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Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions

Wolfgang W. Weisser[a,*], Christiane Roscher[b,c], Sebastian T. Meyer[a], Anne Ebeling[d], Guangjuan Luo[a], Eric Allan[e], Holger Beßler[f], Romain L. Barnard[g,h], Nina Buchmann[h], François Buscot[c,i], Christof Engels[f], Christine Fischer[j,k], Markus Fischer[e], Arthur Gessler[l], Gerd Gleixner[m], Stefan Halle[d], Anke Hildebrandt[j,m], Helmut Hillebrand[n], Hans de Kroon[o], Markus Lange[m], Sophia Leimer[p], Xavier Le Roux[q], Alexandru Milcu[r,s], Liesje Mommer[t], Pascal A. Niklaus[h,B], Yvonne Oelmann[u], Raphael Proulx[v], Jacques Roy[r], Christoph Scherber[w], Michael Scherer-Lorenzen[x], Stefan Scheu[y], Teja Tscharntke[z], Michael Wachendorf[A], Cameron Wagg[B], Alexandra Weigelt[c,C], Wolfgang Wilcke[p], Christian Wirth[c,C], Ernst-Detlef Schulze[m], Bernhard Schmid[B], Nico Eisenhauer[c,D]

[a]*Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, School of Life Sciences Weihenstephan, Technical University of Munich, Hans-Carl-von-Carlowitz-Platz 2, D-85354 Freising, Germany*
[b]*UFZ, Helmholtz Centre for Environmental Research, Physiological Diversity, Permoserstrasse 15, 04318 Leipzig, Germany*
[c]*German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany*
[d]*Institute of Ecology, Friedrich Schiller University Jena, Dornburger Str. 159, 07743 Jena, Germany*
[e]*Institute of Plant Sciences, University of Bern, Altenbergrain 21, 3013 Bern, Switzerland*
[f]*Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, Plant Nutriton and Fertilisation, Albrecht-Thaer-Weg 4, Humboldt Universität zu Berlin, 14195 Berlin, Germany*
[g]*Agroécologie, INRA, AgroSup Dijon, Univ. Bourgogne Franche-Comté, F-21000 Dijon, France*
[h]*Institute of Agricultural Sciences, ETH Zurich, LFW C56, Universitätsstr. 2, 8092 Zurich, Switzerland*
[i]*UFZ, Helmholtz Centre for Environmental Research, Department of Soil Ecology, Permoserstrasse 15, 04318 Leipzig, Germany*
[j]*Institute of Geoscience, Friedrich Schiller University Jena, Burgweg 11, 07749 Jena, Germany*
[k]*UFZ, Helmholtz Centre for Environmental Research, Department of Conservation Biology, Permoserstrasse 15, 04318 Leipzig, Germany*
[l]*Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland*
[m]*Max Planck Institute for Biogeochemistry, POB 100164, 07701 Jena, Germany*
[n]*Plankton Ecology, Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossietzky University Oldenburg, 26382 Wilhelmshaven, Germany*
[o]*Institute for Water and Wetland Research, Radboud University Nijmegen, Heyendaalseweg 135, P.O. Box 9010, 6500 GL Nijmegen, The Netherlands*

*Corresponding author. Fax: +49 8161 714427.
E-mail address: wolfgang.weisser@tum.de (W.W. Weisser).

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[p]*Institute of Geography and Geoecology, Karlsruhe Institute of Technology (KIT), Reinhard-Baumeister-Platz 1, 76131 Karlsruhe, Germany*
[q]*Microbial Ecology Centre, INRA, CNRS, Université de Lyon, Université Lyon 1, UMR CNRS 5557, UMR INRA 1418, bât. G. Mendel,43 boulevard du 11 novembre 1918, 69622 Villeurbanne, France*
[r]*CNRS, Ecotron (UPS-3248), Campus Baillarguet, F-34980, Montferrier-sur-Lez, France*
[s]*Centre d'Ecologie Fonctionnelle et Evolutive, CEFE-CNRS, UMR 5175, Université de Montpellier – Université Paul Valéry – EPHE,1919 route de Mende, F-34293, Montpellier Cedex 5, France*
[t]*Plant Ecology and Nature Conservation Group, Wageningen University, P.O. Box 47, 6700 AA, Wageningen, The Netherlands*
[u]*Geoecology, University of Tübingen, Rümelinstraße 19-23, 72070 Tübingen, Germany*
[v]*Canada Research Chair in Ecological Integrity, Centre de recherche sur les interactions bassins versants-écosystèmes aquatiques, Université du Québec à Trois-Rivières, 3351 Boul. des Forges, CP 500, Trois-Rivières, QC, G9A 5H7, Canada*
[w]*Institute of Landscape Ecology, Animal Ecology and Multitrophic Interactions, University of Münster, Heisenbergstr. 2, 48149 Münster, Germany*
[x]*Geobotany, Faculty of Biology, University of Freiburg, Schänzlestr. 1, D-79104 Freiburg, Germany*
[y]*J.F. Blumenbach Institute of Zoology and Anthropology, Georg August University Göttingen, Berliner Strasse 28, 37073 Göttingen, Germany*
[z]*Department of Crop Science, Agroecology, University of Göttingen, Grisebachstrasse 6, 37077 Göttingen, Germany*
[A]*Department of Grassland Science and Renewable Plant Resources, University of Kassel, Steinstrasse 19, 37213 Witzenhausen, Germany*
[B]*Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstr. 190, CH-8057 Zurich, Switzerland*
[C]*Institute of Biology, Leipzig University, Johannisallee 21, 04103 Leipzig, Germany*
[D]*Institute of Biology, Leipzig University, Deutscher Platz 5e, 04103 Leipzig, Germany*

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Abstract

In the past two decades, a large number of studies have investigated the relationship between biodiversity and ecosystem functioning, most of which focussed on a limited set of ecosystem variables. The Jena Experiment was set up in 2002 to investigate the effects of plant diversity on element cycling and trophic interactions, using a multi-disciplinary approach. Here, we review the results of 15 years of research in the Jena Experiment, focussing on the effects of manipulating plant species richness and plant functional richness. With more than 85,000 measures taken from the plant diversity plots, the Jena Experiment has allowed answering fundamental questions important for functional biodiversity research.

First, the question was how general the effect of plant species richness is, regarding the many different processes that take place in an ecosystem. About 45% of different types of ecosystem processes measured in the 'main experiment', where plant species richness ranged from 1 to 60 species, were significantly affected by plant species richness, providing strong support for the view that biodiversity is a significant driver of ecosystem functioning. Many measures were not saturating at the 60-species level, but increased linearly with the logarithm of species richness. There was, however, great variability in the strength of response among different processes. One striking pattern was that many processes, in particular belowground processes, took several years to respond to the manipulation of plant species richness, showing that biodiversity experiments have to be long-term, to distinguish trends from transitory patterns. In addition, the results from the Jena Experiment provide further evidence that diversity begets stability, for example stability against invasion of plant species, but unexpectedly some results also suggested the opposite, e.g. when plant communities experience severe perturbations or elevated resource availability. This highlights the need to revisit diversity–stability theory.

Second, we explored whether individual plant species or individual plant functional groups, or biodiversity itself is more important for ecosystem functioning, in particular biomass production. We found strong effects of individual species and plant functional groups on biomass production, yet these effects mostly occurred in addition to, but not instead of, effects of plant species richness.

Third, the Jena Experiment assessed the effect of diversity on multitrophic interactions. The diversity of most organisms responded positively to increases in plant species richness, and the effect was stronger for above- than for belowground organisms, and stronger for herbivores than for carnivores or detritivores. Thus, diversity begets diversity. In addition, the effect on organismic diversity was stronger than the effect on species abundances.

Fourth, the Jena Experiment aimed to assess the effect of diversity on N, P and C cycling and the water balance of the plots, separating between element input into the ecosystem, element turnover, element stocks, and output from the ecosystem.

While inputs were generally less affected by plant species richness, measures of element stocks, turnover and output were often positively affected by plant diversity, e.g. carbon storage strongly increased with increasing plant species richness. Variables of the N cycle responded less strongly to plant species richness than variables of the C cycle.

Fifth, plant traits are often used to unravel mechanisms underlying the biodiversity–ecosystem functioning relationship. In the Jena Experiment, most investigated plant traits, both above- and belowground, were plastic and trait expression depended on plant diversity in a complex way, suggesting limitation to using database traits for linking plant traits to particular functions.

Sixth, plant diversity effects on ecosystem processes are often caused by plant diversity effects on species interactions. Analyses in the Jena Experiment including structural equation modelling suggest complex interactions that changed with diversity, e.g. soil carbon storage and greenhouse gas emission were affected by changes in the composition and activity of the belowground microbial community. Manipulation experiments, in which particular organisms, e.g. belowground invertebrates, were excluded from plots in split-plot experiments, supported the important role of the biotic component for element and water fluxes.

Seventh, the Jena Experiment aimed to put the results into the context of agricultural practices in managed grasslands. The effect of increasing plant species richness from 1 to 16 species on plant biomass was, in absolute terms, as strong as the effect of a more intensive grassland management, using fertiliser and increasing mowing frequency. Potential bioenergy production from high-diversity plots was similar to that of conventionally used energy crops. These results suggest that diverse 'High Nature Value Grasslands' are multifunctional and can deliver a range of ecosystem services including production-related services.

A final task was to assess the importance of potential artefacts in biodiversity–ecosystem functioning relationships, caused by the weeding of the plant community to maintain plant species composition. While the effort (in hours) needed to weed a plot was often negatively related to plant species richness, species richness still affected the majority of ecosystem variables. Weeding also did not negatively affect monoculture performance; rather, monocultures deteriorated over time for a number of biological reasons, as shown in plant-soil feedback experiments.

To summarize, the Jena Experiment has allowed for a comprehensive analysis of the functional role of biodiversity in an ecosystem. A main challenge for future biodiversity research is to increase our mechanistic understanding of why the magnitude of biodiversity effects differs among processes and contexts. It is likely that there will be no simple answer. For example, among the multitude of mechanisms suggested to underlie the positive plant species richness effect on biomass, some have received limited support in the Jena Experiment, such as vertical root niche partitioning. However, others could not be rejected in targeted analyses. Thus, from the current results in the Jena Experiment, it seems likely that the positive biodiversity effect results from several mechanisms acting simultaneously in more diverse communities, such as reduced pathogen attack, the presence of more plant growth promoting organisms, less seed limitation, and increased trait differences leading to complementarity in resource uptake. Distinguishing between different mechanisms requires careful testing of competing hypotheses. Biodiversity research has matured such that predictive approaches testing particular mechanisms are now possible.
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Keywords: Complementarity; Selection effect; Biomass; Nutrient cycling; Carbon storage; Multi-trophic interactions

Introduction

In the past 20 years, there has been an increasing interest in the question of how important a diverse biotic community is for the functioning of an ecosystem (e.g. Schulze & Mooney 1993; Loreau, Naeem et al. 2001; Kinzig, Pacala, & Tilman 2002; Hooper et al. 2005; Cardinale et al. 2012; Naeem, Duffy, & Zavaleta 2012). This question, and the research underpinning it, represent a paradigm shift in ecology (Naeem 2002; Hillebrand & Matthiessen 2009). Traditionally, biodiversity research has sought to understand the regulation and maintenance of diversity, i.e. the drivers of biodiversity, and biodiversity was considered to be the consequence of the abiotic and biotic factors regulating a community (Chesson 2000). This emphasis has shifted towards understanding the consequences of biodiversity changes for ecosystem functions and services (Hooper et al. 2005; Naeem, Bunker, & Hector 2009; Cardinale et al. 2012), thus considering biodiversity itself as a driver and not only as a dependent variable in ecosystems, as first pointed out explicitly in the seminal book edited by Schulze and Mooney (1993). This paradigm shift is important as many processes in an ecosystem are vital for mankind: if biodiversity is needed for the provisioning of services by nature, then this provides a further reason for conserving biodiversity (MEA 2005; Mace 2015). Furthermore, understanding how biodiversity influences ecosystem functioning and the delivery of services

could pave the way to a sustainable management of biodiversity, for reinforcing ecosystem performance and resilience, and for developing locally attuned systems. Ecosystem services in this context are, e.g. production, maintenance of soil fertility, water purification, pollination and many others (MEA 2005; Balvanera et al. 2006).

An increasing number of studies have been performed in the past two decades to analyse the relationship between biodiversity and ecosystem functioning (Balvanera et al. 2006; Schmid et al. 2009; Cardinale et al. 2012; Hooper et al. 2012). One approach that has been increasingly used is to manipulate biodiversity directly as an independent variable. Such *biodiversity experiments* allow for a decoupling of manipulated aspects (mostly plant diversity) and environmental factors (e.g. site fertility), often correlated in natural ecosystems, and therefore allow a test of causal relationships between biodiversity and ecosystem variables (Schmid & Hector 2004). Early and very influential biodiversity experiments were set up in the US by David Tilman in Cedar Creek (Tilman et al. 2001), by a consortium of scientists in Europe in 1996 (BIODEPTH: Hector et al. 1999), and in the UK Ecotron, which manipulated not just plant species richness but an entire food web (Naeem, Thompson, Lawler, Lawton, & Woodfin 1994). These and subsequent biodiversity experiments (e.g. CLUE, the Jena Experiment, van der Putten et al., 2000; Roscher et al. 2004) were successful in revealing that many ecosystem variables have a lowered mean, and an increased variance, in low diversity communities, while high diversity communities often provided higher and more stable levels of ecosystem functioning (Loreau, Naeem et al. 2001; Loreau, Naeem, & Inchausti 2002; Hooper et al. 2005; Roscher et al. 2005; Allan, Weisser et al. 2013). This is supported not only by individual experiments but also by meta-analyses of the results (Balvanera et al. 2006; Cardinale et al. 2006; Worm et al. 2006; Cardinale et al. 2007; Isbell et al. 2011; Hooper et al. 2012; Allan, Weisser et al. 2013; Isbell, Craven et al. 2015). Today, it is the general consensus that biodiversity has manifold effects on processes at the ecosystem level, and that this conclusion holds not only for biodiversity experiments, but also for real-world ecosystems (but see Eisenhauer et al. 2016; Wardle 2016). The history of biodiversity experiments, and a summary of the main debates have recently been reviewed by Tilman, Isbell, and Cowles (2014).

The Jena Experiment, a large grassland biodiversity experiment in central Germany that was set up in 2002, has contributed substantially to debates on the importance of biodiversity for ecosystem functioning. Like the Cedar Creek experiments, it is a long-running biodiversity experiment. The Jena Experiment was set up with a broad ecosystem perspective, to investigate plant diversity effects on water, carbon (C), nitrogen (N), and phosphorus (P) cycling, as previous biodiversity experiments had mostly investigated only snapshots of element cycling. The Jena Experiment has also allowed comparing the responses of a broad range of taxa, from bacteria to mammals, to the manipulation of plant diversity. This allowed the exploration of how multi-trophic species interactions changed along the gradient of plant diversity and how these changes in species interactions underlie the observed changes in ecosystem functioning.

In this paper, we summarize and integrate the results obtained from studies conducted within the Jena Experiment. Previous synthesis articles have largely focussed on summarizing the evidence for an effect of biodiversity on ecosystem functioning, rather than on describing in detail the response of an ecosystem to a manipulation of plant diversity (Hooper et al. 2005; Balvanera et al. 2006; Cardinale et al. 2012; Naeem et al. 2012; Tilman et al. 2014). We believe it is important to detail the variability in responses among different ecosystem variables, to be able to ask more detailed questions about the mechanisms underlying biodiversity–ecosystem functioning relationship. Such an approach is possible in the Jena Experiment, because a wide range of ecosystem variables was measured in the same plots, so that their responses to the manipulation of plant diversity can be directly compared. In contrast, previous syntheses have brought together results from a variety of experiments that differed in experimental detail. This makes it difficult to directly compare the responses of different ecosystem variables, e.g. different groups of organisms or different element cycles, to a manipulation of biodiversity. In addition, there is a large overlap in variables investigated in different experiments (e.g. plant biomass), so that the number of different ecosystem-level variables that could be compared between experiments was limited.

In our review, we focus on the following topics:

(1) the generality of the biodiversity effect on ecosystem variables and variability between different types of variables;
(2) the occurrence of delayed biodiversity effects and transient dynamics, that underscore the importance of the long-term nature of the Jena Experiment;
(3) responses of the plant community itself to the manipulation of plant species richness;
(4) changes in the community composition of other organisms, from bacteria to mammals, along the gradient of plant species richness;
(5) a detailed summary of how the different components of the N, P, C and water cycles respond to plant species richness;
(6) examples of approaches that link changes in multi-trophic species interactions along the gradient of diversity to changes in ecosystem variables mediated by these interactions;
(7) the importance of the results from the Jena Experiment for applied questions related to grasslands, i.e. grassland management and bioenergy production;
(8) challenges to the interpretation of the biodiversity experiments and how these can be addressed, in particular the necessity of weeding the plots and the low performance of low-diversity plots;

(9) mechanisms underlying the biodiversity–ecosystem functioning relationship, as in the Jena Experiment many hypotheses on these mechanisms have been tested, in particular with respect to the positive effect of plant species richness on aboveground plant community biomass;
(10) future avenues for functional biodiversity research, based on the insights gained in the Jena Experiment.

We start by describing the scientific questions open at the time the Jena Experiment was founded, as these were important for the design of the experiment (Section "The Jena Experiment, its origin and design"). This section also includes a detailed description of the experimental design of the Jena Experiment, including the statistical considerations that have guided both the experimental design and the analysis of data from the Jena Experiment in the past years. We then describe the establishment and development of the plant communities in the diversity plots (Section "Plant community dynamics during the long-term experiment"), as the dynamic nature of the plant communities in biodiversity experiments is rarely reported, yet the plant community forms the basis for the observed biodiversity effects.

The review of the ecosystem-level responses to the manipulation of plant species richness starts with a section that summarizes the results of the Jena Experiment with respect to four major topics in biodiversity research (Section "The footprint of diversity"), i.e. (a) the generality of the biodiversity effect on ecosystem functioning as well as the differences in this effect between different categories of ecosystem variables, (b) the effect of plant species richness on ecosystem stability, (c) the occurrence of delayed biodiversity effects, and (d) the question whether dominant individual plant species drive or counteract the effect of plant species richness on ecosystem variables.

The following sections (Sections "Responses of individual plant species to plant species richness", "Invasion into the target communities" and "Responses of other trophic levels to plant biodiversity") then describe the responses of plants and other organisms to the manipulation of plant species richness, starting with the responses of individual plant species to the changes in the competitive environment when plants are sown in different mixtures.

After discussing effects of plant diversity on the various organisms in the grassland ecosystem, we review the effect of plant species richness on primary productivity and water and element cycling (Sections "The effects of plant species richness on plant community biomass", "The effects of diversity on biogeochemical cycling"). The effect of plant species richness on plant community biomass, above- and belowground, is singled out as a separate section (Section "The effects of plant species richness on plant community biomass"), because this variable has been the focus of most biodiversity experiments. Studies on ecosystem variables that represent animal-mediated processes, e.g. herbivory, are summarized in the following section (Section "Animal-mediated ecosystem processes"), which also reports on efforts to link the observed changes in such processes to changes in the community of organisms along the gradient in plant species richness.

After outlining the applied implications of the results of the Jena Experiment for grassland management and conservation (Section "Grassland management, productivity and bioenergy production"), we discuss in detail potential side-effects of weeding on the plant communities and reasons for the low performance of low-diversity plots (Section "Weeding issues and monoculture performance").

Finally, we illustrate the mechanistic approach taken in the Jena Experiment, by outlining how functional traits have been used to understand the mechanisms underlying biodiversity–ecosystem functioning relationships (Section "Mechanisms underlying the biodiversity–ecosystem functioning relationships"). Using the example of the plant species richness–plant community biomass relationship, the section also illustrates how detailed studies have been carried out to confirm or refute the various hypotheses that have been proposed to underlie this relationship. The final section (Section "Discussion and conclusions") concludes the paper by deriving a number of future avenues for functional biodiversity research, based on the insights gained in the Jena Experiment.

In this paper, we will use the term 'ecosystem functioning' to generally refer to the 'joint effects of all processes that sustain an ecosystem' (Reiss, Bridle, Montoya, & Woodward 2009). In the biodiversity literature, 'ecosystem process' and 'ecosystem function' are often considered to be synonymous (Reiss et al. 2009), and are often used to denote any variable that can be measured in an ecosystem. More narrow definitions of ecosystem functions have the disadvantage that they disagree on which processes they do not consider to be ecosystem functions. A more narrow definition of ecosystem function also does not solve the problem of whether two ecosystem variables describe the same function or not, a problem frequently encountered in meta-analyses of many ecosystem variables (e.g. Allan, Weisser et al. 2013; Meyer et al. 2016). We will therefore use the more general term 'ecosystem variable' throughout this paper, as a property that can be compared among different ecosystems. These ecosystem variables quantify ecosystem functions either directly ('the changes in energy and matter over time and space through biological activity', sensu Reiss et al. 2009), or indirectly ('key ecosystem properties affected by ecosystem functions', sensu Jax 2010).

The Jena Experiment, its origin and design

Biodiversity-ecosystem functioning research around 2000

The biodiversity experiment in Jena was established in 2002 (Fig. 1) by the research unit 456 'The role of

Fig. 1. Impressions from the Jena Experiment. (A) View of field site before sowing, (B) Sowing of large plots, (C) Sowing of small plots, (D) Weeding, (E) Mowing, (F) Aerial view of the field site in 2006. The trait-based experiment (TBE) was established in 2010 in the area next to the river (mown area in the left, cf. Fig. 3). ©The Jena Experiment.

biodiversity for element cycling and trophic interactions — an experimental approach in a grassland community', funded by the German Research Foundation (DFG), and supported by the Max Planck Society and the Friedrich Schiller University of Jena, Germany. First thoughts about the experiment date back to the year 2000, and the application to the DFG was submitted in 2001.

At the time the Jena Experiment was conceived, there was some controversy about the results obtained from early biodiversity experiments (Grime 1997; Kaiser 2000). The criticisms ranged from poor study design (Huston 1997) and failure to account for statistical artefacts (Doak et al. 1998), to the choice of inappropriate model communities (Thompson, Askew, Grime, Dunnett, & Willis 2005). A review on biodiversity effects published at the time, initiated as a consensus paper among different schools of thought (Loreau, Naeem et al. 2001), did not succeed to bridge the different interpretations of the results.

One important debate was whether the results obtained in the early biodiversity experiments represent a 'true' biodiversity effect, i.e. an emerging property due to species interactions that positively affect processes at the ecosystem level. With respect to plant biomass production, there was the important observation that more diverse mixtures have a higher chance of containing the most productive plant species from the species pool of the experiment (Wardle 1999), such that the higher biomass in more diverse mixtures could simply be the consequence of this 'sampling effect' (later more clearly defined as 'selection effect', Loreau 1998), i.e. not resulting from positive interactions between species. Related to this question was the question of how to correctly compare the observed biomass (yield) of a diverse plant species mixture to the yield of the species in monocultures, when calculating the biodiversity effect as overyielding, i.e. the difference between the observed and expected yield. Some authors argued that the mixture yield needs to be compared to the yield of the most productive monoculture among the species that occur in a mixture, rather than the average productivity of the monocultures (Huston 2000). The theoretical framework understanding biodiversity effects was only beginning to be developed at the time, e.g. the additive partitioning method that allows separating a net biodiversity effect into contributions of complementarity and selection effects (Loreau & Hector 2001). It is now accepted that complementarity occurs if the performance of species in mixture is on average higher than expected from their monoculture yields, while the selection effect explains higher productivity of mixtures by the dominance of individual, highly productive species.

