) UK Ministry for Agriculture Fisheries and Food (MAFF) for the recreation of moderately diverse grassland on land taken out of agricultural production. The recommended seed mixtures were all composed of species from the relevant regional species pool, which were also appropriate for the type of local environment to be restored. The "high-diversity" treatments (25–41 species) were supplemented with species found in diverse hay meadows typical of the region, soils, and hydrology of each site. The high-diversity mixtures therefore contained species appropriate for each site but which had been omitted from the recommended mixture and which were therefore presumably thought to be redundant for restoration purposes. The key result was a linear relationship between hay yield and the number of additional species added to the high-diversity treatments which was consistent across all sites and which persisted for at least eight years after the initial establishment season (Bullock *et al.*, 2007) (**Fig. 6**). The selection of a wider diversity of species increased yield by up to 60% and by nearly 50% even in the eighth year after seeding. For all the seven sites, the seed mixtures thought to be sufficient for restoration aims led to levels of hay production which were far below those achievable at the same site by more diverse mixtures that contained a wider selection of appropriate species. The increase in yield seemed not to be due to selection effects, given that the communities did not become dominated by one or a few species, though rigorous testing of the underlying mechanisms was not possible without monocultures of all species involved.

Furthermore, a continental-scale coordinated experiment, conducted at 30 sites across Europe, assessed how many species, which combinations of species, and what planted proportions of each of these species would maximize hay yields (Kirwan *et al.*, 2007; Finn *et al.*, 2013). It found overyielding benefits of mixing different plant species and functional groups.

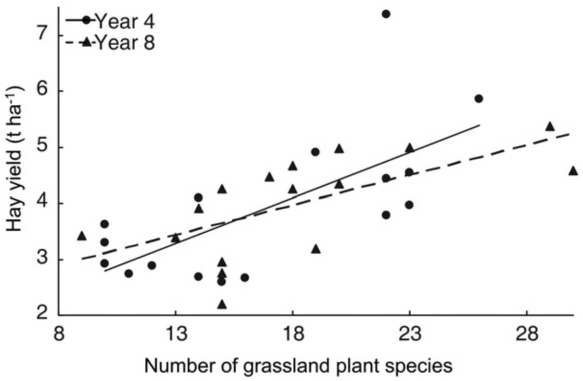

Fig. 6 Increases in hay production through the augmentation of agriculturally recommended seed mixtures with additional species. Graph by F. Isbell. Data from Bullock, J. M., Pywell, R. F., Walker, K. J. (2007). Long-term enhancement of agricultural production by restoration of biodiversity. Journal of Applied Ecology, 44, 6–12.

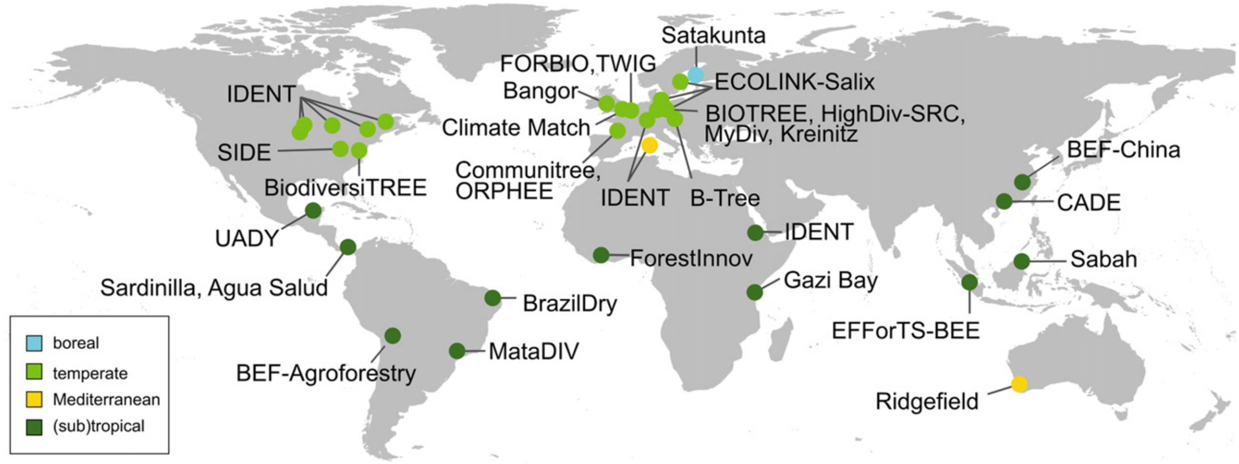

Fig. 7 A global network of tree diversity experiments, TreeDivNet. Map by Els Dhiedt.