Despite these theoretical advances, there were a number of potential issues with the design of the early experiments that could not be ruled out *a posteriori*, e.g. that monocultures were available for only a subset of species, or that some covariates had not been measured, such as initial soil conditions, so that their influence on the results could not be tested (Huston 1997, 2000; Doak et al. 1998; Wardle 1998; Schmid et al. 2002; Schmid & Hector 2004). The critiques of the early experiments have been important promoters of more refined studies and methods of analysis, and have led to higher awareness of potential artefacts (Schmid et al. 2004). For the design of the Jena Experiment, it was clear that it had

to take into account the statistical issues unravelled by the debate.

A second major point at that time was that the early experiments only focussed on a small number of ecosystem variables that hindered a comprehensive understanding of the role of biodiversity at the ecosystem level. In particular, ecosystem ecology was not well represented in many previous experiments, as measurements of element cycles were often only snapshots in time and/or represented only particular components of the C, N, P and water cycle (e.g. only nitrate in soil water). As a consequence, it was decided that the Jena Experiment should measure both the biotic component of the ecosystem, i.e. all organisms from bacteria to mammals, as well as variables related to element cycling, in much detail.

Design features of the Jena Experiment

The design of the Jena Experiment was chosen to address the various statistical issues, real or perceived, of previous biodiversity experiments. In addition, it was decided to measure important potential covariates such as soil conditions. Important aspects of the design were

(1) a large species pool of 60 species, to reduce the similarity of mixtures at high plant species richness;
(2) a division of plants into functional groups based on a multivariate trait analysis;
(3) an almost orthogonal combination of plant species richness and functional group richness, i.e. there are plots with 8 or even 16 species, all of the same plant functional group;
(4) an even representation of the different functional groups at each diversity level, allowing the partitioning of biodiversity effects on ecosystem functioning into the effects of species richness, functional group richness, and the contribution of particular functional groups (Roscher et al. 2004);
(5) the establishment of monocultures of all 60 plant species in two replicates, to allow the partitioning of net diversity effects into selection and complementarity effects (Loreau & Hector 2001);
(6) the establishment of several sub-experiments. In addition to the '*main experiment*' with its large species gradient (1–60) species and large plots (originally 20 × 20 m), an experiment with dominant species only ('*dominance experiment*') and small (3.5 × 3.5 m) replicates of the main experiment were established. Several split-(split-)plot experiments in the main experiment served to test particular questions. A *trait-based experiment* (TBE) was established in 2010 based on the results obtained from the other experiments.

In the following, we provide the necessary details for the understanding of the experimental design of the main and other experiments. A full description is given in Roscher et al. (2004).

Study site and species pool

The experimental site is located on the floodplain of the Saale river at the northern edge of Jena (Thuringia, Germany, 50°55′N, 11°35′E, 130 m a.s.l.). Mean annual air temperature is 9.9 °C (1980–2010), and mean annual precipitation is 610 mm (Hoffmann, Bivour, Früh, Koßmann, & Voß 2014). The site was used as an arable field for 40 years. It was converted from grassland in the early 1960s and was used to grow vegetables and cereals until the onset of the Jena Experiment.

Before the start of the experiment, the soil of the field site was surveyed (Roscher et al. 2004). The soil of the experimental site is an Eutric Fluvisol developed from up to 2 m-thick loamy fluvial sediments that are almost free of stones. Surface stone cover (0–23%), sand content (45–628 g kg^{-1}), and $CaCO_3$ concentrations (40–391 g kg^{-1}) varied considerably in the field site (coefficient of variation, CV >65%) whereas the variation in pH (7.1–8.4) was smaller, as was the variation in C_{org} (5–33 g C kg^{-1}), N_{tot} concentrations (1.0–2.7 g N kg^{-1}), and $\delta^{13}C$ of the organic matter (−27 to −26‰, CV <21%). The high variation in stone cover was attributable to a small area with high stone cover in the south-eastern corner of the field. As expected for a lowland river floodplain setting, sand content correlated with distance from the Saale river (r = 0.95). Close to the river, the topsoil consisted of sandy loam, and gradually changed into a silt loam with increasing distance to the river. For the selection of the experimental plots, the areas with the highest sand contents (and lowest carbonate concentrations), and the small area with high stone coverage were excluded. To account for the spatial variation in soil properties, a block design was used (see below, Roscher et al. 2004).

The target plant community of the experiment was a semi-natural species-rich mesophilic grassland (Molinio-Arrhenatheretea meadows, Arrhenatherion community, Ellenberg 1996), typical for fertile floodplain sites of the study region. Traditionally, these grasslands are managed as hay meadows mown two or three times a year. The selection of a species pool was based on data bases of the German flora, existing phytosociological studies in the Jena alluvial plain, and knowledge of scientists on the regional grassland vegetation. The 60 plant species selected for the experiment are all able to grow well under the conditions of the field site, although they may not all occur together in a semi-natural grassland (Table 1).

The plant species were divided into four functional groups based on a classification of 18 functional traits, with the constraint that more than four functional groups would be difficult to handle in an experimental design requiring large plots, and that each functional group should comprise a

Table 1. Plant species used in the Jena Experiment. Nomenclature follows Jäger (2001).

Grasses	
Alopecurus pratensis L. (Poaceae)	*Festuca rubra* L. (Poaceae)
Anthoxanthum odoratum L. (Poaceae)	*Helicototrichon pubescens* (Huds.) Pilg. (Poaceae)
Arrhenatherum elatius (L.) J. Presl et C. Presl (Poaceae)	*Holcus lanatus* L. (Poaceae)
Bromus erectus Huds. (Poaceae)	*Luzula campestris* (L.) DC. (Juncaceae)
Bromus hordeaceus L. (Poaceae)	*Phleum pratense* L. (Poaceae)
Cynosurus cristatus L. (Poaceae)	*Poa pratensis* L. (Poaceae)
Dactylis glomerata L. (Poaceae)	*Poa trivialis* L. (Poaceae)
Festuca pratensis Huds. (Poaceae)	*Trisetum flavescens* (L.) P. Beauv. (Poaceae)
Small herbs	
Ajuga reptans L. (Lamiaceae)	*Primula veris* L. (Primulaceae)
Bellis perennis L. (Asteraceae)	*Prunella vulgaris* L. (Lamiaceae)
Glechoma hederacea L. (Lamiaceae)	*Ranunculus repens* L. (Ranunculaceae)
Leontodon hispidus L. (Asteraceae)	*Taraxacum* Sect. *ruderalia* Kirschner et al. (Asteraceae)
Plantago lanceolata L. (Plantaginaceae)	*Veronica chamaedrys* L. (Plantaginaceae)
Plantago media L. (Plantaginaceae)	*Scorzoneroides autumnalis* (L.) Moench (Asteraceae)
Tall herbs	
Achillea millefolium L. (Asteraceae)	*Geranium pratense* L. (Geraniaceae)
Anthriscus sylvestris (L.) Hoffm. (Apiaceae)	*Heracleum sphondylium* L. (Apiaceae)
Campanula patula L. (Campanulaceae)	*Knautia arvensis* (L.) J.M. Coult. (Dipsacaceae)
Cardamine pratensis L. (Brassicaceae)	*Leucanthemum vulgare* (Vaill.) Lam. (Asteraceae)
Carum carvi L. (Apiaceae)	*Pastinaca sativa* L. (Apiaceae)
Centaurea jacea L. (Asteraceae)	*Pimpinella major* (L.) Huds. (Apiaceae)
Cirsium oleraceum (L.) Scop. (Asteraceae)	*Ranunculus acris* L. (Ranunculaceae)
Crepis biennis L. (Asteraceae)	*Rumex acetosa* L. (Polygonaceae)
Daucus carota L. (Apiaceae)	*Sanguisorba officinalis* L. (Rosaceae)
Galium album Mill. (Rubiaceae)	*Tragopogon pratensis* L. (Asteraceae)
Legumes	
Lathyrus pratensis L. (Fabaceae)	*Trifolium dubium* Sibth. (Fabaceae)
Lotus corniculatus L. (Fabaceae)	*Trifolium fragiferum* L. (Fabaceae)
Medicago lupulina L. (Fabaceae)	*Trifolium hybridum* L. (Fabaceae)
Medicago × varia Martyn (Fabaceae)	*Trifolium pratense* L. (Fabaceae)
Onobrychis viciifolia Scop. (Fabaceae)	*Trifolium repens* L. (Fabaceae)
Trifolium campestre Schreb. (Fabaceae)	*Vicia cracca* L. (Fabaceae)

similar number of species. Three groups of ecologically relevant attributes were distinguished (Roscher et al. 2004):

1. above- and belowground morphological traits that describe plant architecture and define space occupancy;
2. phenological traits that describe plant development and define temporal presence, e.g. the occupancy of seasonal niches by species;
3. the capacity for N_2-fixation by legumes as an important physiological trait (based on experiences of earlier biodiversity experiments).

The application of multivariate cluster analysis and ordination techniques resulted in the following four functional groups (Table 1, Roscher et al. 2004):

Species group 1 (‘*grasses*’, *16 species*): The group contains all species of the Poaceae and *Luzula campestris* (Juncaceae). Common traits are a perennial life cycle (exception *Bromus hordeaceus*), a short-lived primary root system, the prevailing ‘caespitose’ growth and overwintering green leaves.

Species group 2 (‘*small herbs*’, *12 species*): The second species group subsumes herbs of small stature. Besides their low canopy height, all species show the same seasonality of foliage (overwintering green, exception *Leontodon hispidus*) and have a perennial life cycle.

Species group 3 (‘*tall herbs*’, *20 species*): The third species group consists of herbs with medium or tall stature (canopy height of vegetative and flowering plants). Their growth form according to Ellenberg and Mueller-Dombois (1967) is predominantly ‘semirosulata’, but the species are rather variable with regard to other traits.

Species group 4 (‘*legumes*’, *12 species*): All legumes were forced into one group by the double weighting of the legume/nonlegume trait. Therefore they differ markedly with respect to the other characters. Because there is abundant evidence that legumes have disproportionate effects on ecosystem variables (Spehn et al. 2002), it is useful to consider this group of species separately.

Table 2. Species richness and functional group richness of mixtures in the main experiment.

	Species number																								
Functional Group	1	1	1	1	2	2	2	2	2	2	2	2	4	4	4	4	4	4	4	4	4	4	4	4	4
Grasses	1				2				1		1		4				2		2		2	1	1		1
Small herbs		1				2			1			1		4			2			2	1		1	2	1
Tall herbs			1				2			1	1				4			2	2		1	2		1	1
Legumes				1				2		1		1				4		2		2		1	2	1	1
Number of replicates	4	4	4	4	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	4

	Species number																							
Functional Group	8	8	8	8	8	8	8	8	8	8	8	8	8	16	16	16	16	16	16	16	16	16	16	16
Grasses	8				4		4		2	3	3		2	16		8		8		5	5	6		4
Small herbs		8			4			4	3		3	2	2			8			8	5		5	6	4
Tall herbs			8			4	4		3	2		3	2		16		8	8		6	5		5	4
Legumes				8		4		4		3	2	3	2				8		8		6	5	5	4
Number of replicates	1	1	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	4

The main experiment

In the main experiment that was established 2002 and where most research has been conducted, plant species number and the number of functional groups in the species mixtures were varied as independently as possible. Species number increased on a logarithmic scale with levels 1, 2, 4, 8, and 16 species (and 60-species controls, see below). The five levels of species richness were near-orthogonally crossed along a gradient in plant functional group richness (1–4), with the restriction that functional group number cannot exceed species number (Table 2). For the species richness levels 1, 2, 4, 8, 16, all possible combinations 'Species number × Number of functional groups' were present in the design four times. Exceptions were the 16-species mixtures with only one functional group (low number of species in legumes and small herbs Table 1), and the monocultures and 2-species mixtures, where the number of replications (plots) was increased to account for expected higher variability at low diversity levels. All diversity levels were thus represented in 16 plots except for the 16-species mixture (14 plots, Table 2). The restriction to 16 instead of all 60 monocultures was due to the logistic constraints of maintaining the large plots. Random allocation of species to the experimental mixtures including monocultures was restricted by the requirement that each functional group should be equally represented at every species richness level. Species for each particular mixture were chosen randomly (with replacement) from the species pool. Thus, there were 78 plots in total for the diversity levels 1, 2, 4, 8, and 16 species (Table 2). In addition, there were four plots with 60 species (i.e. the full species pool) so that the total number of species mixtures in the main experiment was 82.

The species mixtures were complemented by a number of treatments that served as comparisons: bare ground (4 plots), succession with mowing (2), and succession without mowing (2) (total 8 plots). In addition, there were control plots with, C4 plants only (2), arable fields with conventional farming and rotation, managed by an agricultural cooperative (2) and semi-natural grasslands adjacent to the field site (2) (Fig. 3). The total number of large plots is thus 82 plus $8 + 6 = 14$ additional plots.

A main aim when setting up the Jena Experiment was to enable several research groups to study ecosystem variables on the same plots ('all measurements on all plots') and over a time-scale of more than two years. Thus, the plots needed to be large enough to allow long-term observations. In 2002 plant mixtures were established on plots with a size of 20×20 m (Fig. 2A). The large plot size allowed us to continue the experiment until today. Plot size was reduced to 6×5.5 m in spring 2010 to free space and working time for new experiments (Fig. 2B). In 2012, plot size was extended by reinstating weeding on some former invasion subplots (Fig. 2B).

Replication of the main experiment in smaller plots

In an additional experiment, also set up in 2002, all diversity mixtures from the main experiment were identically replicated in 3.5×3.5 m plots (total 82 plots), to test for the effect of plot size on the results obtained. This experiment also served to assess within-mixture variability and its dependence on diversity (Roscher et al. 2004). In a split-plot design, these replicates were also used to test for the effects of increased sowing density and evenness on the species richness-biomass relationship (Schmitz et al. 2013). The small replicates of the main experiments were given up in 2008.

Monocultures in small plots

Monocultures served as a basis for calculations of overyielding and any further comparisons of the performance of plant species and their interactions with other organisms

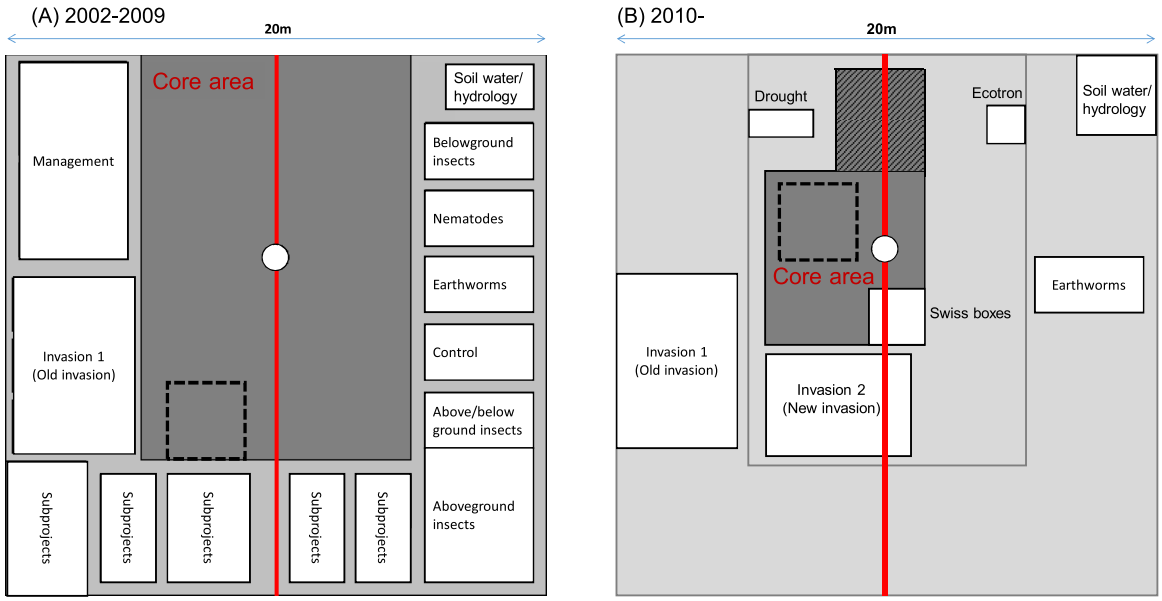

Fig. 2. Layout of a 20 × 20 m plot of the main experiment. (A) Plot outline in 2002: the central core area (dark grey) was left untouched for the first years except for plant biomass harvest immediately before the mowing event and became the plot area for later phases. The area indicated by the broken borders was used for plant cover estimates. To avoid mutual interference between researchers, subprojects of the Jena Experiment carried out their research in allocated subplots. In addition, there were a number of split-plot experiments, e.g. the *Management experiment* (2006–2010) with four levels of fertilization and mowing. The *Drought experiment* was nested within the management experiment (see text). *Invasion 1 (Old invasion)*: various invasion experiments in smaller subsubplots where weeding and seed addition was manipulated. In a number of fauna manipulations, the presence (or density in the case of earthworms) of *Aboveground insects*, *Belowground insects*, both groups, *Nematodes*, *Earthworms* and *Molluscs* (not shown) was manipulated. One *Control* subplot served for comparisons. (B) Plot outline in 2010 after plot reduction to 6 × 5.5 m. The *Soil water/hydrology* (with e.g. suction cup and tensiometer installations) and some manipulated subplots (*Drought*, *Earthworms*) were maintained, i.e. still weeded. The *Invasion 2 (New invasion)* subplot was not weeded since 2009 (see main text). The remainder of the old plot area was mown frequently, at the same time as the paths between plots. All corners are colour-coded in the field to allow for easier orientation. In 2012, the Earthworm plots were given up and monoliths were removed for the Ecotron experiment and the holes filled with local soil. From 2011 onwards, a former Succession subplot (hatched area) next to the drought experiment was weeded again, extending plot size. The red lines represent the location of the electricity/data cables belowground. The open circle represents the local meteorology station with e.g. temperature and relative humidity sensors in various heights. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

between different diversity levels. Because the main experiments only contained monocultures of 16 species due to logistic constraints, all 60 species included in the Jena Experiment were sown in monocultures in 3.5 × 3.5 m plots with two replicates per species (total 120 plots). The monocultures were used for calculations of overyielding (e.g. Roscher et al. 2005) for trait measurements, and for life-history studies (e.g. Heisse, Roscher, Schumacher, & Schulze 2007; Roscher, Scherer-Lorenzen et al. 2011). One plot per species was given up in 2007, and the second was reduced to 1 × 1 m weeded area in spring 2009, to reduce the weeding effort.

In 2010, new monocultures were established for all species, for additional trait measurements, as in several of the original monocultures cover had become very low (see Section "Weeding issues and monoculture performance" on monoculture performance).

In spring 2005, a common garden was established at the field site, where 5–10 individuals of each experimental species, pre-grown in a greenhouse, were planted in a seed bed. This common garden was used for trait measurements and determination of plant biomass production of individuals growing without competition. The common garden was maintained until autumn 2007.

The dominance experiment

This experiment on 3.5 × 3.5 m plots was set up in 2002, to disentangle the effects of species richness *per se* from the presence/absence of particular dominant species or particular pairs of dominant species. Studying the effects of species along the diversity gradient, and of particular plant-plant interactions, was not possible in the main experiment, as in the main experiment each plant species only occurred in a small subset of plots, a consequence of the large species pool (the most frequent species was the small herb *Plantago lanceolata* which occurred in 18 large plots). Using dominance of species in semi-natural grasslands in the vicinity of the Jena Experiment as a selection criterion, the nine species of the dominance experiment were: the herbs *Anthriscus sylvestris* and *Geranium pratense*, the legumes *Trifolium pratense* and *Trifolium repens*, and the grasses *Alopecurus pratensis*,

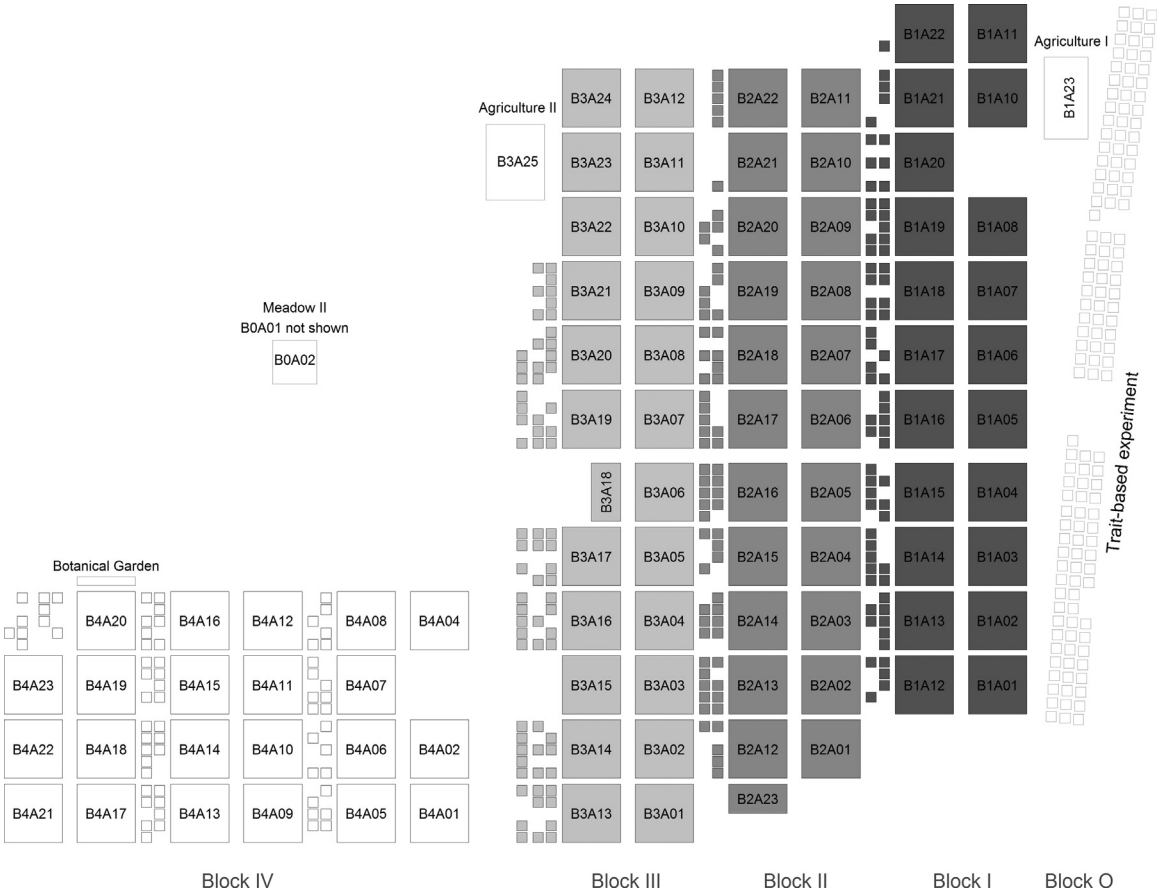

Fig. 3. Layout of the field site in 2015. The Saale river is East of the field site i.e. to the right. The coordinates were fixed at the beginning of the experiment, and to store sampling location precisely in the database. Plots are arranged in four blocks. The figure shows the locations of plots; large plots were 20 × 20 m. Small plots (originally 3.5 × 3.5 m) were placed between rows of large plots. Small plots that were given up, e.g. the second replicate of the original monocultures, are not shown. The trait-based experiment (TBE, Ebeling, Pompe et al., 2014) is a separate experiment almost parallel to the Saale river. B1A23 and B3A25 are agricultural control plots with a crop rotation. B2A23 and B3A18 are half-plots used to grow C4-plants. The Botanical Garden holds individuals of all species which are used to train student helpers for weeding. B0A01 (not shown) and B0A02 are plots placed in adjacent managed grasslands.

Arrhenatherum elatius, *Dactylis glomerata*, *Phleum pratense* and *Poa trivialis*. Emphasis on detecting effects of individual species was reflected in a different experimental design. Species richness levels varied from 1, 2, 3, 4, 6, to 9, and each species occurred in eight mixtures at every diversity level. Additionally, each possible two-species combination appeared equally often at each diversity level, and each species also occurred in duplicated monocultures. All species mixtures were replicated once (i.e., two plots) with the exception of the 9-species mixture that was replicated eight times (Roscher et al. 2004). In total, there were 188 plots for the 2- to 9-species mixtures in dominance experiment (2 × 36 plots = 72 plots for 2-species mixtures, 2 × 24 plots = 48 plots for 3-species combinations, 2 × 18 plots = 36 plots for 4-species combinations, 2 × 12 plots = 24 plots for 6-species combinations, and 8 plots with the 9-species mixture), plus 9 × 2 plots = 18 plots monocultures, which were also part of the monoculture experiment. The 206 plots were reduced in size to 2.5 × 2.5 m in 2008 and to 1 × 1 m in 2010.

Blocking and soil samples

Because of the gradient in soil parameters perpendicular to the Saale river (e.g. sand, silt and clay content, see below), the experiments (main experiment, dominance experiment, small replicates of the main experiment, monocultures) were set up in four blocks. For the main experiment each block contained an equal number of plots per species richness × functional group number combinations, corresponding to a randomized block design (Fig. 3). The soil samples that were taken from every plot of the main experiment also served as covariates in analyses of the influence of soil parameters on ecosystem variables (Huston & McBride 2002).

Split-plot treatments in the main experiment

The plots of the main experiment were large enough to establish split-plot experiments to test particular questions. This possibility was used since the beginning of the Jena

Experiment. *Invasion experiments* tested the resistance of the plant communities against plant invasion (e.g. Roscher, Beßler et al. 2009; Roscher, Schmid, & Schulze 2009). A split-split plot experiment with various genotypes of *Lolium perenne* was set up to test for interactions of within-plant genetic diversity with plant species richness (Roscher, Schumacher, Weisser, & Schulze 2008; Nestmann et al. 2011).

In manipulations of above- and belowground fauna, the densities of particular consumer groups were reduced on subplots. These manipulations were designed to investigate how these consumer groups affect the relationship between plant species richness and ecosystem variables, such as productivity and decomposition. The split-plot manipulations included the manipulation of the presence of aboveground insect herbivores, belowground insects, molluscs and nematodes, and combinations of these manipulations, using targeted insecticides/molluscides as well as manipulation of earthworm densities (Eisenhauer, Milcu, Allan et al. 2011).

To investigate how biodiversity may mitigate effects of climate change, a split-plot was established, to test for the resistance and resilience of the plant communities against drought (*Drought experiment*, Vogel, Scherer-Lorenzen, & Weigelt 2012). In an additional experiment, the effects of land-use intensification on the biodiversity-productivity relationship was tested (*Management experiment*, Weigelt, Weisser, Buchmann, & Scherer-Lorenzen 2009). These experiments were also used to test for the interaction between management and drought (Vogel et al. 2012).

All these experiments are described in more detail below.

Plant invasion experiments

Different invasion experiments were conducted in the area of the original 20 × 20 m plots of the main experiment for various lengths of time. These experiments were established in all weeded plots of the main experiment, but studies of community assembly also included the four unweeded control plots (succession with mowing; succession without mowing; Roscher et al. 2016). The experiments were placed in two larger invasion areas in each plot, the so-called '*old*' and '*new*' invasion subplots (Fig. 2). The *old invasion* subplot had an overall area of 6.5 × 4.5 m, subdivided into six subplots of 2 × 2.25 m with differing weeding history. Two subplots and thus a total area of 2 × 4.5 m were never weeded since the establishment of the main plots in 2002 ('s' — subplots in Roscher, Schumacher, Gerighausen, & Schmid 2014). In 2004, weeding was stopped in two additional and adjacent subplots of in total 2 × 4.5 m size ('c' — subplots) and in 2008, weeding was stopped in a third pair of subplots ('w' — subplots). In 2005, viable seeds of all 60 species of the experimental species pool were added to one of the two subplots of the three above-mentioned pairs with different weeding history ('s+', 'c+', 'w+'), while the second subplots served as control without seed addition ('s−', 'c−', 'w−'). The number of added seeds was 1000 viable seeds per m^2 distributed equally among the added 60 species (Roscher, Schmid et al. 2009). Between 2005 and 2007, all species not belonging to the initially sown species combinations were regularly weeded in the 'w−' subplots, while all species not belonging to the sown species pool were weeded in the 'w+' subplots. From 2008 onwards the whole area of the old invasion experiment was not weeded anymore.

The *new invasion* experiment was established in 2009 with an area of 5 × 3 m. From 2002 until June 2009 this area was part of the core area of the main plots and thus regularly weeded twice a year. In July 2009, weeding was stopped on this subplot of the main experiment.

In addition, 31 monocultures of common invasive species were established in 2010 on small 3.5 × 3.5 m plots for analyses of overyielding in the invasion experiment. These monocultures were regularly weeded.

Thus, the invasion experiments included treatments where weeding or not weeding was practiced for different durations, and treatments with and without seed addition.

Community history experiment ('Swiss boxes')

In 2011 a *community history* experiment was started in 48 experimental plant communities (twelve monocultures, twelve two-species mixtures, twelve four-species mixtures and twelve eight-species mixtures), to test if evolutionary processes during the first eight years of the Jena Experiment had led to measurable evolutionary changes in the plant communities (van Moorsel, Schmid, Hahl, Zuppinger-Dingley, & Schmid 2017). Two community selection treatments were used: plants with eight years of shared community selection in the Jena Experiment (selected communities) and plants without a common selection history in the Jena Experiment (unselected communities). Seeds of selected communities were produced in an experimental garden in Zurich, Switzerland, from cuttings that had been made in the Jena Experiment and were then planted in the original species combination in fenced plots in Zurich to reduce pollination between communities. A small number was additionally collected directly in the plots of the Jena Experiment. All these seeds were thus offspring of plant populations that had been sown in 2002 and grown until 2010 in plots of the Jena Experiment. Seeds of unselected communities were produced by the original suppliers of seeds for the Jena Experiment (Rieger Hoffmann GmbH, Germany). This supplier collected plants of the different species at field sites in Germany and propagated them for at least five years in monoculture.

The seeds of selected and unselected communities germinated and were transplanted to 2 × 2 m subplots in March 2011. There were four 1 × 1 m quadrats with different soil types in each subplot ('*Swiss boxes*', Fig. 2). The original plant cover was removed in September 2010 and the soil was excavated to a depth of 0.35 m and sieved. Half of the soil was gamma-sterilized to remove the original soil community, and the other half was not sterilized. Of the sterilized soil, half was inoculated with 4% (by weight) of live original soil of the corresponding plot and 4% and of live sugar-beet soil ('native

soil' obtained by inoculation). The other half of the sterilized soil was inoculated with 4% (by weight) of live sugar-beet soil and 4% of sterilized original soil of the corresponding plot. The other half of the original soil was not sterilized and was used for the other two soil treatments. Half of this soil was filled back into one quadrat of the corresponding plot ('native soil'). The other half of the unsterilized soil was mixed among all 48 plots and filled into the remaining quadrats. The borders of the quadrats and the subplot were separated by plastic sheets. Each 1 × 1 m quadrat was split into two 1 × 0.5 m halves. Seedlings of selected communities were transplanted into one half of each quadrat, and seedlings of unselected communities into the other half. Plant communities in the Swiss boxes were weeded three times a year and 'mown' by cutting the plants to 3 cm aboveground twice a year at the same time the other experiments were mown.

Plant genetic diversity manipulations

This experiment was set up with 15 genetically different cultivars of *L. perenne* L. (Poaceae), a common grassland species not included in the experimental species pool, in all plots of the main experiment. This split-split-plot experiment was used to study how genetically different lines of a species vary in their response to plant species richness (Roscher, Schumacher et al. 2008) and whether plant species richness affects genetic differentiation and diversity (Nestmann et al. 2011).

Fauna manipulations

Invertebrates influence a wide range of ecosystem variables and themselves react to the manipulation of plant species richness (Scherber, Eisenhauer et al. 2010). To experimentally investigate how different invertebrate groups affect (a) plant community development, (b) individual plant performance, and (c) the relationship between plant species richness and plant biomass, the presence of invertebrate groups was manipulated using pesticides. Five groups of invertebrates were excluded from subplots within plots of the main experiment (Eisenhauer, Milcu, Allan et al. 2011):

a) molluscs (2005–2009);
b) aboveground insects (2003–2009);
c) belowground insects (2003–2009);
d) earthworms (2003–2011);
e) nematodes (2005–2008).

In some of the subplots, two taxa were excluded in combination, to test for interactive effects on ecosystem variables. For example, above- and belowground insects were manipulated individually, but were both excluded from one additional subplot. The same was true for aboveground insects and molluscs. All subplots were located outside the original central core area of each plot and the position of the subplots was randomized between plots (Fig. 2).

Molluscs were excluded on a 2 × 2 m subplot by application of slug pellets (active substance: 4% metaldehyde ($C_8H_{16}O_4$); Spiess-Urania, Hamburg, Germany) at a dose of 0.9 g m^{-2} and at monthly intervals between April and September (approximately 0.18 g active ingredient (a.i.) m^{-2} with 5 applications per year).

Belowground insects were excluded on a 2 × 5 m subplot by application of the insecticide 'Hortex' (active substance: 2% chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$); Celaflor, Dow AgroSciences LCC, USA) as an aqueous solution (40.188 g/l^{-1} water) at a dose of 125 ml m^{-2} and at monthly intervals between April and November. Application was scheduled prior to forecast precipitation events in order to increase incorporation into the soil (approximately 0.7 g a.i. m^{-2} with 7 applications per year).

Aboveground insects were excluded on a 5 × 5 m subplot by application of the insecticide 'Perfekthion' (active substance: 40% dimethoate ($C_5H_{12}NO_3PS_2$); BASF, Ludwigshafen, Germany) as an aqueous solution (0.1%) at a dose of ca. 30 ml m^{-2} (ca. 50 ml m^{-2} in 2008) and at monthly intervals between April and September (approximately 0.061 g a.i. m^{-2}, with 5 applications per year).

Interactions between *aboveground insects* and *belowground insects* and between *molluscs and aboveground insects* were investigated in two additional subplots. The aboveground insects × mollusc exclusion subplot (2 × 2 m) and the aboveground insects × belowground insects exclusion subplot (2 × 5 m) were both treated at the same doses and intervals as subplots with exclusion of a single group (see above). An untreated subplot (2 × 5 m) served as control for all treatments where no water was added due to the small amounts of water used in the pesticide treatments.

Nematode densities were manipulated on all experimental plots starting in 2005. On each plot two randomly selected subplots of 1 × 1 m were used to establish a 'nematicide' and a 'control' treatment. The widely used nematicide fosthiazate was applied to nematicide subplots as a granulate (Nemathorin, Syngenta Agro GmbH, Maintal, Germany) three times a year using a sieve. To facilitate homogeneous application the nematicide was mixed with soil from the Jena Experiment field site (10% w/w fosthiazate, 3 g/m^2 mixed with 97 g Jena soil). The control subplots received 100 g Jena soil per application to control for the addition of soil (Eisenhauer, Ackermann et al. 2010).

Earthworm densities were manipulated on the 1- (16 plots), 4- (16) and 16-plant species plots (14) starting September 2003. On each plot, two randomly selected subplots of 1 × 1 m were used to initially establish 'earthworm' and 'earthworm reduction' treatments. Subplots were enclosed with PVC shields aboveground (20 cm) and belowground (15 cm) to reduce colonization by earthworms. In the first three years of the experiment, 'earthworm' subplots received 25 adult individuals of *Lumbricus terrestris* L. (average fresh weight with gut content 4.10 ± 0.61 g (mean ± s.e.)) per year (15 individuals in spring and 10 in autumn) as earthworm density was low after establishment of the Jena Experiment. Earthworm addition was stopped in 2006 as colonization of the field by earthworms had reached

equilibrium level, as indicated by similar earthworm densities in control and earthworm addition subplots (Eisenhauer, Milcu, Sabais, & Scheu 2008). To reduce earthworm density in 'earthworm reduction' subplots, earthworms were extracted twice a year (spring and autumn) by electro-shocking (for details, see Eisenhauer, Milcu, Nitschke et al. 2009). The success of earthworm density manipulations was proven by measuring the soil surface activity of *L. terrestris*, which was significantly lower (38%) in the earthworm reduction treatment than in the earthworm treatment (Eisenhauer et al. 2008).

Management experiment

The standard management in the Jena Experiment was two cuts per year with biomass removal and no fertilisation. To test if the results obtained under this type of management, which is less intensive than in many grasslands in the area that are managed for high productivity, could be extrapolated to grasslands managed more intensively, a gradient in management intensity was established in 80 plots of the main experiment (the two monocultures of *Bellis perennis* and *Cynosurus cristatus* were not used due to very low cover (<10%)). Five subplots were established and management varied in mowing regime (one (M1), two (M2) or four (M4) cuts per year) and the amount of NPK-fertilizer application (no fertilizer (F0); 100 (F100) or 200 (F200) kg N ha^{-1} yr^{-1}) and similar increases in P and K additions (Weigelt et al. 2009). Mowing and fertilisation were combined as follows to yield five management treatments: M1F0, M2F0, M2F100, M4F100, M4F200. All three fertilizer treatments were arranged randomly on an area of each 1.6 m × 4 m within the main plots (20 × 20 m), while the M2F0 treatment was always located in the central core area of the plots, representing the standard management of the Jena Experiment. The M1F0 subplots was always placed at the plot margins due to logistical constraints. Fertilization was done twice a year (31 March and 23 June 2008, 31 March and 16 June 2009) and mowing took place in spring (end of April, only M4- subplots), in early summer (beginning of June, all subplots), end of July (M4-subplots) and in late summer at the beginning of September (all subplots). The management experiment was stopped after the growing season of 2009.

Drought experiment and management × drought interaction

Prolonged periods of summer drought is one of the consequences of global climatic change and the question how biodiversity mitigates effects of such climatic extremes is an important question in global change research (e.g., Isbell, Craven et al. 2015). The effect of plant species richness on drought resistance was tested in an experiment where drought was artificially induced (Vogel et al. 2012). The *drought experiment* was set up in 2008 using transparent rain shelters. Every year subplots (1.6 × 2 m) were shielded from rain for six weeks, before the second cut of the plots.

Rain shelters were made of LDPE greenhouse film (www.dm-folien.com) in 2008 and of PVC sheets (www.paruschkekunststoffe.de, product code: PVCSPK7018K10) since 2009 because of its higher durability. Rain shelters were inclined at a height of 1.3–1.5 m to enable ventilation and runoff of rain water in one direction 1 m away from the core area. Control subplots were part of the core area of the main experiment. They remained unsheltered and therefore received ambient precipitation.

When the drought experiment was started, there was strong interest in the interaction between drought resistance and land use intensification. Thus, one sheltered and one unsheltered subplot was established for each management treatment in each of the 80 plots covering the whole diversity gradient. In each subplot a central area of 1 × 1 m was marked for measurements.

After the management experiment was stopped in 2009, only the drought treatment in the M2F0 treatment (i.e. the standard management of the Jena Experiment) was kept and maintained. This area was enlarged in 2009 to establish an additional roof control in all plots, e.g. a sheltered subplot where collected rain water was added after each rain event, to separate the pure roof effect (heat, altered light conditions) from effects of reduced water availability on our response variables (Vogel et al. 2012). Thus, starting in 2009, the drought experiment consisted of three plots of 1 × 1 m size in each plot of the main experiment: (i) one marked plot in the core area of the main experiment under ambient conditions (ambient), (ii) one plot below the rain shelter without addition of rain water (drought) and (iii) one plot below the rain shelter with addition of rain water (roof control). The current rain shelters are 2.6 × 3.0 m in size to avoid edge effects below the roofs.

Trait-based experiment (TBE)

In 2010, a new diversity experiment, the *Trait-based experiment (TBE)*, was initiated, where plant community selection was based on plant traits. This experiment addressed the question whether functional diversity — measured as variation in trait values — is underlying the functioning of communities. In the TBE plant communities were established with different *a priori* functional diversity (Ebeling, Pompe et al. 2014). The experiment maximizes the coverage of trait space by a community that is predicted to lead to higher ecosystem functioning (Dimitrakopoulos & Schmid 2004). This predictive approach was possible because a large number of plant traits had been measured in the Jena Experiment and allowed for hypothesis-driven biodiversity–ecosystem functioning research. A full description of the design is given in Ebeling, Pompe et al. (2014).

In summary, 480 plots (90 plots for the main experiment, 82 plots for the small replicates of the main experiment, 120 monocultures, 188 mixtures for the dominance experiment) were established in 2002. Through the various amendments,

Table 3. Numbers of plots/subplots in the Jena Experiment at the end of 2016. Note that in the main experiment the two monocultures of *Bellis perennis* and *Cynosurus cristatus* were given up due to very low cover (<10%). Numbers in bold for plot/subplot sizes give the overall plot-area for each single experiment. Number in bold in the plot number column give the number of plots, numbers in standard fonts are subplot numbers in the main experiment.

Experiment/plot/subplot	Plot/subplot size	Number of plots (in bold), subplots
Main experiment (2002-today)	**104.75 m^2** (originally 400 m^2)	**80 + 8 + 6 = 14 controls** (originally 82 + 14)
Core area	6 × 5.5 m + 3 × 3.5 m = 43.5 m^2 (originally 10 × 15 m = 150 m^2)	88
Soil science corner	3 × 3 m = 9 m^2	88
Drought experiment	1 × 1 m = 1 m^2 (×2)	80 × 2 = 160
Old invasion	2.25 × 2 m = 4.5 m^2 (×6)	86 × 6 = 516
New invasion	5 × 3 m = 15 m^2	82
Community history experiment ('Swiss boxes')	1 × 1 m = 1 m^2 (×4)	52 × 4 = 208
Management experiment (2006–2009)	1.6 × 4 m = 6.4 m^2 (×4)	0 (originally 80 × 4 = 320)
Small replicates of the main experiment (2002–2010)	3.5 × 3.5 m = **12.25 m^2**	**0** (originally 82 + 12 controls, e.g. succession)
Dominance experiment (2002-today)	1 × 1 m = **1 m^2** (originally 3.5 × 3.5 m)	**188** (2- to 9-species mixtures)
Monocultures 2002 (2002-today)	1 × 1 m = **1 m^2** (originally 3.5 × 3.5 m)	**60** (originally 2 × 60 = 120)
Monocultures 2010 (2010-today)	1 × 1 m = **1 m^2**	**60**
Monocultures invaders 2010 (2010-2014)	1 × 1 m = **1 m^2**	**0** (originally 31)
Trait-based experiment (2010-today)	3.5 × 3.5 m = **12.25 m^2**	**138**
Total plots		**534**

the number increased to 534 plots in 2016, with more than 1100 subplots as part of the main experiment (Table 3).

Plot establishment and maintenance

The field was ploughed after the last harvest in autumn 2000 and kept fallow throughout 2001. In order to reduce the weed pressure the field was harrowed bimonthly (June, August, October) and treated with glyphosate (*N*-(Phosphonomethyl)-glycine, Roundup) in July 2001. In spring 2002, the experimental area was harrowed twice within 5 weeks before the plots were established.

Seeds were obtained from commercial suppliers. The desired seedling density was 1000 seedlings per m^2. In mixtures, species were sown at equal proportions. The number of seeds taken for each species was adjusted according to the observed germination rate during a laboratory study. Because many grassland species have dormant seeds, various pre-treatments of seeds were conducted in the laboratory to test their effects on germination rates. Scarification increased germination rates successfully in seven species, and pre-treatment with gibberellic acid improved germination in eight species. The seed material was therefore treated accordingly (see Roscher et al. 2004 for details). The species mixtures of the main experiment, the small replicates the dominance experiment and the monocultures were sown from 11 to 16 May 2002 (Fig. 1A–C).

Similar preparations of the ground were made for the establishment of plots set up after 2002, including the trait-based experiment except that the area to be sown was covered with black plastic foil for several months instead of using glyphosate.

All plots except the four non-weeded control (succession) plots of the main experiment were weeded twice a year during a 3-week interval (Fig. 1D, since 2012: three times a year) and mown twice a year (Fig. 1E). Mowing took place in early June and early September, corresponding to the usual management of extensively used hay meadows in the region. The first weeding period was in spring (April), the second after the first mowing in July, when the canopy was not fully closed and the vegetation was low enough to minimize damage. In order to reduce soil disturbance, the roots of the weeds were cut with a knife just below the soil surface and removed. To combat noxious weeds and to reduce the enormous work load of weeding, it was decided to selectively use herbicides in addition to weeding. Individual applications to creeping thistle *Cirsium arvense* (L.) Scop. (with Roundup) and sorrel (broad-leaved dock) *Rumex obtusifolius* L. and curled dock *Rumex crispus* L. (with Banvel M, Syngenta) were successful and virtually eradicated these species by 2004. To combat *Taraxacum officinale*, large individuals were individually removed using bulb trowels since 2007. In the period from 2006 to 2009, herbicide against dicotyledonous plants (Banvel M) was applied on a number of plots with grasses only. In the same period, Select 240 EC was used

to reduce invading grasses, such as *P. trivialis*, in purely herbaceous communities. From the possible plots, only a subset was chosen for each application with the herbicides, to avoid confounding effects of herbicide application with occurrences of particular functional groups. Since 2010, herbicides were not used any more with the exception of spot treatments of *C. arvense*. Starting in 2010, a third weeding campaign was organized every autumn to remove weeds germinating later in the season. All management activities were done blockwise, and the order in which the blocks were treated was rotated. Fig. 1F shows an aerial view of the field site in 2006.