The study design did not consider very high levels of plant diversity, but rigorously crossed two dimensions of diversity: temporal development (fast-establishing or persistent) and N-fixing legume or grass. Management such as fertilization and the frequency of hay cutting varied substantially among sites, reflecting the local agricultural practices. The pool of four species also varied among geographical and climatic regions: Mid-European, Northern European, Moist Mediterranean, and Dry Mediterranean. Unlike in some of the large biodiversity experiments described above, plots were not weeded to maintain levels of plant diversity and composition after sowing, given that it would be impossible to hand-weed at agricultural spatial scales. Despite all these differences among sites and regions, as well as several differences between these agricultural studies and the large ecological experiments described above, results were surprisingly similar to those of other biodiversity experiments. For example, across multiple years, the total biomass (including weeds) in mixtures exceeded that of the average monocultures in 97% of comparisons (Finn *et al.*, 2013). It also exceeded that of the single most productive species in monoculture at about 60% of the sites. If weed biomass was ignored, given that it may be lower quality forage, mixtures exceeded the single most productive species in monoculture at about 70% of sites. Mixtures also exhibited a resistance to invasion over at least three years. Together these results show that, under agriculturally-relevant conditions, strategically diversifying forage production systems could increase yields and reduce invasion by weeds.

Tree Diversity Experiments

Forest ecosystems, particularly in the tropics, are important both for the conservation of biodiversity and the provision of important ecosystem services. Forest ecosystems around the globe that have previously been clear felled or selectively logged are being replanted providing an opportunity to test the importance of restoring levels of biodiversity for the functioning of the rehabilitated ecosystems. There is now a global network of forest biodiversity experiments, TreeDivNet, that currently includes studies on all habitable continents (Verheyen *et al.*, 2016; Grossman *et al.*, 2018) (**Fig. 7**). Some of these tree diversity experiments are designed to disentangle several dimensions of biodiversity, including testing the contributions of taxonomic, functional trait, and phylogenetic diversity (Grossman *et al.*, 2017). Remote sensing studies in one of these tree diversity experiments showed that the canopies of diverse tree communities fit together like puzzle pieces, efficiently and fully packing the aboveground space (Williams *et al.*, 2017), a mechanism hypothesized to also explain the positive effect of tree diversity on productivity found in the Sabah Biodiversity Experiment in Malaysian Borneo (Wu *et al.*, 2020). In the BEF China experiment, which was described above and which is part of TreeDivNet, functional diversity consistently increased productivity and became a more reliable predictor of productivity than traits (Bongers *et al.*, 2021). In other words, forest productivity seems to depend at least as much on the diversity as on the types of plants. Similar results have also been found in grasslands (Hector *et al.*, 2011). These experiments signal a new direction in research on biodiversity and ecosystem functioning which moves beyond general tests to large-scale trials in threatened and managed ecosystems that are known to provide important ecosystem services to people.

Concluding Remarks

At last count, there were 374 published biodiversity experiments, coming from grasslands and forests, as well as freshwater and marine ecosystems (O'Connor *et al.*, 2017). The designs of biodiversity experiments have been gradually improved over the past three decades, but results have remained surprisingly consistent. That is, the vast majority of results from biodiversity experiments suggest that loss of half the species would lead to loss of 16.5% (95% CI: 10.5–22.6%) of ecosystem functioning (O'Connor *et al.*, 2017). Thus far, these experiments have focused on the consequences of changes in biodiversity at the relatively small local scales at which individuals interact with one another. An important next step will be for new experiments to additionally quantify the consequences of the spatial homogenization of biodiversity at the larger spatial scales over which organisms disperse. Despite enormous progress in studying the consequences of biodiversity loss with a combination of experiments, theory, and observations, we still see only a part of the picture of how biodiversity contributes to ecosystem functioning, stability, and services.

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