Technical installations

A weather station was established on the field site in June 2002, for continuous measurements of 43 standard meteorological variables including air temperatures at various heights, relative humidity, precipitation, photosynthetic active radiation, albedo, saturation and actual water vapor pressure, wind velocity, air pressure, as well as soil temperatures and soil moisture in various depths. In all plots of the main experiment, sensors for soil temperature (-5 cm, -15 cm), and suction plates at -30 cm depths were installed in 2002, that were later complemented by additional suction plates at -20 cm depth. In addition, access pipes for soil moisture measurements were installed (Kreutziger 2006; Leimer, Kreutziger et al. 2014). Block II was designated as area of intensive measurements. Here, additional sensors (e.g. air temperature and humidity (30 cm), soil temperature (-60 cm), infrared temperature sensors Heitronics KT 15), soil moisture loggers, and additional suction plates (-10 cm, -60 cm) were installed (cf. Roscher et al. 2004).

The Jena Ecotron Experiment

In addition to field manipulations, an additional experiment was designed to take advantage of a unique controlled environment facility for ecosystem research, the CNRS European Ecotron at Montpellier (www.ecotron.cnrs.fr), to further the understanding of the role of plant species richness for carbon, nutrient and water fluxes. The macrocosms platform of the CNRS Ecotron facility was specifically designed for continuous and automatic high-frequency measurements of ecosystem-level carbon and water fluxes while allowing for the control and measurement of environmental variables and the deployment of multiple isotopic tracers (^{13}C, ^{15}N and ^{2}H) — approaches, that are challenging to set up in the field. The *Jena Ecotron Experiment* used large soil monoliths (2 m^2, diameter of 1.6 m and 2 m depth, weighing 7–8 t) that were taken from six different plots in each of two diversity levels (4 and 16 species). The monoliths were excavated from the plots, placed in lysimeters and transported to the Ecotron facility at the end of March 2012, where they were hosted in the Ecotron until the end of July, when the experiment finished with a destructive harvest (Fig. 4). During the experiment, continuous measurements of ecosystem evapotranspiration, carbon net ecosystem exchange (NEE), night-time ecosystem respiration (Reco-night) and gross primary productivity (GPP) were automatically performed at a time interval at 12 minutes (Milcu et al. 2014). Furthermore, high frequency carbon and water fluxes allowed for the modeling of day-time ecosystem respiration (Reco-day) as well as the partitioning of evapotranspiration in to evaporation and transpiration (Milcu et al. 2014, 2016). These variables permitted the estimation of water, nitrogen and light use efficiencies, three parameters of carbon uptake efficiency that are not typically measured in plant diversity experiments. In addition to the ecosystem-level carbon and water fluxes, a suite of multi-trophic response variables were measured, including but not limited to: microbial biomass C, microbial PLFAs, soil mesofauna and macrofauna, multiple plant functional traits, the incorporation of ^{13}C and ^{15}N in plants and soil fauna as well as the root water uptake patterns using ^{2}H tracing and modelling. Taken together, the Jena Ecotron Experiment allowed to answer questions raised in the field studies, in particular the importance of plant species richness and plant functional diversity for the ecosystem-level energy and mass fluxes that are underpinning primary productivity.

Statistical analyses

The Jena Experiment was primarily designed to test for the effects of plant species richness and functional group richness on a variety of ecosystem variables (biomass, soil, producers, consumers). Missing combinations of plant species number × number of functional groups (because the number of functional groups cannot exceed the number of species in a plot) means that an ANOVA Type I Sum of Squares analysis is appropriate (Schmid et al. 2002; Schmid, Baruffol, Wang, & Niklaus 2017). The general order of terms was block, species richness, functional group richness, the interaction between species richness and functional group richness and then the presence of up to three of the four functional groups, and possibly interactions. Covariates such as plant biomass were generally included before the species richness term.

Depending on the type of response variables other, more appropriate techniques were used as well (e.g. generalized linear models with Poisson error distribution for count data). Using plant species richness as a continuous variable (rather than as a factor) on arithmetic or log scale improved the explanatory power and resulted in a greater flexibility in data analysis (e.g. parameter estimates using generalized linear models (GLM) or tree models). In more recent years, analyses have been conducted using linear mixed effect models. In addition, complex interrelationships are increasingly being modelled using structural equation modelling (e.g. Scherber,

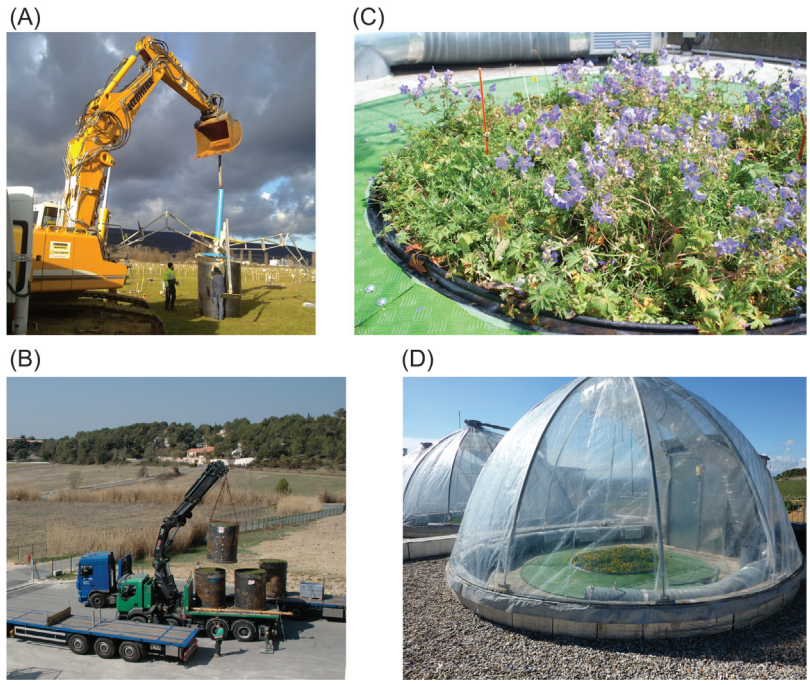

Fig. 4. The Jena Ecotron Experiment. (A) Extraction of lysimeters at the Jena Experiment site, (B) unloading of lysimeters after the transport to France, (C) close-up of a plant community after installation at the CNRS European Ecotron of Montpellier, (D) view of the macrocosms. Photos by Markus Lange (A), Jacques Roy (B), Alexandru Milcu (C, D).

Eisenhauer et al. 2010; Ebeling, Meyer et al. 2014; Fischer et al. 2014; Lange et al. 2015; Hertzog, Meyer, Weisser, & Ebeling 2016). For some analyses, the realized number of species was used, in particular when small subplots were used and when only a subset of the species was considered to affect the process investigated, as in the case of grasshopper feeding (e.g., Specht, Scherber, Unsicker, Köhler, & Weisser 2008; Ebeling et al. 2013), or in analyses of pollination (e.g. Ebeling, Klein, Schumacher, Weisser, & Tscharntke 2008). Generally, sown species richness and realized species richness were highly positively correlated.

Due to non-orthogonality, species number and number of functional group terms were not completely statistically independent. Of particular interest are the functional forms of the dependence. Linearity (log-linearity) could be tested using respective contrasts. In the presence of significant interactions between species number and number of functional groups, the functional form of the dependences was assessed separately within the levels of the respective other factor. All effects of particular functional groups or combinations of functional groups were tested in sets of orthogonal contrasts. A simulation study supported the view that the statistical power of the experiment was sufficient to detect a number of biodiversity effects (Roscher et al. 2004). For example, the experimental design had a high power to detect complementarity effects between functional groups. With an effect size of 0.3 standard deviations, which is well within expectations based on previous biodiversity experiments, the corresponding power was 80%. The targeted design and analysis was successful, as reports on the results of the Jena Experiment have not been clouded by discussions over the statistical approach used.

Plant community dynamics during the long-term experiment

Establishment of the plant communities

After sowing, all plant species established at the field site in the year of sowing, with the exception of two species that germinated later (*A. sylvestris*, *Heracleum sphondylium*), but the rates of seedling establishment varied strongly among species (Heisse et al. 2007). The following species had very low rates of establishment during the first growing season (<50 seedlings per m^2 in monoculture): *Bromus erectus*, *Campanula patula*, *Cardamine pratensis*, *Carum carvi*, *G. pratense*, *L. campestris*. Eight species which had such low germination rates and/or did not reach a cover >5% in autumn of the first growing season were re-sown in all plots in November 2002 (for details see Roscher et al. 2004). In addition, the legumes *Trifolium campestre* and *T. dubium* were re-sown with 50% density because of their obligate short life cycle (annuals). In the first year after sowing (2003), all species grew in the monocultures on small plots. In the mixtures, 46 out of the 60 species established in all large plots where they were initially sown (survey on 18 m^2 from the total of 400 m^2 plot area per large plot). Only three species occurred on less than 50% of the mixtures into which they were seeded (*C. pratensis*, *Primula veris*, *T.*

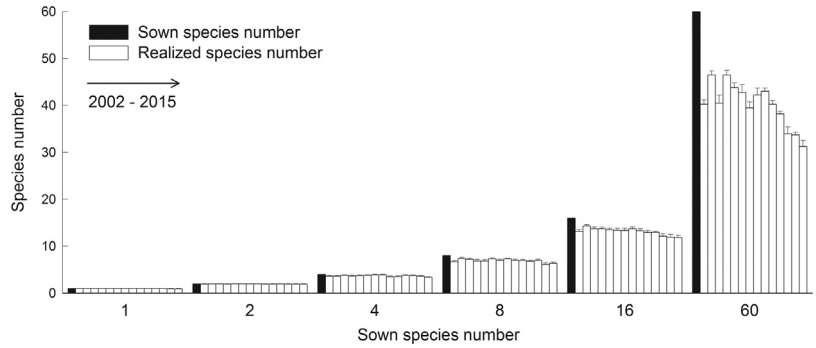

Fig. 5. Realized target species richness of the plant communities in the main experiment over time, based on cover estimates on an area of 9 m^2 in spring (May) and summer (August). Species occurrences from both inventories are summed for realized species richness. Shown are means (+1SE) per species-richness level.

campestre). One species (*L. campestris*) failed to establish completely in mixture plots during the first two years, but became established thereafter. Establishment and weeding was successful. The target plant species richness in plots was closely correlated to the sown plant species richness in all years of the experiment (Fig. 5, Marquard, Weigelt, Temperton et al. 2009).

Plant community development: the role of environmental variation, competition and historical contingency

Weeding of the plots aimed at removing species that are not part of the seeded plant community, but did not manipulate target species sown into a particular mixture. Hence target plant species composition was allowed to change over time. Individual species abundances increased or decreased over the years, or fluctuated among years. The temporal stability in plant populations based on biomass data differed greatly among species, but it was generally higher for small herbs and grasses than for tall herbs and legumes (Roscher, Weigelt et al. 2011). Fluctuations among species assigned to different plant functional groups were often asynchronous, while species dynamics within functional groups were more correlated, suggesting that functionally similar species showed similar responses to environmental variation or changes in resource availability (Roscher, Weigelt et al. 2011). Interspecific interactions between plants, foremost plant–plant competition, but also facilitation, and interactions with other organisms such as herbivores or pathogens are likely to have affected the temporal development of the plant communities, as temporal dynamics of species grown in monocultures differed from the dynamics of the same species in mixtures (Marquard et al. 2013). At the functional-group level, the abundance of legumes decreased over time, while grasses and small herbs increased in abundance (Roscher, Schumacher et al. 2013). Along with these changes in functional group proportions, abundance-weighted community means (CWM) of several traits related to plant nutrition shifted over time from high abundances of fast-growing, exploitative species in the early years, to higher abundances of slow-growing, conservative species in later years (i.e. decrease of CWM in leaf N concentrations, specific leaf area; increase in CWM in foliar $\delta^{15}N$, biomass:N ratios) (Roscher, Schumacher et al. 2013). Communities became more similar with respect to the CWMs of several growth-related traits, suggesting that environmental filtering and the exclusion of weaker competitors occurred during community assembly, which was reflected in a gradual loss of species at the highest sown species-richness levels (Fig. 5). At the same time, functional trait diversity within mixtures increased over time, suggesting that community assembly was controlled by niche differentiation and limiting similarity (Roscher, Schumacher et al. 2013). The prevalence of limiting similarity in structuring species abundance distributions was also shown by increasing phylogenetic overdispersion in abundance over time. This means that plots became dominated by increasingly distantly related species over time. Interestingly, this only occurred in communities sown with a mix of close and distant relatives and was not observed in plots with only close or only distant relatives (Allan, Jenkins et al. 2013). This might suggest that negative interactions occur between close relatives (perhaps due to sharing of specialist pathogens), while less negative or positive interactions occur between more distantly related species (perhaps due to sharing of mutualists).

Evenness increased during the first six years (2002–2007) (Fig. 6), but substantially declined in many mixtures around 2008, at a time when functional group proportions shifted in favour of small herbs and grasses, while proportions of legumes declined (Roscher, Schumacher et al. 2013). Afterwards, evenness increased again (Fig. 6).

The effect of plant species richness on community assembly was also studied in subplots where plant species composition was allowed to reassemble in subplots which were never weeded or where weeding stopped three or six years after sowing. Half of these subplots were also sown with all 60 experimental species (Invansion experiments, Roscher, Schmid et al. 2009). Opening the experimental

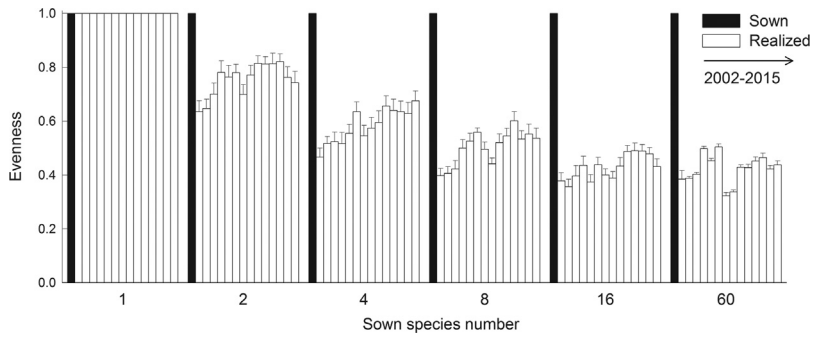

Fig. 6. Realized evenness (Simpson) in the plant communities of the main experiment, based on cover estimates on an area of 9 m^2 in spring (May) and summer (August). Evenness was averaged over spring and summer inventories. Shown are means (+1SE) per species-richness level and year.

communities to invasion led to an increase in plant species richness, functional richness and phylogenetic diversity, which was stronger in plots with seed addition (Petermann, Fergus et al. 2010; Allan, Jenkins et al. 2013; Roscher et al. 2014). Over a 5-year study period, functional evenness (i.e. the evenness of the abundance distribution of functionally different species) generally declined. Functional richness (i.e. the amount of niche space spanned out by a community) peaked two years after seed addition and then declined (Roscher et al. 2014). These results suggest that weaker competitor exclusion and limiting similarity played a role during assembly. Similar results were found for phylogenetic overdispersion in abundance, which was stronger in many communities immediately after invasion before declining (Allan, Jenkins et al. 2013). This was because close relatives could co-occur in communities but could not both reach high abundance. Because communities initially sown with a higher number of species were more resistant to invasion of new species, unweeded communities converged in species richness, functional trait composition (Petermann, Fergus et al. 2010; Roscher et al. 2014), and phylogenetic diversity (Allan, Jenkins et al. 2013) over time. However, historical contingency with regard to species composition, which remained distinct after cessation of weeding and enforced invasion through seed addition, showed that priority effects were still visible even after several years (Roscher et al. 2014). The role of priority effects was also shown by a lower gain in species and functional richness in communities with a longer colonization period before adding seeds (Roscher et al. 2014). Interestingly, the increasing species richness and high levels of functional richness resulting from invasion led to a decay in the positive diversity–productivity relationships (Roscher, Temperton, Buchmann, & Schulze 2009; Petermann, Fergus et al. 2010; Roscher et al. 2016; Steinauer et al. 2016). Instead, three years after opening communities to invasion the realized proportions of particular functional groups were more important in explaining above-average (legumes) or below-average (small herbs) community biomass production (Petermann, Fergus et al. 2010).

To summarize, while weeding ensured that only target species occurred in the plant communities, plant species composition within communities showed complex dynamics. It has been suggested that biodiversity experiments are unrealistic because they use 'immature' communities where processes such as competitive exclusion have not taken place (Thompson et al. 2005). Our results show that synthesized plant communities are clearly shaped by assembly processes that are likely to affect the interactions with organisms other than plants and hence processes such as biomass production or element cycling.

The footprint of diversity

In this section, we summarize the results of synthesis works from the Jena Experiment (e.g., Scherber, Eisenhauer et al. 2010; Allan, Weisser et al. 2013; Meyer et al. 2016) as well as from a number of individual papers that directly addressed the following fundamental questions:

1) How general is the biodiversity effect on ecosystem functioning?
2) Are there systematic differences in this effect between different categories of ecosystem variables?
3) What is the effect of biodiversity on ecosystem stability?
4) Are there delayed biodiversity effects or transients?
5) Do dominant individual plant species drive or counteract the effect of plant species richness on ecosystem variables?

Generality and variability of the biodiversity effect on ecosystem functioning

When functional biodiversity research started, the emphasis was on showing that biodiversity can affect processes at the ecosystem level, and early works focussed on studies of one or only a few variables, mostly including plant community biomass (e.g., Naeem et al. 1994; Tilman & Downing 1994; Hector et al. 1999). As the number of studies increased, more and more ecosystem variables were measured in different experiments, and first reviews appeared that summarized effects of biodiversity on ecosystem

Table 4. Ecosystem variables measured in the Jena Experiment and stored in the Jena Experiment database as of 10.2.2017. Each of the ecosystem variables was measured in each of the 82 plots of the main experiment, or in all plots of the dominance experiments, or the monocultures. Derived variables (e.g. diversity indices) are included in this list.

Category	Variables	# Variables	Examples
Plant	Plant cover	27,699	Cover of *Poa trivialis* spring 2015
	Plant biomass	16,831	Biomass of *Poa trivialis* spring 2015, sample 1
	Plant height	4,663	Height of *Poa trivialis*, spring 2015
	Plant phenology	1,509	Flowering of *Trifolium pratense*
	Plant species presence and abundance	11,130	Presence of *Poa trivialis* 2015
	Plant trait	3,887	Length of *Trifolium repens* petiole
	Plant elemental concentrations	3,300	Oxygen isotopic composition of root crown water of *Lotus corniculatus*
Consumer	Macrofauna, Mesofauna, Earthworms, Arthropods, Pollinators	1,722	Number of pollinator species 2006
	Pathogens, Fungal infection, Herbivory	3,754	Minimal percentage of downy mildew infected plant tissue in *Achillea millefolium* 2006
	Predation	981	Number of biting marks left by mammals on dummy 2014
Soil	Soil elemental concentrations, Soil temperature, Soil moisture, Soil physical properties	9,585	Bulk soil density in 2008
Other	Other variables	2,492	Forage gas production in management plots
Total		87,553	

variables in different compartments of the ecosystem (e.g., Loreau et al. 2002; Hooper et al. 2005; Balvanera et al. 2006; Cardinale et al. 2006). While reviews often made qualitative consensus statements such as 'There is now unequivocal evidence that biodiversity loss reduces the efficiency by which ecological communities capture biologically essential resources, produce biomass, decompose and recycle biologically essential nutrients' (Cardinale et al. 2012), or 'Twenty years of intense theoretical and empirical research have shown that such biotic impoverishment can markedly alter the biogeochemical and dynamic properties of ecosystems' (Naeem et al. 2012), the increased availability of data including different biodiversity experiments and a range of ecosystem variables have also led to an increasing number of studies that investigate quantitatively the magnitude of the effect of biodiversity on ecosystem functioning, using formal meta-analysis (Balvanera et al. 2006; Cardinale et al. 2006; Cardinale et al. 2012; Allan, Weisser et al. 2013). Such quantitative analyses are important to put the biodiversity effect into perspective, in particular in comparison with other drivers of ecosystem functioning. For example, focussing on productivity and decomposition, a recent meta-analysis comparing effects of species loss to other global change drivers found that 'an intermediate level of species loss (20–40%) reduced plant production by 5–10%, comparable to previously documented effects of ultraviolet radiation and climate warming' (Hooper et al. 2012). The study also found that 'Higher levels of extinction (41–60%) had effects rivalling those of ozone, acidification, elevated CO_2 and nutrient pollution', and that for decomposition, intermediate species loss had effects at least as large as those of realistic levels of elevated CO_2 and nitrogen addition.

The Jena Experiment is in the unique position to analyse the average effect of biodiversity on ecosystem functioning quantitatively, due to the large number of variables investigated. At the time of writing, more than 85,000 variables were measured in plots of the main experiment (Table 4). In an analysis comprising 418 ecosystem variables of 38 different types of ecosystem processes, 45% were found to be significantly affected by plant species richness (Allan, Weisser et al. 2013). Thus, in line with other synthesis papers, the overall result from the Jena Experiment is that biodiversity significantly influences a considerable fraction of ecosystem processes. This result also means that about half of the processes were not affected by plant species richness, at least in the years when they were investigated. Understanding this variability in the response of ecosystem variables to the manipulation in plant species richness may point to the underlying mechanisms in the effects of biodiversity on ecosystem function. Because, in contrast to other meta-analyses, all ecosystem variables in the Jena Experiment were obtained from the same plots, a more direct comparison of the strength of biodiversity effects across different functions can be performed.

In a first study, data on abundance and diversity of above- and belowground organisms, and on interspecific interactions, were summarized in a meta-analysis (Scherber, Eisenhauer et al. 2010). Abundance and diversity of both above- and belowground organisms generally responded positively to an increase in plant species richness (Fig. 7). Negative effects of increasing plant species richness

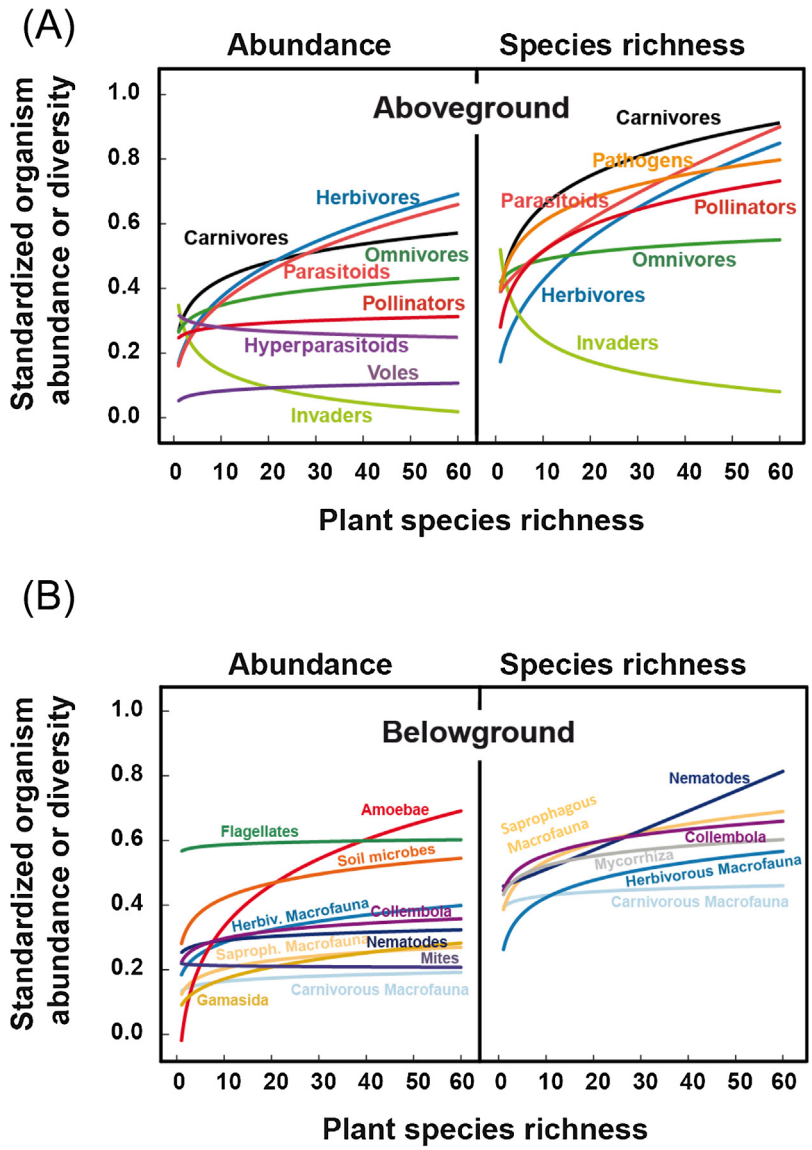

Fig. 7. Effects of plant species richness on above- and belowground organisms in temperate grassland (modified from Scherber, Eisenhauer et al. 2010). (A) Abundance and species richness of aboveground organisms. (B) Abundance and species richness of belowground organisms. All response variables scaled to [0,1]. Every curve is fitted using a power function with covariates. Identical colours in each pair of panels indicate identical groups of organisms.

were found for biological invasion, i.e. plants invading the experimental plots, for pathogen infestation, and for hyperparasitism. When the responses of above- and belowground-dwelling consumer species were classified as a function of trophic level, the responses of herbivore species richness to a loss in plant species richness was stronger than the response of carnivore and omnivore species richness (Fig. 7, Scherber, Eisenhauer et al. 2010). Independent of trophic level, aboveground organisms showed a stronger response to a loss in diversity than belowground organisms (Fig. 7). In a separate analysis using a number of consumer-mediated ecosystem variables, pathogen resistance, weed suppression, decomposition, microbial respiration, parasitism, and pollination all showed positive, saturating relationships with plant species richness, but with different slopes (Fig. 8, Scherber, Eisenhauer et al. 2010). The analysis by Scherber, Eisenhauer et al. (2010) thus provided the first comprehensive analysis of biodiversity effects on multi-trophic interactions, clearly indicating that the effects of plant species richness resonate in the entire ecological community. Importantly, effects on adjacent trophic levels were strongest, while effects on higher trophic levels were mostly indirect and propagated up the trophic levels as bottom-up effects. The results also showed that there is substantial variability among different types of ecosystem variables that needs to be explained. While experts predicted a linear or an even stronger effect of plant species richness on the processes investigated (Schläpfer, Schmid, &

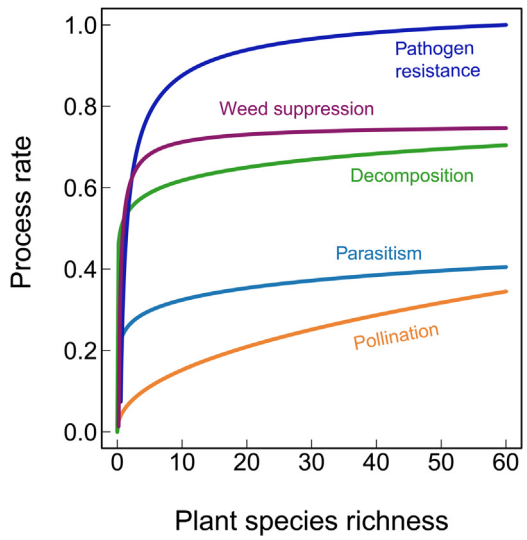

Fig. 8. Effects of plant species richness on standardized process rates of processes mediated by organism interactions (modified from Scherber, Eisenhauer et al. 2010).

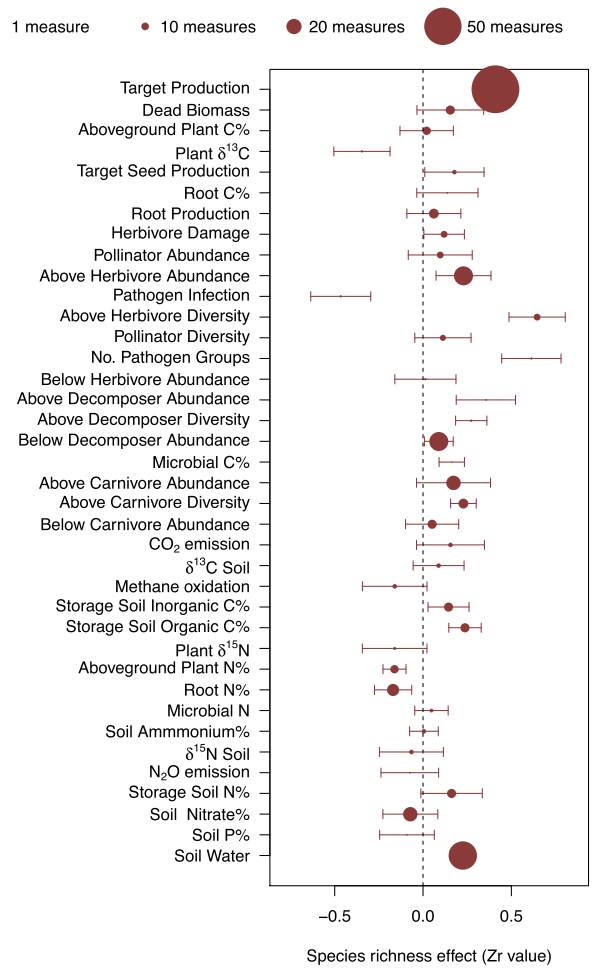

Fig. 9. Results of a meta-analysis on 418 ecosystem variables (modified from Allan, Weisser et al. 2013). All variables were grouped according to 38 ecosystem processes with which they are associated. Effect sizes, measured as Zr values, are shown for the different ecosystem processes with 95% confidence intervals: ecosystem processes whose confidence intervals do not include 0 can be considered to be significantly affected by species richness. The size of the points is scaled according to the total number of variables ('measures') taken per ecosystem process. Target production are measures of plant biomass, of species belonging to the plant species mixtures (not weeds).

Seidl 1999), many of them increased linearly with log species richness.

In a more comprehensive meta-analysis, focussing not only on organismic abundances and diversity, but including ecosystem variables from all compartments and all element cycles, a stronger effect of diversity on above- than belowground processes was confirmed (Allan, Weisser et al. 2013). In fact, the diversity effect decreased in strength with increasing soil depth, showing that it is not a simple above-/belowground contrast that is important for biodiversity effects. The analysis also found that there was great variability in the effect of plant species richness on the different ecosystem variables (Fig. 9). Interestingly, ecosystem variables associated with the C cycle (e.g. C sequestration, C in biomass, but also e.g. herbivore abundances) were affected more strongly by plant diversity loss than variables associated with nutrient cycling (e.g. microbial N, soil nitrate, soil P, Allan, Weisser et al. 2013). In particular, plant species richness had strong positive effects on carbon variables and overall neutral effects on N measures (the nutrient measures were dominated by N-associated variables as there were far fewer measures of the P cycle). This result has been controversial, because there is as yet no clear explanation why this should be the case. It strongly suggests that the C and N cycles are less closely coupled than generally thought, and a number of mechanisms may contribute to this effect. For example, herbivore/pathogen attack may lead to a loss of C in monocultures or to plants investing in C-rich stems under conditions of high light competition in diverse communities. This analysis by Allan, Weisser et al. (2013) is the first comprehensive analysis of biodiversity effects on ecosystem variables across different compartments and processes, allowing for a direct quantitative comparison of the strength of the biodiversity effect. Further work is needed to test whether these patterns occur in other ecosystems. Several of the results demand further theoretical and empirical work. In particular, it might be predicted that stronger diversity effects on C would only be expected in more fertile systems, which are not strongly limited by N. Strong effects on N cycling would be expected in N limited systems.

In summary, the results of the Jena Experiment support the consensus statement that plant species richness affects the mean of many ecosystem variables. The meta-analyses conducted within the Jena Experiment also quantified the variability in the response of ecosystem variables to changes in plant species richness. Several patterns stood

out: responses were stronger aboveground than belowground, herbivores were more strongly affected than carnivores or omnivores, abundances responded less strongly than taxonomic diversities, and there were differences among the element cycles with the C cycle being affected more strongly than the N cycle.

Diversity–stability relationships

The question whether diversity begets stability precedes the question whether biodiversity affects the mean of ecosystem process rates (McCann 2000). Before the 1970s, the prevailing view, prominent among eminent ecologists, such as Elton, Odum and McArthur, was that a loss in diversity would cause ecosystems to show stronger fluctuations in ecosystem variables, in particular population abundances (McCann 2000). This view was challenged by modelling work of Robert May in the early 1970s, who showed that a more complex (more diverse) ecosystem could in fact be more prone to population fluctuations (May 1972). Subsequently, there have been further theoretical works and first empirical studies which found that increasing diversity can increase ecosystem stability, but that details of the interactions among species determine whether such stabilizing effects are observed (McCann 2000). In addition, stability has many components, e.g. variability, resistance or resilience, and different components of stability may be differentially affected by diversity (e.g. Yachi & Loreau 1999; McCann 2000).

In the Jena Experiment, a number of studies tested the effect of diversity on ecosystem stability. In a study on the temporal stability of 42 variables characterizing twelve processes, the diversity–stability hypothesis was supported for many of these variables, including the abundance of parasitic hymenoptera (as a proxy for food-web complexity), the suppression of non-resident plant species (invasion resistance), vegetation structure and biomass (primary productivity), the abundance of aboveground invertebrates (secondary consumers), and trace gas/matter fluxes (Proulx et al. 2010). The results also showed that variables below the community level, i.e. at the population level, were also temporally stabilized, which is contrary to previous findings, which have shown that the population level CV (Coefficient of Variation) is higher, i.e. less stable, in more diverse communities (e.g. Caldeira, Hector, Loreau, & Pereira 2005; Tilman, Reich, & Knops 2006; Flynn, Schmid, He, Wolfe-Bellin, & Bazzaz 2008). The analysis of Proulx et al. (2010) did, however, include not only measures of resident plants, but also measures of earthworms, hymenoptera and invasive plants, which contributed to differences in the results. Thus, the population CV of the constituent plant species in a community may be destabilised (cf. Allan et al. 2011) but the populations of associated species such as earthworms can be stabilised. In another analysis of the plant community itself, Roscher, Weigelt et al. (2011) found no effect of species richness on the temporal stability of individual species biomass, categorized according to their proportional contribution to community biomass. Temporal population stability was in fact higher in plant species that contributed a larger proportion to community biomass production.

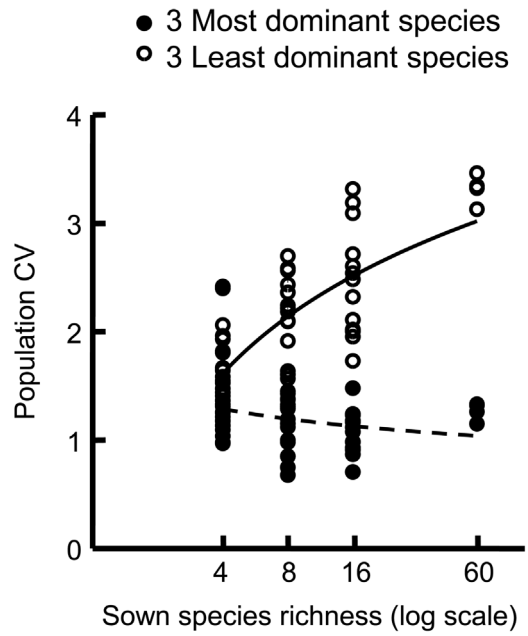

Fig. 10. Diversity–stability relationships in the Jena Experiment for the biomass of individual plant species in mixtures. The population CV of each experimental plot was obtained by averaging across selected species the ratio of standard deviation to mean of plant biomass (years 2003–2008, Weigelt et al. 2010). The open circles (◯) represent the population CV obtained using the three least dominant (least productive) species, while the closed circles (●) represent the CV obtained using only the three most dominant (most productive) species per plot. The least dominant species in each plot were those with the lowest mean biomass calculated on time-series containing at least three non-zero harvests. Similarly, the most dominant species were those with the highest mean biomass. Therefore, the identities of the three least dominant species and the most dominant species change from plot to plot. When selecting only the three most productive species per plot, the overall sample size is reduced to 50 plots after excluding lower species richness treatments. In the 4- and 8-species plots, the three most productive species form more than two thirds of the total biomass, while they only form more than half and one third of the total biomass in the 16- and 60-species plots, respectively.

Further analyses showed that the population CV of plant biomass is sensitive to a diversity-induced change in plant biomass itself. When analysing the plant biomass data using a framework developed by Thibaut and Connolly (2013), the population CV for plant biomass was less stable at higher diversity, because of a reduction in individual species biomass with increasing species richness that led to a reversal of the diversity–stability relationship (Fig. 10). On average, the three most abundant (=dominant) species in species-rich communities fluctuated less than the three most

dominant species in species-poor communities. The situation is reversed when considering only the three least dominant species, i.e., they fluctuate more in species-rich communities. These contrasting results illustrates the sensitivity of the population CV to individual species biomass and unevenness. These relationships imply that unevenness can alter the way in which population variability changes with species richness, even when plant species are assembled randomly into communities with respect to their mean abundances.

A further analysis explored whether diverse communities have consistently higher functioning over time, as compared to less diverse communities (Allan et al. 2011). The number of years in which plots had biomass production exceeding a threshold was calculated. Species-rich and functionally diverse communities exceeded the threshold in more years than species poor communities. This could be because diverse communities are more likely to contain a species which produces consistently high biomass (a temporal sampling effect) or because there is functional turnover in species in diverse communities, meaning that different species provide function in different years, resulting in more stable delivery of a particular ecosystem process over time. To test these hypotheses a 'functional turnover' was calculated for each plot, in the following way: for each year and plot, functionally important species were defined as those species that were needed to achieve 50% of the biomass production. This was done by ranking species by biomass and adding species to the list of functionally important species starting from the most productive species, until the threshold of 50% was reached (i.e. the minimum set of species needed). The more years were included in the analysis, the more species are potentially needed, if species contributing most to biomass changed between years. The dependence of functional turnover on the number of years considered and on plant diversity was then investigated in a separate analysis. In species-rich, and more even, communities, there was much higher turnover in the identities of these functionally dominant species. High functional turnover meant that more than twice as many species were required to sustain functioning across seven years as opposed to in one year, which agrees with results of Isbell et al. (2011). This functional turnover also promoted high levels of functioning over time and was associated with high levels of complementarity in communities. Interestingly, both these effects were only apparent in functionally diverse communities (those with 3 or 4 functional groups present). This indicates that a high turnover of functionally similar species does not stabilise biomass production. Instead, a high turnover of functionally complementary species is needed to promote high levels of ecosystem functioning over time. An increase in asynchrony among species with increasing diversity has been shown to increase the stability of biomass production over time also in meta-analyses that included data from the Jena Experiment (de Mazancourt et al. 2013).

Yet another aspect of the biodiversity–stability relationship is the stability against perturbations, such as climatic changes or changes in land-use. In a combined manipulation of prolonged summer drought and increased management intensity (see Section "The Jena Experiment, its origin and design": Drought and management experiment), resistance (the degree of change after perturbations) and resilience (time until pre-perturbation levels are regained) of aboveground biomass production against drought were highly dependent on the management intensity and only partly on species richness (Vogel et al. 2012). Resistance of grassland communities against drought was mainly affected by mowing frequency, while species richness partly decreased resistance. Resilience was higher in species-rich communities, but only in highly managed grasslands. Nevertheless, species richness and aboveground biomass were positively related even under drought conditions (Vogel et al. 2012). The drought experiment was also used to test the assumed buffering capacity of plant diversity to disturbances, with litter decomposition and soil microbial processes being the target ecosystem variables. Decreasing plant species richness and induced summer drought both reduced mass loss of a standard litter material (wheat straw), and basal respiration and microbial biomass (Vogel, Eisenhauer, Weigelt, & Scherer-Lorenzen 2013). However, drought reduced decomposition rates and microbial properties irrespective of plant species richness, i.e. there were no interactive effects between plant species richness and summer drought, and thus no evidence for a buffering effect of diversity.

Plant biomass data from the Jena Experiment also contributed to a more general analysis where productivity data from 46 experiments that manipulated grassland plant diversity were aggregated, to test whether plant diversity provides resistance and resilience after climate events (Isbell, Craven et al. 2015). Climate data from the different locations were analysed for the occurrence of extreme events, wet or dry, moderate or extreme, brief or prolonged. In this meta-analysis, plant diversity generally increased ecosystem resistance across experiments (Isbell, Craven et al. 2015). There was no effect on resilience, because productivity had mostly recovered to normal levels, or overshot, independent of diversity.

In Jena itself, a more recent extreme disturbance event, a 200-yr flood in June 2013, challenged common diversity–stability theory, but also illustrated the complexity of processes that occur in an ecosystem after a disturbance (Wright et al. 2015). Stability was assessed by analyzing absolute change in aboveground live biomass over time, for the period during the flood (comparison of biomass in May 2013, i.e. directly before the flood, and in July 2013, i.e. directly after the flood, referred to as resistance), for the two-month period following the flood, by measuring biomass again in September 2013 (referred to as initial recovery), and for the entire season before and after the flood (referred to as resilience). Flooding increased the availability of resources after the flood. Microbial biomass was 1.5 times higher after the flood, and inputs of dead organic material increased soil nitrate after the flood, in particular in the

plots that experienced the most severe flooding (measured in the number of days a plot was flooded, i.e. 1–24 days, depending on the location of the plot). Water availability was also increased after the flood had receded. On the other hand, increasing flooding increased stress levels, e.g. by reducing oxygen availability, and responses of the plant communities depended on the level of stress experienced (Wright et al. 2015). Higher diversity communities were most capable of taking advantage of the resource influxes and thus were more productive than before, but only when stress levels were low. Because increased productivity constituted a large deviation from baseline productivity, higher diversity communities were also less stable (Wright et al. 2015). Thus, high-diversity plant communities responded in a very pronounced way to the perturbation, by showing a strong increase in plant biomass production when soil water and nutrient availability was high, and a drastic reduction in plant biomass production in those plots with higher stress levels (Wright et al. 2015).

Taking a multi-trophic perspective, the effects of the presence of aboveground and belowground invertebrates on the temporal and spatial stability of plant productivity were studied in subplots of the main experiment, where these groups were excluded (Eisenhauer, Milcu, Allan et al. 2011). Aboveground insects generally increased temporal stability, but impacts of both earthworms and aboveground insects depended on plant species richness and the presence of grasses. Complementary greenhouse studies further supported the role of consumers for the stability of plant productivity (Eisenhauer & Schädler 2011). These results suggest that inconsistent results of previous studies on the diversity–stability relationship may have been in part due to neglecting higher-trophic level interactions governing ecosystem stability, as they affect plant-plant competition (Eisenhauer, Milcu, Allan et al. 2011).

Starting with Elton (1958), the diversity–stability debate also included the question whether more diverse communities are more resistant against invasion by species not present in the community, in particular alien species (McCann 2000). Several studies in the Jena Experiment tested this hypothesis and generally found that the more diverse plant communities are more resistant against plant invasion (e.g., Mwangi et al. 2007; Roscher, Beßler et al. 2009). These results are discussed in detail in Section "Invasion into the target communities".

To sum up, the results from the Jena Experiment provide evidence that diversity begets stability (expressed as variability), with somewhat less evidence for diversity effects on resistance or resilience. As the outcome of the various analyses depended on the measures used to assess stability, the results of the Jena Experiment also highlight the need to revisit diversity–stability theory and to re-consider common measures and the implication of stability for ecosystem service provisioning (Wright et al. 2015).

Effects of individual species on ecosystem variables

In several of the early biodiversity experiments, the species pool was relatively small, so that the similarity in plant species composition was often higher among high-diversity communities than among low-diversity mixtures (Roscher et al. 2004). As a consequence, certain very productive plant species occurred in almost all high diversity mixtures, but only in some of the low-diversity mixtures, and could thus exert a disproportionate effect on the relationship between plant species richness and plant community biomass. For example, the legume red clover, *T. pratense*, was a very productive species in the BIODEPTH study and strongly affected community biomass (Hector et al. 1999). Despite the development of *a posteriori* methods to disentangle the complementarity and selection effects in biodiversity experiments (Loreau 1998; Yachi et al. 1999), disentangling the role of individual species from the effect of biodiversity *per se* requires some adjustments of the experimental design.

In the main experiment, a particular focus was to study effects of diversity in terms of plant species richness and functional group number and composition, and to avoid overriding effects of individual species. The large species pool for the main experiment and the random allocation of species with replacement to the mixtures had the consequence that individual species were not very common in the experimental plots and did not occur in equal frequency. The most frequent species was the small herb *P. lanceolata* that occured in 18 large plots and was present in plots of each of the six species-richness levels. The least frequent species was the tall herb *Pastinaca sativa* that occured in six large plots. Because of the strong focus on making results as independent as possible from the effects of individual species, the main experiment was not suitable to disentangle species identity effects and diversity effects.

Nevertheless, some species had strong effects on many ecosystem variables. Explorative analyses showed that *Onobrychis viciifolia*, a tall legume species, which was among the most dominant species over several years, occurred more often than expected by chance in highly productive mixtures (Roscher, Scherer-Lorenzen et al. 2011) suggesting a strong sampling effect (Huston 1997). Nevertheless, irrespective of the effect of *Onobrychis*, sown species richness and functional trait diversity had positive effects on community biomass production in all years from 2003 to 2009 (Roscher, Schumacher et al. 2013), and therefore it is unlikely that the occurrence of a dominant key species explains positive diversity–productivity relationships in the main experiment.

In contrast to the main experiment, species identity effects could be tested in the dominance experiment where all of the nine species and all possible species pairs were equally represented at all diversity levels. The dominance experiment showed that strong effects of individual species,

and an overall biodiversity effect, do not need to be mutually exclusive, or be confounded such that the biodiversity effect becomes an artefact of the higher probability of a dominant (and productive) species to occur in more diverse communities, as has been suggested repeatedly (Wardle 1998). In the dominance experiment, five species had higher, and four species had lower observed than expected relative yields averaged across all mixtures. One species, the grass *A. elatius*, illustrated well the strong effect of a single species on an ecosystem process, here plant biomass production. *A. elatius* was the most productive species in monoculture, but its relative yield (RY) continually increased along the diversity gradient, while RY of the remaining eight species either decreased with species richness (three species) or was not affected by species richness (five species) (Roscher, Schumacher, Weisser, Schmid, & Schulze 2007). Apart from the positive effects of sown species richness on *A. elatius* itself, the very high biomass production of this species also resulted in higher community biomass production, while having negative effects on RYT (Relative Yield Total), and RY of other species. Obviously, *A. elatius* benefited from reduced intraspecific competition at increased species richness, while the strong niche overlap in the species pool of the dominance experiment reduced the performance of the competitively inferior species. *A. elatius* was also superior in using aboveground space, i.e. it grew highest and had the largest module density across all strata of the canopy profile (Lorentzen, Roscher, Schumacher, Schulze, & Schmid 2008). It also had the largest biomass:N ratios in shoot material (i.e. the highest amount of biomass produced per unit N; Roscher, Thein, Schmid, & Scherer-Lorenzen 2008), which is consistent with resource-competition theory predicting the dominance of species with greatest ability to efficiently acquire and use limiting resources (Tilman 1990; Fargione & Tilman 2006). Overall, aboveground community biomass in the dominance experiment increased strongly with the transition from monocultures to two-species mixtures but only slightly from two- to nine-species mixtures (Roscher, Schumacher, Weisser et al. 2007).

To summarize, the results of the Jena Experiments indicate strong effects of individual species on ecosystem variables such as community biomass production, yet these effects do not negate or invalidate effects of species richness on the same processes. Thus, the interesting question is not whether biodiversity or individual species affect a particular process, but how large the contribution of biodiversity is in relation to other factors such as individual species.

Delayed biodiversity effects

The Jena Experiment is now at the point where it enters the long-term time scale compared with other grassland experiments (currently only experimental plots in Cedar Creek, Minnesota, have longer time scales; Reich et al. 2012). This makes it possible to investigate effects of plant species richness on community assembly processes, including responses of fauna and microorganisms, and their relations to productivity and C, N, and P cycling. In fact, short-term plant biodiversity experiments have been criticised because the plant communities are 'immature' shortly after sowing (Thompson et al. 2005), and such claims can only be ruled out if experiments are run for a longer time. Recent results indicate that the diversity effect of ecosystem functioning becomes stronger with time rather than weaker (Reich et al. 2012), and the Jena Experiment has contributed to unravelling this effect (Eisenhauer, Reich, & Scheu 2012; Meyer et al. 2016).

Results in the Jena Experiment strongly emphasize that biodiversity effects need time to develop, such that effects observed in the first few years after establishment of the diversity mixture may be transients. For example, we initially found that biodiversity effects on plant community productivity increased over the years and thought this to be caused by a deterioration of monocultures (Marquard, Weigelt, Temperton et al. 2009). However, after the first six years this trend could no longer be observed, and instead positive complementarity effects were increasingly compensated by negative selection effects. Many plant species with low monoculture performance turned out to be low-performing also in mixtures if observed over more than six years (Marquard et al. 2013).

Delayed biodiversity effects were most obvious in the soil compartment. Belowground organisms generally have low dispersal capabilities which is consistent with the observation that soil microorganisms (Fig. 11A, Eisenhauer, Bessler et al. 2010; Strecker, González Macé, Scheu, & Eisenhauer 2016), and soil fauna (Eisenhauer, Milcu, Sabais et al. 2011) only responded significantly to the manipulation of plant species richness after a delay of 3–5 years. However, such delayed responses in the soil compartment were not restricted to soil organisms, but were also seen in a number of ecosystem variables measured belowground. One prominent example is the development of a plant species richness effect on root standing biomass, where no effect of plant species richness on belowground plant biomass was observed in the first years after establishment of the experiment (Fig. 11B, see also Section "The effects of plant species richness on plant community biomass", Ravenek et al. 2014).

Another important example is C-storage in the soil. After the land-use change from an agricultural field to grassland, the homogeneous C concentrations in the ploughing horizon developed slowly towards a distribution typical for grasslands, namely very high C concentrations in topsoil with a pronounced decrease with depth (Steinbeiss, Temperton, & Gleixner 2008), as observed in real-world cropland to grassland conversions (Attard et al. 2016). This restructuring of the C depth profile was positively influenced by plant species richness by higher accumulation in the top soil and decreased loss in the deeper soil layers. Thus, it took at least four years until a plant diversity effect on C-storage was observed. Over time, this positive plant species richness

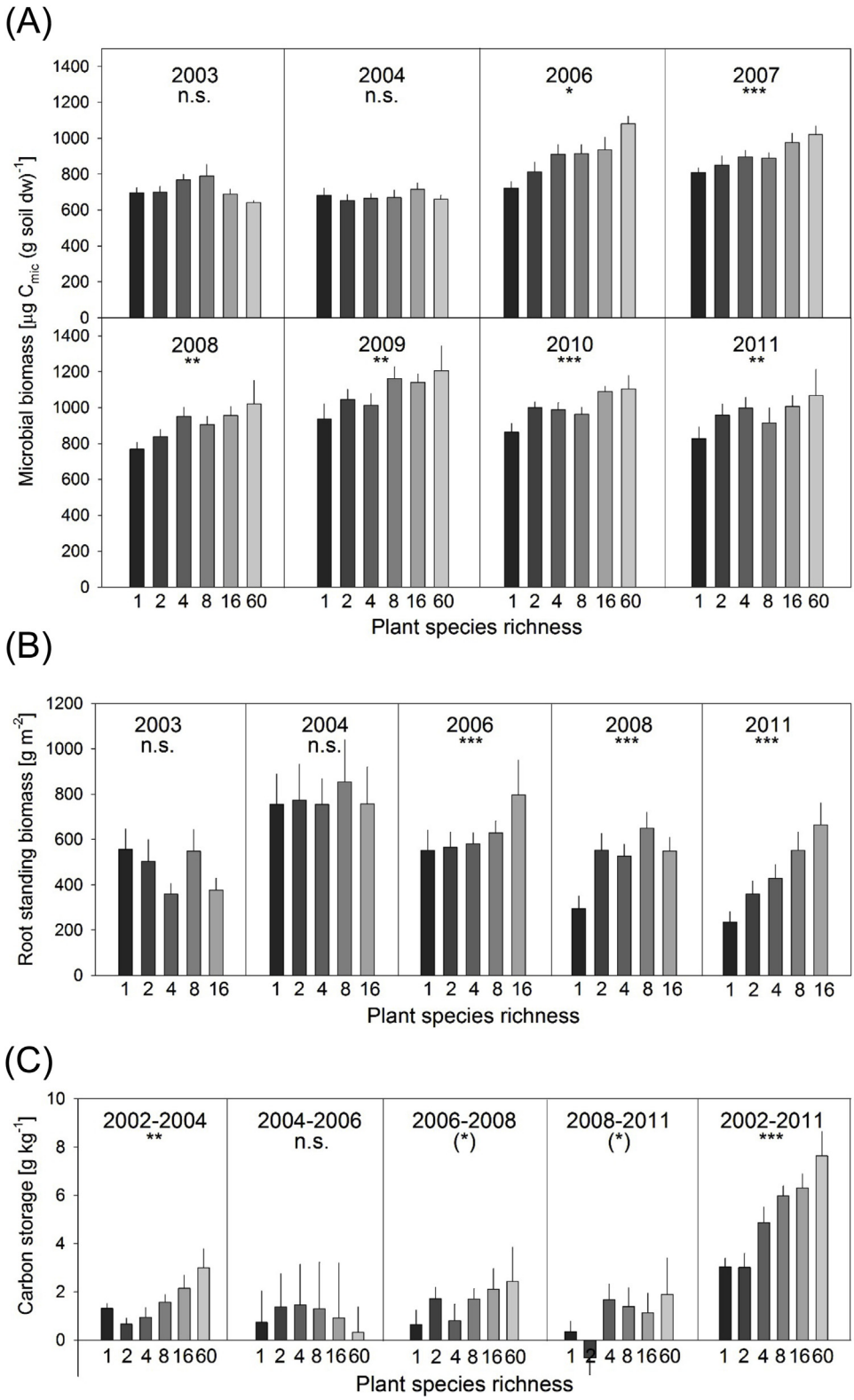

Fig. 11. Delayed biodiversity effects: (A) development of soil microbial biomass over time in the main experiment, estimated through substrate-induced respiration (Eisenhauer, Bessler et al. 2010). The positive species richness effect was significant from 2006 onwards. Redrawn from Strecker et al. (2016). (B) Development over time of the relationship between plant species richness and root standing biomass (0–30 cm) in the main experiment. A positive species richness effect was significant from 2006 onwards (modified from Ravenek et al. 2014). (C) Development over time of the relationship between species richness and carbon storage in the top 5 cm over time in the main experiment (Lange et al. unpubl.). For methods see Steinbeiss, Bessler et al. (2008).

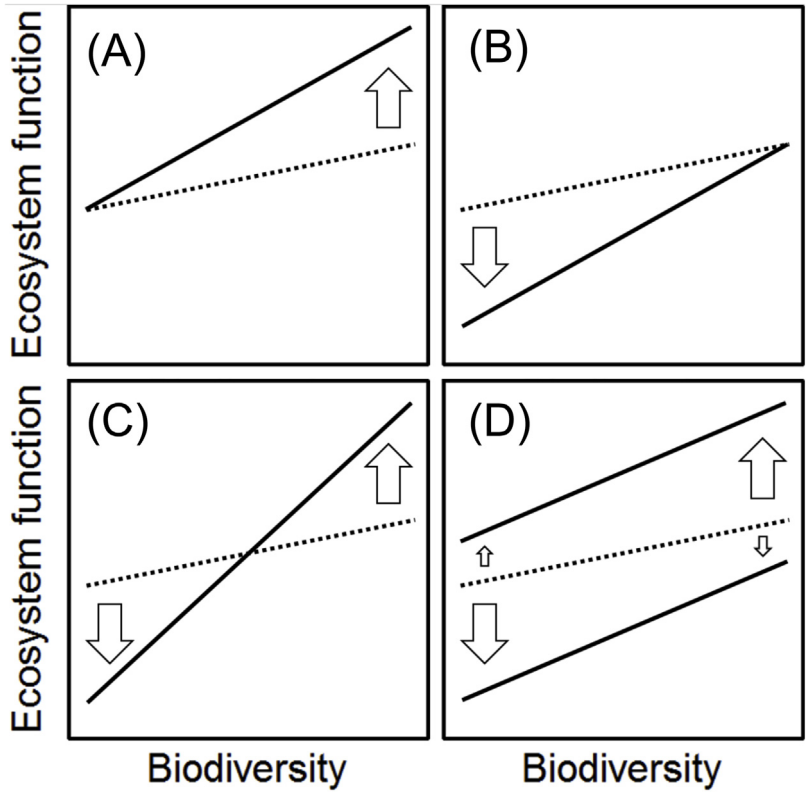

Fig. 12. Schematic illustrating how the strength of the biodiversity effects can become stronger over time. Change over time is visualized by arrows. The dotted line visualizes the relationship between the ecosystem function and biodiversity at the onset of the experiment. The biodiversity effect in later years (solid line) can be strengthened by an increasing performance at high biodiversity (A) or a decreasing performance at low biodiversity (B). Both effects can occur simultaneously (C). For biodiversity effects to strengthen, it is sufficient if the increase is stronger at high, or the decrease is stronger at low biodiversity, in the case that the performance at both ends of the biodiversity gradient changes in the same direction (D). In the case of negative biodiversity effects that occur if the measured variable decreases with increasing biodiversity, the logic applies accordingly with inverted signs. Modified from Meyer et al. (2016).

effect became stronger (Fig. 11C, Lange et al. unpubl.) and this effect is still increasing. At present, it is not clear whether more diverse grassland mixtures store more C than low-diversity mixtures, or whether a fixed C-storage limit is reached faster in high-diversity mixtures.

More generally, a biodiversity effect can strengthen over time by an increasing performance of high-diversity communities, by a decreasing performance of low-diversity communities, or a combination of both processes (Fig. 12, Meyer et al. 2016). Which of these two mechanisms are more common had been unclear, as had been the question whether an increase in the biodiversity effect over time is a general property of many functions. In a meta-analysis of 50 ecosystem variables measured repeatedly over 11 years in the Jena Experiment, Meyer et al. (2016) found strong evidence that the increase of the biodiversity effect over time is a general phenomenon (Fig. 13). This increase in the strength of the biodiversity effect was independent of the ecosystem compartment (above- or belowground), organizational level (ecosystem variables associated with the abiotic habitat, primary producers, or higher trophic levels such as herbivores and pollinators), and variable type (measurements of pools or rates). Importantly, biodiversity effects strengthened because of both, a progressive decrease of functioning in species-poor, and a progressive increase of functioning in species-rich communities. This study, the first of its kind, provides evidence that negative feedback effects at low biodiversity are as important for biodiversity effects as complementarity among species at high biodiversity.

To summarize, results from the Jena Experiment show that some biodiversity effects on ecosystem variables need time to develop, in particular in the belowground compartment. The implication is that short-term experiments are likely to under-estimate the importance of biodiversity for ecosystem functioning. This emphasises the importance of long-term experiments to distinguish trends from transient dynamics. More generally, the implication is that a loss of species will result in a future impairment of ecosystem functioning, potentially decades after the event of species extinction. This type of ecosystem-service debt has so far been overlooked (Meyer et al. 2016), and, importantly, it occurs in addition to the ecosystem-service debt due to the extinction debt, i.e. the delayed extinction of species after a habitat change or habitat loss (Isbell, Tilman, Polasky, & Loreau 2015).

Compartment	Level	Ecosystem function	Effects of time: Slope of biodiversity effect	Effects of time: Performance at low biodiversity	Effects of time: Performance at high biodiversity
Aboveground	Animals	Herbivory rate	↘	↗ 39%	↘ 61%
	Plants	Drought resilience	↗	↗ 0%	↗ 100%
		Drought resistance	↗	↗ 0%	↗ 100%
		Dead plant biomass	↘	↘ 0%	↘ 100%
		Leaf area index	↗	↗ 19%	↗ 81%
		Reproductive plant height	↘	↗ 3%	↘ 97%
		Target plant cover	↗	↘ 100%	↘ 0%
		Target plant K	↗	↗ 43%	↗ 57%
		Target plant N	↗	↗ 0%	↗ 100%
		Target plant Na	↗	↗ 0%	↗ 100%
		Vegetative plant height	↘	↘ 0%	↘ 100%
		Weed plant cover	↗	↘ 100%	↘ 0%
		Weed plant species richness	↗	↘ 100%	↘ 0%
Belowground	Animals	Soil basal respiration	↗	↗ 52%	↗ 48%
		Earthworm abundance	↘	↗ 100%	↗ 0%
		Soil microbe biomass	↗	↗ 0%	↗ 100%
	Plants	Root biomass	↗	↗ 35%	↗ 65%
	Environment	Inorganic soil C	↘	↗ 11%	↘ 89%
		Organic soil C	↗	↘ 25%	↗ 75%
		Soil N	↗	↘ 100%	↘ 0%
		Soil ^{15}N	↗	↗ 6%	↗ 94%
		Soil surface water content	↗	↗ 0%	↗ 100%
		Soil water content	↗	↗ 0%	↗ 100%

Fig. 13. Change in slope of biodiversity effects and performance at low and high biodiversity over time. Upwards arrows depict steeper slopes and higher performance; downwards arrows depict shallower slopes and lower performance. Black arrows show significant changes over time while grey arrows show non-significant trends. The percentages state how much of the chance in slope over time is caused by the respective changes in performance at low and high biodiversity. Note that an increase in performance at low biodiversity causes the slope of biodiversity effects to become shallower. A number of additional ecosystem variables were tested for changes in the strength of biodiversity effects over time, but no significant effects were detected: Bare ground cover, Consumed plant biomass, Down flow soil water, Earthworm biomass, Evapotranspiration, Fauna soil surface abundance, Fauna soil surface species richness, Fauna vegetation abundance, Fauna vegetation species richness, Frequency pollinator visits, Pollinator abundance, Rain throughfall, Soil bulk density, Soil larvae abundance, Soil macrofauna abundance, Soil mesofauna abundance, Soil NH_4^+, Soil NO_3^-, Soil NO_3^- leaching, Soil P, Soil respiratory quotient, Target plant biomass, Target plant C, Target plant Ca, Target plant Mg, Target plant P, Up flow soil water. Modified from Meyer et al. (2016).

In the coming sections, we review the specific results of the Jena Experiment in more detail, starting with the response of the plants themselves to the manipulation of the richness of the plant community.

Responses of individual plant species to plant species richness

It is well-known that plants respond to changes in their biotic and abiotic environment. Such responses can result from phenotypic plasticity, i.e. genotype × environment interactions that result in physiological or morphological changes in the plant individual. The biotic and abiotic environment is, however, also a selective environment that may favor certain genotypes over others. Thus, the plant individuals of the same species may differ between plant communities of different diversities (Zuppinger-Dingley et al. 2014), and studying this variation can help understanding the effects of biodiversity on ecosystem functioning. In the Jena Experiment, a suite of studies have addressed phenotypic and genetic changes in the synthetic plant communities.

Plant physiological and morphological responses to increasing plant species richness

The environment an individual plant experiences in a highly diverse plant community differs from the environment in a plant community with few or just a single species. This is due to a greater diversity and density of neighboring plants (Roscher, Schumacher, Weisser et al. 2007; Marquard, Weigelt, Roscher et al. 2009), a more complete consumption of essential resources, such as light, space (Lorentzen et al. 2008) and nutrients (Oelmann, Buchmann et al. 2011), and more diverse interactions with organisms at higher trophic levels, such as herbivores, pollinators or pathogens (Scherber, Eisenhauer et al. 2010). Species performance is the consequence of the functional characteristics of a species, comprising morphological, physiological and phenological traits that operate at different levels of plant organization (Violle et al. 2007). The set of trait values characterizing a species results from trade-offs between different functional requirements and from the species-specific ability to respond to variation in the biotic or abiotic environment.

The extensive study of functional traits of all 60 experimental species of the Jena Experiment showed remarkable intra-specific trait variation in response to increased community species richness in grasses (Gubsch, Buchmann et al. 2011), legumes (Roscher, Schmid, Buchmann, Weigelt, & Schulze 2011) and herbs (Lipowsky et al. 2015). In general, plasticity in shoot traits related to light acquisition indicated greater efforts for light acquisition at increased plant species richness. In particular, species in high-diversity mixtures species formed longer shoots with elongated internodes, increased biomass allocation to supporting tissue at the expense of leaf mass, and reduced branching. The reduction in branching resulted from both a decrease in the number of shoots per plant individual and a decrease in the number of secondary axes per shoot. In addition to these shade-avoidance responses, species may also compensate for reduced light availability by plastically adjusting leaf morphology and physiology. In all functional groups, species formed leaves with higher specific leaf area (SLA) and lower foliar $\delta^{13}C$ values when growing in communities of increased species richness (Dassler, Roscher, Temperton, Schumacher, & Schulze 2008; Gubsch, Buchmann et al. 2011; Roscher, Schmid, Buchmann, Weigelt, & Schulze 2011; Lipowsky et al. 2015). Foliar stable-C isotope ratios ($\delta^{13}C$) are known as an integrative long-term measure related to stomatal conductance and photosynthetic activity, which are dependent on light, water and nutrient availability (Farquhar, Ehleringer, & Hubick 1989). Close relationships between SLA and foliar $\delta^{13}C$ suggested that canopy characteristics and changes in light availability and air humidity, rather than differences in N nutrition, determined variation in foliar $\delta^{13}C$ in the species of the Jena Experiment (Gubsch, Buchmann et al. 2011; Lipowsky et al. 2015), although we cannot disentangle the effects of varying $\delta^{13}C$ of source CO_2 within the canopy (Buchmann, Brooks, & Ehleringer 2002). For non-legume species, the presence of legumes generally induced plastic responses in light-acquisition traits in the same direction as observed for increased species richness, and trait variation in response to legume presence was even stronger than the effects of increasing species richness (Gubsch, Buchmann et al. 2011; Lipowsky et al. 2015).

Analysis of intraspecific trait variation indicated that the plant species of the Jena Experiment use similar strategies to optimize light acquisition. However, the extent of trait variation varied greatly among species. A comparative study of four legume species with similar morphology including the tall-growing *O. viciifolia* and three species of medium or short growth stature showed little variation in shoot morphology in *O. viciifolia*, while species with shorter growth stature showed greater trait variation in response to increased species richness (Thein, Roscher, & Schulze 2008). As a consequence, intraspecific trait variation either increased or decreased functional differences among species at increasing plant species richness. For example, Lipowsky et al. (2015) showed that the greater plasticity in SLA and foliar $\delta^{13}C$ values in small-statured herb species, which cannot escape shading, increased the functional difference to tall-statured forb species that forage vertically for light.

Analyses of traits related to N acquisition and N use provided further evidence for physiological responses to increased plant species richness. First, analysis of ^{15}N natural abundances showed that the proportion of N that legumes derive from the atmosphere ($\%N_{dfa}$) increased with increasing species richness in the years following the establishment of the Jena Experiment, i.e. there was a

positive species richness effect on atmospheric N_2 fixation through bacterial symbionts. Among legumes, the tallest species that reached the upper canopy layers were least dependent on atmospheric N_2 fixation (Roscher, Schmid et al. 2011; Roscher, Thein et al. 2011). The legume-reliance on symbiotic N_2 fixation first increased over time, but became independent of species richness in later years. Second, non-legume species adjusted their uptake of different N forms depending on species richness and the presence of legumes, indicating complementary resource use in diverse communities. In grass species, foliar $\delta^{15}N$ and $\Delta\delta^{15}N$ values (=foliar $\delta^{15}N$ − soil $\delta^{15}N$) decreased at higher species richness, indicating an enhanced uptake of N depleted in ^{15}N. In communities containing legumes, the reduced foliar $\Delta\delta^{15}N$ values of grasses also suggested a direct use of legume-derived atmospheric N. In contrast, increased leaf N concentrations without changes in foliar $\delta^{15}N$ in small and tall herbs implied that these species received additional mineral N not fixed by legumes (Gubsch, Roscher et al. 2011).

Physiological and morphological adjustments to increased plant species richness are, however, not necessarily sufficient for species to avoid resource limitation and competitive exclusion in multi-species mixtures. For example, the subordinate grass, *L. perenne*, sown as an additional species into all communities of the main experiment, had a declining performance (smaller population and plant individual sizes) at increased species richness (Roscher, Schumacher et al. 2008). This species showed morphological (increased SLA), physiological and biochemical adjustments (decreased rates of photosynthesis, increased chlorophyll concentrations) to canopy shade, and had a better N supply in communities with legumes, as indicated by increased leaf N concentrations. Nevertheless, analyses of leaf blade carbohydrates and nitrate as indicators for nutritional status indicated (1) enhanced light limitation in communities of increased species richness and with increased legume proportion, and (2) enhanced N-limitation with increasing proportions of non-legumes, both of which resulted in a decreased performance of this species at higher plant species richness (Roscher, Kutsch, & Schulze 2011). This view is also supported by untargeted metabolite profiling of the small-statured herb species *B. perennis* and *Scorzoneroides autumnalis*. When the canopy was fully developed, key-metabolites indicated C- and N-limitation (Scherling, Roscher, Giavalisco, Schulze, & Weckwerth 2010). In contrast, the small-statured legume *Lotus corniculatus* was facilitated by increased legume proportions, and tall-statured dominant species, such as *Knautia arvensis* (tall herb) and *Medicago × varia* (legume), did not show altered metabolite profiles with increasing plant species richness (Scherling et al. 2010).

Nevertheless, small-statured herbs did not go extinct in the high-diversity communities of the Jena Experiment and even increased their abundances over time. Analyses of seven small-saturated herbs showed that their net C assimilation balance just compensated the cost of maintenance respiration during the period of low light shortly before spring mowing (estimated to be <5% deep in the canopy compared to the above-canopy level) (Roscher, Kutsch, Kolle, Ziegler, & Schulze 2011). Close relationships between SLA and seasonal light availability in these species (Roscher, Kutsch, Kolle et al. 2011), and low diversity-effects on daily C turnover in two of the small-statured species (*Plantago media*, *Veronica chamaedrys*) when the canopy was not fully developed (Dassler et al. 2008), suggested that small-statured herbs are capable of exploiting seasonal niches and grow when light supply is better.

To summarize, the plant species in the Jena Experiment showed species-specific physiological and morphological responses to the increase in diversity of the plant communities, many of which indicated an increasingly competitive environment. These results strongly question the use of monoculture or 'average' trait values for predicting community effects on ecosystem variables, as it is common in trait-based ecology (see also Section "Mechanisms underlying the biodiversity–ecosystem functioning relationships"). Niche differentiation among species is hypothesized to be a key for species coexistence and requires functional differences among species. The observed trait plasticity in response to increased species richness may, however, increase or decrease niche overlap among species (Lipowsky et al. 2015) and could therefore have important implications for the functioning of diverse plant communities. Using 'realized' trait values as predictors of ecosystem functioning will be an important step forward to evaluate the reliability of trait-based approaches.

Plant genetic responses to increasing plant species richness

Intraspecific variation in plant individual performance as well as in the expression of functional traits may not only be due to phenotypic plasticity but also to genetic variation. Although populations of all species were established from the same seed source in all communities of the Jena Experiment, diversity-induced selection may have caused genetic differentiation and changes in genetic diversity over time (Zuppinger-Dingley et al. 2014). A first transplant-replant study using seed families of five species (*Cirsium oleraceum*, *Crepis biennis*, *P. lanceolata*, *P. media* and *Rumex acetosa*), collected five years after the start of the experiment in monocultures and 60-species mixtures, already indicated differential selection and some local adaptation, even though plastic responses to the environment into which species were planted (monocultures vs. 60-species mixtures) dominated plant responses (Lipowsky, Schmid, & Roscher 2011). In a more recent experiment using the plants selected over eight years in species mixtures, relative differences in plant height and SLA among species selected in mixture (mixture types) were greater than among species selected in monoculture (monoculture types), suggesting a selection

for niche differentiation with increasing duration of the biodiversity experiment (Zuppinger-Dingley et al. 2014).

Plant genetic differentiation in response to plant species diversity was also shown for *L. perenne* that was sown in subplots (3 × 4 m size) of the main experiment with the same initial sowing density (100 viable seeds per m^{-2}) irrespective of plant species richness (see Section "The Jena Experiment, its origin and design": Plant genetic diversity manipulations). Four years after sowing, genetic distances between the field populations and the initially sown seed populations increased with increasing plant species richness, based on single-nucleotide polymorphisms (SNPs). This was mostly explained by a plant species richness effect on *L. perenne* population sizes, i.e. smaller populations of *L. perenne* at increasing plant species richness. In addition to genetic drift, the genetic differentiation of *L. perenne* populations was affected by plant community functional composition, suggesting a selection through genotype-specific interactions with other species (Nestmann et al. 2011). A more recent experiment that tested the performance of plant communities either selected or not selected in the Jena Experiment (see Section "The Jena Experiment, its origin and design": Community history experiment) is currently being analysed (van Moorsel et al., 2017).

To summarize, the plant species in the synthetic communities of the Jena Experiment not only showed phenotypic responses to increasing plant species richness but also genotypic sorting and possibly trans-generational selection. While the studies in the Jena Experiment indicate that genetic diversity in species-rich communities is lower than in low-diversity communities when compared to the initial diversity in the seed material, the question whether plant genetic diversity and plant species richness are indeed inversely correlated is still open (Vellend & Geber 2005).

Invasion into the target communities

More diverse communities are thought to be locally more resistant against invasion by additional species (Elton 1958; McCann 2000). Many plant diversity experiments have therefore tested the invasibility of plant communities and most have found that more diverse communities are less susceptible to invasion (Hooper et al. 2005). Open questions therefore concern the understanding of the mechanisms underlying this resistance (Roscher, Beßler et al. 2009). The diversity-invasion resistance hypothesis and the underlying mechanisms of invasion resistance were tested in several studies in the Jena Experiment, capitalizing on the necessity that the experimental plant communities had to be weeded in order to maintain the species composition (see Section "The Jena Experiment, its origin and design": Invasion experiments).

Colonization of new species was studied in regularly-weeded subplots as well as in subplots which were never weeded since sowing the experimental communities, or where weeding ceased after several years (Roscher, Beßler et al. 2009; Roscher, Temperton et al. 2009). A seed addition experiment, adding seeds of all 60 experimental species to subplots with a different colonization history (i.e. weeded for a different number of years), complemented studies of spontaneous colonization (Roscher, Schmid et al. 2009; Petermann, Fergus et al. 2010). In addition, seedlings of different plant species were transplanted into all main plots of the experiment ('phytometers'), to study the effects of the resident community on invaders after germination (Temperton, Mwangi, Scherer-Lorenzen, Schmid, & Buchmann 2007; Nitschke et al. 2010). Animal exclusion experiments were used to determine the role of belowground invertebrates, in particular earthworms and springtails (Eisenhauer et al. 2008; Eisenhauer, Milcu, Nitschke et al. 2009; Eisenhauer, Sabais, Schonert, & Scheu 2010), and insect herbivores (Nitschke et al. 2010), for preventing or facilitating invasion. The overall result of the various invasion studies is that increasing plant species richness decreases success of invading plants.

Performance of invader transplants belonging to the Jena Experiment species pool was reduced at higher species richness (Scherber, Milcu, Partsch, Scheu, & Weisser 2006; Temperton et al. 2007; Nitschke et al. 2010), although effects varied between different invader species. Scherber, Milcu et al. (2006) found slightly decreased mortality in low-diversity communities for the transplant *R. acetosa*, while Nitschke et al. (2010) reported strongly reduced survival of *Centaurea jacea* transplants at high plant species richness. For growth-related variables (e.g. plant mass, branch and leaf number, relative growth rates) and fitness measures (e.g. inflorescence length (*R. acetosa*), number of flower heads (*C. jacea*)) there was a general decrease with increased species richness (Scherber, Milcu et al. 2006; Temperton et al. 2007; Eisenhauer, Milcu, Nitschke et al. 2009; Nitschke et al. 2010).

For invaders immigrating naturally into the plant communities, seedling number as well as the number, density and biomass of established plants decreased with increasing sown species richness (Roscher, Beßler et al. 2009; Roscher, Schmid et al. 2009; Petermann, Fergus et al. 2010). Invaders with early successional traits (i.e. annual life cycle, reproduction by seeds, small and long-lived seeds, earlier start of a longer flowering period) were particularly successful in the newly established plant communities or directly after weeding was stopped experimentally (Roscher, Gerighausen, Schmid, & Schulze 2015). In contrast, when plant communities were not weeded for four, or even seven years, these invaders were replaced by plant species with mid-successional traits (i.e. taller growth, perennial life cycle, vegetative reproduction) (Roscher, Gerighausen et al. 2015), and these species were also the main invaders in weeded plots later in the Jena Experiment. These changes in the functional characteristics of invader species corresponded to a shift from high abundances of 'external invader' species (i.e. species not belonging to the experimental species pool) during the first years of the experiment, to the dominance of 'internal

invader' species (i.e. species being part of the experimental species pool) in the following years (Roscher, Beßler et al. 2009; Roscher et al. 2016). Species with early-successional traits were also more successful invaders in experimental communities sown with a lower number of species, while mid-succession traits characterised invaders at higher species richness (Roscher, Gerighausen et al. 2015).

The 'filtering' of invader species along a colonization-competition trade-off with increasing species richness of the invaded community even resulted in selection among different clones of the small herb dandelion (*T. officinale*), the most frequent internal invader species in the Jena Experiment. Offspring originating from seeds and cuttings collected in invader and 'resident' (i.e. sown) populations of the five-year old biodiversity experiment were grown under standardized conditions in the greenhouse. R strategy traits (i.e. traits related to high reproductive output) were observed when plants were selected in weedy (=invader) populations. In contrast, higher plant species richness supported the selection of clones with K-strategy traits (higher root and leaf mass, fewer flower heads, higher individual seed mass) in resident populations (Lipowsky, Roscher, Schumacher, & Schmid 2012).

In addition to species richness, increased plant functional group number increased invasion resistance. However, more important was functional group composition, in particular the presence of legumes and grasses, which had large effects on plant community invasibility (Roscher, Schmid et al. 2009; Roscher et al. 2014) and the performance of invader transplants (Scherber, Milcu et al. 2006; Nitschke et al. 2010). The presence of legumes had negative effects on the survival of invader transplants, while their effects on growth of the transplants depended on invader species identity (Scherber, Milcu et al. 2006; Temperton et al. 2007; Nitschke et al. 2010). The effect of legumes on invasion changed during the time-course of the experiment: early after establishment of the biodiversity experiment, the presence of legumes in fact increased the number, density and biomass of naturally dispersed invader species, in particular external invader species (Roscher, Beßler et al. 2009). After several years, however, effects of legumes reversed, and fewer species invaded communities with legumes (Roscher, Gerighausen et al. 2015). In contrast to legumes, grasses often decreased the growth of invader transplants (Scherber, Milcu et al. 2006; Nitschke et al. 2010). Species number, density and biomass of naturally dispersed colonizers were also lower when communities contained grasses (Roscher, Beßler et al. 2009; Roscher, Gerighausen et al. 2015). Grass and legume presence also filtered for a shift in functional trait composition of invader species. Legume presence in the sown communities favored invaders with traits characteristic for rapid nutrient uptake and cycling (higher specific leaf area). In the presence of grasses, invaders with traits indicative of nutrient retention or with the ability to symbiotically fix N_2 were particularly successful, suggesting that resource competition limited the success of invaders (Roscher, Gerighausen et al. 2015). The role of resource competition for invader success was also supported by the fact that the success of naturally dispersed invaders was negatively affected by the cover (aboveground competition) as well as root length density (belowground competition) of the established vegetation (Roscher, Beßler et al. 2009). More directly, Scherber, Mwangi et al. (2010) demonstrated in an experiment where shading by neighbors was manipulated, that both above- and belowground competition was important for the performance of *K. arvensis* transplants. However, resource availability only explained some of the diversity effects on plant community invasibility. Several invasion studies in the Jena Experiment demonstrated that resident (i.e. originally sown) species exerted a strong negative effect on invader species of the same functional group (Mwangi et al. 2007; Roscher, Schmidet al. 2009; Petermann, Fergus et al. 2010). These results support the view that invasion resistance is related to the degree of niche overlap between resident and invader species, however, not only with respect to resource use, but also through other mechanisms, e.g. pathogen-driven negative soil feedbacks (Petermann, Fergus et al. 2010).

Overall, the number and cover of invader species increased with increasing time after sowing the biodiversity experiment (Roscher, Fergus et al. 2013). For example, averaged across all diversity levels, 13, 17 and 22 invader species were found in 1-, 4- and 7-year-old communities, respectively, on a subplot of 4.5 m^2 size one year after cessation of weeding (Roscher, Gerighausen et al. 2015). In particular, communities sown with a lower initial plant species richness became less invasion-resistant with increasing time since the establishment of the biodiversity experiment (Roscher, Gerighausen et al. 2015). Therefore, it is likely that the lower invasibility early in the experiment was not only due to seed limitation (Roscher, Beßler et al. 2009). Instead, imbalanced depletion of resources and the accumulation of soil-borne pathogens led to a more pronounced decline of invasion resistance in plant communities sown with lower diversity after several years. Importantly, plant invasion was also found to be affected by multitrophic interactions, such as the activity of decomposers, herbivores, and pathogens, often in interaction with plant species richness (Scherber, Milcu et al. 2006; Eisenhauer et al. 2008; Nitschke et al. 2010).

To summarize, the Jena Experiment has contributed to the body of literature that shows that plant species richness and functional group composition of the resident community strongly affect the colonization of new species into the community. Results from the Jena Experiment show that a number of mechanisms contribute to invasion resistance, in particular several niche dimensions including overlap in resource use and pathogens. The Jena Experiment was the first to show that invasion resistance is changing over time and it emphasized the role of trophic interactions. The results also point to the research needs in this area: because resource competition and biotic interactions both affect the success of a species invading a community, more studies are needed

that disentangle these processes, focussing in particular on belowground interactions.

Responses of other trophic levels to plant biodiversity

Autotroph higher plants fix most of the carbon in terrestrial ecosystems and therefore provide the resources used by other organisms, from microbes to mammals. As a consequence, a number of hypotheses predict that heterotroph biomass and diversity should increase with increasing plant diversity (Siemann 1998). A major aim of the Jena Experiment was to investigate the consequences of increasing plant species richness for trophic interactions. Thus, from the beginning of the experiment, the aim was to comprehensively sample the responses of the communities of heterotrophic organisms along the plant diversity gradient. The large experimental plots of the main experiment allowed for representative sampling of below- and aboveground living organisms, as well as for the study of species interactions, such as pollination.

Overall, increasing plant species richness increased the abundance and species richness of almost all taxa investigated (Scherber, Eisenhauer et al. 2010). As detailed in Section "The footprint of diversity", the response of aboveground organisms was on average stronger than that of belowground organisms, and consumer species richness responded more strongly than consumer abundances (Scherber, Mwangi et al. 2010; Allan, Weisser et al. 2013). Below we summarize the results for the different taxa.

Aboveground consumers

Aboveground invertebrates, in particular insects, spiders and other arthropod groups (isopods and myriapods) were sampled from 2003 onwards, mainly by suction sampling and pitfall trapping. In addition, the occurrence of plant-dwelling fungi was assessed repeatedly. Mammals, in particular voles were assessed using life-trapping and the counting of holes.

Until 2010, about 1000 species were collected by pitfall sampling and suction sampling alone. Across all years, increasing plant species richness increased the abundance and species richness of most invertebrate consumers (herbivores, carnivores, parasitoids, omnivores, aboveground decomposers) (Scherber, Eisenhauer et al. 2010; Ebeling, Meyer et al. 2014; Hertzog et al. 2016). The saturating shape of responses was similar for the observed trophic groups, but plant species richness effects dampened with increasing trophic level and for omnivores (Scherber, Eisenhauer et al. 2010). Not only consumer abundance and species richness changed across the plant species richness gradient, but also the complexity of multitrophic functional group interaction webs (Rzanny & Voigt 2012). Compared to high plant diversity plots, interaction webs occurring in plots with low plant diversity were characterized by significantly lower interaction diversity, connectance and mean interaction strength (Rzanny & Voigt 2012).

Changes in the abundance of different trophic groups were mainly driven by bottom-up effects, tested using structural equation models based on either top-down, bottom-up, or both concepts (Scherber, Eisenhauer et al. 2010). Similar mechanisms hold for interaction webs and arthropod functional group composition, where the mean interaction strength between different arthropod functional groups was highest between adjacent trophic levels (e.g. plant-herbivore or herbivore-carnivore interactions) (Rzanny & Voigt 2012). The highest fraction of variance in arthropod functional group composition was explained by plant diversity, in addition to effects of plant biomass and legumes (Rzanny & Voigt 2012; Rzanny, Kuu, & Voigt 2013).

In a more detailed analysis, Hertzog et al. (2016) explored the mechanisms by which plant species richness affected abundance and species richness of arthropod herbivores and carnivores. Structural equation modelling showed that the increase in species richness in herbivores was caused by an increase in plant species richness, and not by the concomitant increase in plant biomass, while increases in carnivore richness were driven by increases in plant productivity (Hertzog et al. 2016). Evenness of herbivore communities did not change along the gradient in plant species richness, whereas evenness of carnivores declined with increasing species richness. The abundance of dominant herbivore species showed no response to changes in plant species richness, but the dominant carnivores were more abundant in species-rich plant communities. With respect to plant functional groups, there was little effect of the functional composition of plant communities on herbivore communities, whereas carnivore communities were affected by forbs of small stature, by grasses and by legumes. These contrasting patterns in the abundance of dominant species imply different levels of resource specialization for dominant herbivores (narrow food spectrum) and carnivores (broad food spectrum).

In addition to analyses of the entire arthropod community, a number of studies focused on single taxa, or on the performance of selected consumer species, across the gradient in plant species richness. For aphid herbivores (specialized plant suckers) it was found that aphid load (individuals/plant biomass) was highest at intermediate plant species richness (Petermann, Müller, Weigelt, Weisser, & Schmid 2010). When only the subset of plants on which these aphids can feed was considered (host plants), aphid load was negatively affected by both host plant biomass and host plant species richness (Petermann, Müller, Weigelt et al. 2010). Plant species richness also affected life history traits of aphids, e.g. the proportion of winged morphs (Petermann, Müller, Roscher et al. 2010). The density and species richness of aphid (primary) parasitoids was also highest at intermediate levels of plant species richness, while the densities and species richness of secondary

parasitoids (hyperparasitoids) declined linearly with plant species richness (Petermann, Müller, Roscher et al. 2010).

In contrast to aphid performance, survival and fecundity of the grasshopper *Chorthippus parallelus* did not significantly increase with increasing plant species richness (Specht et al. 2008), even though such generalist species should also benefit from increased plant species richness through increased opportunities for diet mixing, as shown in a laboratory study (Unsicker, Oswald, Köhler, & Weisser 2008). The performance of *C. parallelus* strongly differed between mixtures, even between plant communities consisting of grass species only, the preferred food of grasshoppers, in particular when only one or two grass-species were present in the mixture (Specht et al. 2008). One hypothesis explaining the lack of plant species richness effects is that beyond a certain threshold of species, there is sufficient opportunity for diet mixing, such that a further increase in plant species richness does not further increase grasshopper fitness (Franzke, Unsicker, Specht, Köhler, & Weisser 2008). The contrasting patterns for specialists and generalists show that taking consumer traits into account is an important next step to understand how consumers respond to changes in diversity.

Pollinators were quantified by direct observations of species visiting the plots. Overall, a positive relationship between the number of flowering plant species and measures of pollinator abundance, species richness and activity was found (Ebeling et al. 2008). In detail, pollinator visits to plants and pollinator species richness increased with increasing plant diversity, and the same was true for trap-nesting bees, wasps and their parasitoids. However, the shape of responses differed. Plant diversity increased the species richness and abundance of trap-nesting pollinators and the number of pollinator visits linearly, but the number of pollinator species followed a saturating curve (Ebeling et al. 2008). Plant diversity also changed the structure of plant-pollinator interactions and the composition of pollinator communities. High plant diversity led to more complex plant-pollinator interaction webs (higher linkage density) and increased the flower resource specialisation of solitary bees (Ebeling, Klein, Weisser, & Tscharntke 2012). These results indicate a higher stability of diverse pollinator communities, and that was in line with a higher temporal stability in the frequency of pollinator visits in plots with a high number of flowering plant species, high blossom cover and high interaction diversity (Ebeling et al. 2008, 2012). These results suggest that grasslands with high plant diversity enhance and stabilize pollinator communities and plant-pollinator interaction webs, which is likely to sustain effective pollination and plant reproduction in diverse grasslands.

To summarize, the invertebrate, in particular, arthropod community responded to changes in plant species richness. Studies on individual species suggest species-specific responses, pointing to the need to study in more detail the responses of species with different response traits. Because the individual study of interactions among more than 1000 species across the gradient of diversity is not feasible, a next step currently carried out is to construct food webs for each plot based on literature information on feeding behaviour. The analysis of these food webs can then be used to derive testable hypotheses about how plant species richness affects food web structure (Hines et al. 2015). The predictions can be tested in the field.

Among vertebrates, the dominant species present on the field site was the common vole, *Microtus arvalis*, whose abundance and behavior was studied using life-trapping, radio-tracking as well as by counting the number of holes. There was a weak positive effect of plant species richness on the number of voles trapped and the number of holes in plots (Scherber, Eisenhauer et al. 2010), as the species preferred to nest in plots with good cover from predation, mainly those with sufficient vegetation height, and with legumes present. Radio-tracking confirmed higher vole activity on plots with legumes, but no effect of plant species richness. The preference for legume-rich habitats was probably due to feeding preferences, with *O. viciifolia* and *Medicago* × *varia* as the most attractive food plants (Nitschke, unpubl. data).

With respect to pathogenic fungi, total fungal species diversity at the community level increased with increasing plant, i.e. host species diversity, whereas the per plot ratio of pathogen species per host species and the mean pathogen load per host species decreased (Rottstock, Joshi, Kummer, & Fischer 2014). This was most pronounced for legumes and least pronounced for small herbs. The presence and impact of the different pathogen groups were very similar in spring and early summer, and in summer and fall, respectively. In a separate study, the infection of *L. perenne* was studied, a species that was sown into subplots of all main plots along the diversity gradient (see Section "The Jena Experiment, its origin and design": Plant genetic diversity manipulations). Infection with the rust fungi *Puccinia coronata* and *P. graminis* decreased significantly with increasing species richness. This response to the diversity gradient was related to a decreased density and size of the host individuals with increasing plant species richness (Roscher, Schumacher, Foitzik, & Schulze 2007).

Belowground consumers

Soil fauna was studied using steel soil corers and subsequent heat extraction. Until 2008, 50 nematode genera (Eisenhauer, Migunova, Ackermann, Ruess, & Scheu 2011), 27 Collembola species (Sabais, Scheu, & Eisenhauer 2011), five earthworm species (Eisenhauer, Milcu, Sabais et al. 2009), and 90 other meso- and macrofauna invertebrate taxa were sampled using this method (Eisenhauer, Milcu, Sabais et al. 2011). Plant community properties had large effects on the abundance and, more strongly, on the diversity, of soil animals (Eisenhauer, Milcu, Sabais et al. 2011). While the density and diversity of most soil animal taxa increased significantly with increasing plant species richness

(Scherber, Eisenhauer et al. 2010; Eisenhauer, Milcu, Sabais et al. 2011; Milcu et al. 2013), earthworm performance was mostly influenced by plant functional group composition (Eisenhauer, Milcu, Sabais et al. 2009). A complementary greenhouse study revealed that plant species richness stabilizes belowground processes and animal populations (Milcu, Thebault, Scheu, & Eisenhauer 2010). In the long-term, plant species and functional richness turned out to be the most relevant plant community properties affecting soil biota, surpassing the importance of the presence of plant functional groups and of plant productivity (Eisenhauer, Milcu, Sabais et al. 2011; Milcu et al. 2013). In sum, results from the Jena Experiment suggest that plant species richness loss leads to belowground extinction cascades, which, in turn, lead to pronounced deterioration of fundamental belowground functions after a time-lag of several years, as described below.

Mycorrhiza

Grasslands are arbuscular mycorrhizal plant communities (Öpik, Moora, Liira, & Zobel 2006), and Glomeromycota, the fungal partners of arbuscular mycorrhiza (AM), comprise only several hundreds of taxa, while the diversity of AM plant partners is at least three orders of magnitude higher (Buscot 2015). However, relationships between species richness of plants and arbuscular mycorrhizal fungi (AMF) had been shown both in previous field studies (Borstler, Renker, Kahmen, & Buscot 2006) and in microcosm experiments (Johnson et al. 2004), albeit only for a limited gradient of plant species richness. AMF diversity and species composition were analyzed on all 82 large plots of the Jena Experiment in spring 2007, focusing on bulk soil, as this compartment comprises the largest AMF species richness compared to roots and spores extracted from soils (Hempel, Renker, & Buscot 2007). AMF sequence type richness increased with increasing plant species richness and this effect was not mediated by the higher plant productivity in high diversity plots (Koenig et al. 2010).

Soil microorganisms

Soil microorganisms and microbial growth depended on plant species richness (Eisenhauer, Bessler et al. 2010). Supporting the singular hypothesis for plant diversity, the results suggest that plant species are unique, each contributing to the functioning of the belowground system. Over the years, the microbial community adapted to the prescribed grassland plant communities (Strecker et al. 2016). In the dominance experiment, overyielding in soil microbial biomass occurred in 61% of 24 plots containing six species, and transgressive overyielding (mixure yield higher than the most productive monoculture) occurred in 13–21% of the plots, with some differences among seasons. Plots with nine species showed overyielding in all cases, and 25–50% of these plots showed transgressive overyielding (Guenay, Ebeling, Steinauer, Weisser, & Eisenhauer 2013). These results indicate the importance of plant complementarity effects on soil microbial biomass in diverse plant communities and thus highlight the significant consequences of plant species richness for soil processes.

Alongside with changes in soil microbial biomass along the plant species richness gradient, there were significant shifts in soil microbial (i.e. total bacteria and total fungi) composition (Habekost et al. 2008; Lange et al. 2014). These significant shifts were probably driven by the availability and quality of organic resources (Lange et al. 2015; Mellado-Vazquez et al. 2016). The few significant changes observed in the first four years after the establishment of the experiment point again to a time-lag in belowground responses to the plant diversity manipulations. Structural equation modelling revealed that plant species richness impacts the soil microbial community not only by changes in the availability of organic resources but also via changes in abiotic factors, such as soil water content (Lange et al. 2014). Moreover, bacteria were more strongly affected by changes in abiotic conditions, while soil fungi were more affected by plant-derived organic matter inputs. The changes in microclimatic conditions and resource availability and quality along the plant species richness gradient are likely to underlie the positive effect of plant species richness on bacterial and fungal diversity (Lange et al. 2015).

The shifts in the microbial community also resulted in functional changes with increasing plant diversity. For example, soil bacteria produce the antifungal compounds 2,4-diacetylphloroglucinol (DAPG) and pyrrolnitrin (PRN) that can be used to study the effect of plant species richness on soil suppressiveness (Latz et al. 2012). The abundance of DAPG and PRN producers increased with increasing plant species richness, and that of PRN was increased in the presence of grasses. Moreover, legume species richness and coverage, respectively, decreased the abundance of DAPG and PRN producers, contrary to the beneficial effects of legumes on soil microorganisms reported previously. In turn, soil suppressiveness was at maximum when DAPG and PRN producer abundance was high. These results suggest that plant species richness contributes to plant community resistance against pathogens by fostering beneficial bacterial communities, which may have important implications for developing more sustainable and environmentally friendly agricultural management strategies (Latz et al. 2012). Subsequent studies identified the main groups of soil bacteria driving soil suppressiveness (Latz, Eisenhauer, Rall, Scheu, & Jousset 2016) and the mechanistic links between plant community composition and soil suppressiveness (Latz, Eisenhauer, Scheu, & Jousset 2015).

To summarize, the Jena Experiment has provided evidence that the soil microbial community changes in response to changes in plant species richness. While the identity of the players is still being unraveled using metagenomics approaches, the available evidence already indicates that the changes are substantial and possibly underlie many of

the effects seen in below- and aboveground processes (e.g., poor performance of monocultures known as 'soil fatigue' in agriculture, see Section "Weeding issues and monoculture performance" for more details and Cortois, Schröder-Georgi, Weigelt, van der Putten, & De Deyn 2016; Zuppinger-Dingley, Flynn, De Deyn, Petermann, & Schmid 2016).

The effects of plant species richness on plant community biomass

Aboveground plant biomass

Aboveground plant biomass is the variable measured in all biodiversity experiments where plant diversity is manipulated, and the positive effect of plant species richness on aboveground plant biomass was the first reported effect of biodiversity on ecosystem functioning (e.g., Naeem et al. 1994; Tilman, Wedin, & Knops 1996; Hector et al. 1999). It was therefore reassuring that already in the first year after establishment, a positive effect of plant species richness on aboveground plant biomass was found in the main experiment (Roscher et al. 2005). In fact, aboveground biomass production significantly increased with increasing species richness in all years, in the main experiment (Roscher et al. 2005; Marquard, Weigelt, Temperton et al. 2009; Marquard et al. 2013), in the management and drought subplots (Weigelt et al. 2009; Vogel et al. 2012), and in the dominance experiment (Roscher, Schumacher, Weisser et al. 2007); thus the positive effect of biodiversity on productivity occurred irrespective of plot size, species pool, length of the diversity gradient, resource availability and disturbance regime. Similarly, there was a significant increase in vegetation height and leaf area index (LAI) with increasing species richness in the main experiment and in the dominance experiment (data in Lorentzen et al. 2008; Weigelt et al. 2010).

Species richness effects may comprise several components, in particular effects of species richness, functional group richness and the presence of individual functional groups, when these components of plant diversity are confounded in the experimental design, as had been the case in previous biodiversity experiments (Spehn et al. 2005). The effects of plant functional groups was supposed to be stronger than the effect of species richness, because plants from different functional groups may show higher complementarity, and hence higher biomass production, than plants from the same functional groups (e.g. Hooper et al. 2005). Similarly, individual functional groups such as legumes are known to have a disproportionate effect on plant biomass; hence, if the presence of functional groups is not controlled for by the design, increasing plant species richness can result in a stronger effect of legumes on plant community biomass through the sampling effect. The design of the main experiment allowed us to divide these components into the effects of species richness, functional group richness and the presence of individual functional groups. Species richness significantly affected productivity even after all effects of functional group richness and identity were accounted for (Marquard, Weigelt, Temperton et al. 2009). This supported the conclusion that species within functional groups are often not completely redundant in their functioning (Reich et al. 2004). However, the effect of increased species richness between different functional groups was stronger than within functional groups and led to transgressive overyielding and high (>1) relative yields. Additive partitioning revealed strong complementarity effects that increased over time, while selection effects decreased (Marquard, Weigelt, Temperton et al. 2009).

In the first year of the dominance experiment, aboveground plant community biomass increased strongly from monocultures to two-species mixtures, but only slightly along the rest of the gradient, i.e. from two to nine plant species (Roscher, Schumacher, Weisser et al. 2007). Overyielding and transgressive overyielding occurred more often among dominant species (68%) compared to species in the main experiment (23%) (Roscher et al. 2005), and the additive partitioning method (Loreau & Hector 2001) showed that the complementarity effect was stronger than the selection effect although both contributed to the net diversity effect (Roscher et al. 2005).

Showing the existence of a complementarity effect statistically does not, however, explain how plant species interactions depended on diversity (see also Section "Mechanisms underlying the biodiversity–ecosystem functioning relationships"). For example, positive effects of biodiversity on plant productivity may result from diversity-induced changes in the size or density of individual plants. In the main experiment, diversity-induced increases in productivity were due to increases in plant module density rather than to increases in module size (Marquard, Weigelt, Roscher et al. 2009), but variation in productivity within diversity levels was related to module size rather than module density. Twenty-four out of 26 overyielding species had denser populations, and 25 out of 28 underyielding species had smaller modules in mixtures than in monocultures (Marquard, Weigelt, Roscher et al. 2009), and similar effects were also observed in the dominance experiment (Roscher, Schumacher, Weisser et al. 2007). These observations of positive as well as negative relative yields of individual species stress the importance of compositional effects for mean community productivity, and thus confirm that the positive species richness effect on aboveground productivity was caused by a mixture of complementarity and selection effects (Marquard, Weigelt, Roscher et al. 2009).

To summarize, results from the Jena Experiment not only confirmed previous results on the importance of diversity for plant aboveground biomass, but the design of the Jena Experiment also allowed to disentangle effects on biomass production of different components of diversity. The availability of all monocultures allowed to study the responses of plant species to the manipulation of diversity

and studies conducted in the Jena Experiment could therefore elucidate the positive effect on plant biomass in more mechanistic detail.

Aboveground biomass data of the Jena Experiment were used in a number of international synthesis studies that showed, e.g., that each species contributes to at least one function (Isbell et al. 2011), or that diverse plant communities are more stable against extremes in weather conditions (Isbell, Craven et al. 2015).

Belowground plant biomass

Belowground plant biomass is an important part of plant productivity, yet it is more difficult to measure than aboveground biomass, and hence early biodiversity experiments largely ignored belowground productivity. Those studies that included belowground biomass in their measurements often found that, similar to aboveground biomass, belowground plant biomass increased significantly with species richness, in large-scale biodiversity experiments (Tilman et al. 2001; Reich et al. 2004; Fornara & Tilman 2008; Mueller, Tilman, Fornara, & Hobbie 2013; Cong et al. 2014; Ravenek et al. 2014), in mesocosms (Mommer et al. 2010), and in pot experiments (Dimitrakopoulos et al. 2004; Hendriks et al. 2013). However, there were several exceptions where no such pattern was found (e.g. Gastine, Scherer-Lorenzen, & Leadley 2003).

In the Jena Experiment, root growth and belowground biomass was measured from the beginning of the experiment, using a number of methods including soil coring, in-growth cores and rhizotrons. In the second year (2003), there was no positive biodiversity effect on belowground plant biomass despite a positive effect on aboveground biomass (Bessler et al. 2009). The significant effect of biodiversity on root biomass was only observed in later years: four years after the start of the experiment, the 16-species mixtures had 44% higher standing root biomass than the monocultures (Ravenek et al. 2014). This delayed response may be a reason why some earlier but short-term experiments did not observe a positive plant species richness–root biomass relationship.

However, the results from the Jena Experiment are also in contrast to studies in the outdoor mesocosm facility (Nijmegen Phytotron), where positive interspecific root responses preceded the aboveground biodiversity effect (Mommer et al. 2010; Padilla et al. 2013). One possible explanation is that the soil in which an experiment takes place is important (Jena: clay vs. Phytotron experiments: loamy sand), and hence soil-dependent differences in nutrient and water availability. If there are more nutrients available in a soil, the need to invest in roots may be less, i.e. in Jena. Next to differences in soil resources, soil microbial communities including soil-borne pathogens may also affect belowground biomass. Importantly, when comparing diversity effects on aboveground and belowground biomass, methodological aspects also have to be considered. Whereas aboveground biomass is quantified by measuring the yearly biomass increment, belowground biomass in biodiversity experiments is often quantified by measuring the standing biomass which has accumulated since the start of the experiment (Mueller et al. 2013; Ravenek et al. 2014). Thus, diversity effects on belowground biomass include not only productivity but also root turnover. Root turnover can be reduced by low infection with soil-borne pathogens and low soil concentration of nutrients (Dawson et al. 2003), i.e. soil conditions which are facilitated by high diversity (Roscher, Thein et al. 2008; Maron, Marler, Klironomos, & Cleveland 2011; Oelmann, Buchmann et al. 2011). This is why the measurement of root turnover is currently an active research area in the Jena Experiment. Nutrients and pathogens may also explain the differences in the development of rooting patterns in the different experiments.

To summarize, the Jena Experiment has shown significant plant diversity effects on root biomass, with this effect materializing only after a time-delay of several years. The reasons for the observed time-delay, and for different results between those from the Jena Experiment and other experiments, still need to be unraveled.

The effects of diversity on biogeochemical cycling

The Jena Experiment was also designed to quantify how nutrient and water cycling is affected by plant species richness. The aim of the Jena Experiment was to provide a full quantification of the N, P and C cycles and of the water balance, along with key biological groups associated to these cycles.

Water balance

Assessing the water budget is difficult but a prerequisite for understanding not only plant water acquisition and habitat conditions for the soil fauna and microbial communities, but also for understanding nutrient cycling. The water budget of the plant communities was addressed using a combination of approaches: (1) comparison of precipitation, potential evaporation and evapotranspiration from Bowen ratio measurements, (2) measurements of soil water potential and soil water content and subsequent modelling of the water balance with a soil water balance model, (3) estimation of uptake profiles of communities with lysimeters in the Jena Ecotron Experiment as well as high resolution time series analysis of soil water content and isotope labelling, and (4) measurements of soil hydraulic properties.

The average yearly precipitation at the site of the Jena Experiment in the years 2003–2013 was 598 mm, and was almost balanced by the potential evapotranspiration of 573 mm (calculated according to Priestley & Taylor 1972). In most years, potential evapotranspiration exceeded

rainfall during the main growing season (May–September), by up to 90% (median 146 mm, 48%). Despite this deficit, daily averages of potential and actual evapotranspiration were well correlated ($R^2 = 0.94$, $p < 0.001$, $N = 276$), even in comparatively dry years like 2005, suggesting that overall evapotranspiration on the site is limited by the availability of energy rather than water (Hildebrandt, unpubl. data).

Increasing plant species richness increases aboveground plant biomass, which can increase transpiration and affect evaporation via increased shading. Approach (2) indicated that evaporation in topsoil (0–0.06 m and 0–0.1 m) was decreased in the more diverse mixtures, because of increased shading, resulting in a positive relationship between plant species richness and soil water contents in this top layer (Rosenkranz, Wilcke, Eisenhauer, & Oelmann 2012). Soil evapotranspiration as well as percolation were also estimated using a water balance model based on soil water content measurements from May 2003 to January 2006 (Leimer, Kreutziger et al. 2014). Plant species richness did not significantly affect soil water content in both the 0–0.3 m and the 0.3–0.7 m soil layers, probably because the higher water demand by increased aboveground biomass production in the more diverse mixtures was counterbalanced by the effect of more pronounced shading on evaporation. Grasses generally decreased soil water contents and downward water flux, and increased estimated actual evapotranspiration in the 0–0.3 m soil layer. This is because the extensive rooting system of grasses allows for more exhaustive resource use while grass leaf structure at the same time reduces shading (Leimer, Kreutziger et al. 2014). The opposite was observed for mixtures containing legumes, where increased vegetation cover caused more shading while rooting densities were lower in 0–0.3 m soil depth. With time after establishment, soil water contents and fluxes were increasingly influenced by species-richness induced changes in soil structure (see below, Fischer, unpubl.).

Plant species richness positively affected water use efficiency (WUE), as shown in the lysimeter study, conducted in 2012 on 12 monoliths from the Jena Experiment (see Section "The Jena Experiment, its origin and design": approach (3), The Jena Ecotron Experiment), probably because of higher nutrient-use efficiency in more diverse plant canopies (Milcu et al. 2014). Evapotranspiration increased with leaf area index (LAI), which in turn was positively related to plant species richness (Milcu et al. 2016). The ratio between soil water loss through transpiration and soil evaporation, an important determinant of water use efficiency, was greater in diverse compared to less diverse mixtures. This supports the hypothesis of reduced evaporation and increased transpiration in species-rich mixtures. Variations in the strength of reduction of evaporation and the increase in transpiration over time would explain why the relationship between water contents or fluxes and plant species richness varied with time and soil depth. Interestingly, while root length density was independent of diversity, root water uptake was significantly greater in 0.3 m and sometimes also in 0.6 m depth in diverse mixtures. This was related to increased cover of tall herbs in diverse mixtures, which exhibited also more negative leaf water potentials and higher stomatal conductance, and allowed for a more dynamical adjustment of uptake depth by their tap root system. Overall, this led to more complementary root water uptake in diverse compared to less diverse mixtures during times of high water demand (Guderle, unpubl.). Those results from the Ecotron comply with earlier findings from the Jena Experiment field site, where water contents in the 0.3–0.7 m soil layer and deep vertical downward flux decreased in plant mixtures containing tall herbs compared to those without (Leimer, Kreutziger et al. 2014). In contrast, niche partitioning was not observed during times of low water demand (beginning of the growing season and shortly after mowing), in a labeling study within the main plots of the Jena Experiment field site (Bachmann et al. 2015). This may indicate that niche partitioning for water is not omnipresent, but reduced to times of high water demand.

Plant species richness and specific functional groups also affected soil properties important for water holding capacity and soil water flow, in addition to the effects on root water uptake and shading (Fischer et al. 2014). Using approach (4), Fischer et al. (2015) found that soil bulk density and porosity were positively related to plant species richness through an increase in soil organic C in the more diverse mixtures. This had an effect on soil hydraulic conductivity in the topsoil, which increased with plant species richness in spring and fall 2012. Also in deeper soil layers (0.2–0.4 m depth), soil water contents were affected by changes in soil structure, namely increase of soil organic carbon and decrease of the soil bulk density, both decreasing soil water contents due to enhanced drainage (Fischer, unpubl.). Also, the presence of grasses decreased and the presence of legumes increased earthworm activity and macropore flow (Fischer et al. 2014).

In summary, plant species richness was shown to affect several properties of the water cycle, including soil water content, water fluxes and soil hydraulic properties. Interestingly, the effect of plant species richness extended as far as changing soil properties, i.e. soil structure, within a rather short time period. Probably due to the mesic climate, resource partitioning for water was not very pronounced. However, in the Jena Ecotron Experiment, greater uptake at intermediate depth in more diverse mixtures was observed, probably related to the presence of certain functional groups (Milcu et al. 2014).

Nitrogen

Fig. 14 summarizes our current knowledge of plant species richness effects on the N cycle in the Jena Experiment. Overall, the net N budget was negative ($-6.3 \pm 1.1\ g\,N\,m^{-2}\,yr^{-1}$) (Oelmann, Kreutziger, Temperton et al. 2007), due to the annual harvest of biomass.

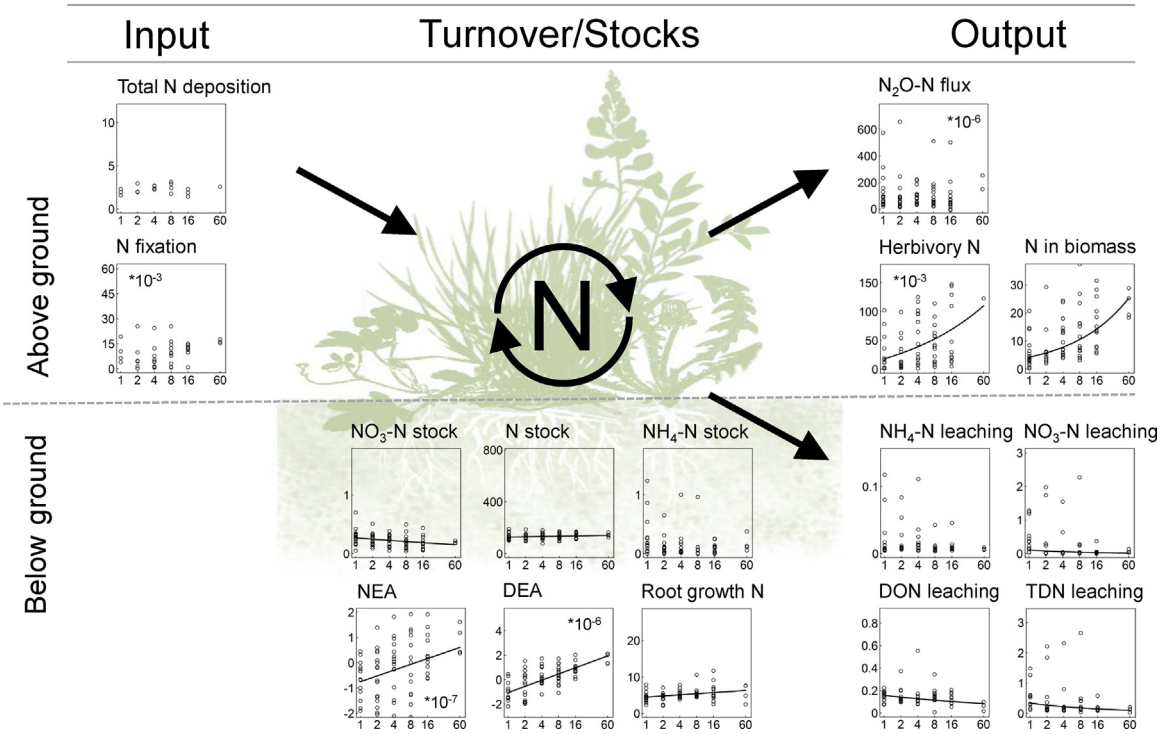

Fig. 14. Effects of plant species richness on different components of the nitrogen cycle. We fitted the same statistical model to all datasets. Block was fitted first, then log-transformed species richness. If necessary, dependent variables were log-transformed, as indicated by '[log]' in the legend. If the effect of species richness was significant in every year, we selected the most recent year for which data were available. If the effect of species richness was only sometimes significant, we selected the last year when the effect was significant. If the effect of plant species richness was never significant, we selected the most recent year for which data were available. Each graph is scaled by dividing all values by the mean value of the monocultures. The y-axis is scaled to the maximum effect of species richness on any of the processes presented in the figure, and the labelling of the y-axis gives the correct absolute values. Black regression lines are only shown when the effect of species richness was significant. When the dependent variable was log-transformed for analysis, back-transformation of data resulted in curved regression lines in the graph. If the model was not significant, no line is drawn. Extreme outliers are not shown in the graphs, in some cases, because they would have changed strongly the scale of the y-axis. Any further scaling is indicated within the graph (e.g. $*10^{-6}$, i.e. all numbers of the y-axis need to be multiplied by 0.000001).
Input: *Total N Deposition* [g m^{-2} yr^{-1}] from continuous collection of incident precipitation and throughfall in Block 2 in 2003 (particulate dry deposition estimated with the canopy budget model of Ulrich (1983) using chloride as tracer). *N fixation* [g m^{-2} yr^{-1}]: N_2 fixed by plant-associated rhizobia in 2006.
Turnover/Stocks: Plant-available (KCl-extractable) [log] *NO_3-N stock* [g m^{-2}] in the 0–15 cm soil depth layer in 2009. Total *N stock* [g m^{-2}]: amount of N in the 0–5 cm soil depth layer in 2011. Plant-available *NH_4-N stock* [g m^{-2}] in the 0–15 cm soil depth layer in 2012. *Root growth N* [g m^{-2} yr^{-1}]: N input by root turnover. *DEA* [g N (g soil dry weight)$^{-1}$ h^{-1}]: denitrifying enzyme activity. *NEA* [g N (g soil dry weight)$^{-1}$ h^{-1}]: nitrifying enzyme activity. NEA was positively related to plant species richness, but note that this effect disappeared when the positive effect of legumes in mixtures was taken into account (Le Roux et al. 2013). DEA was strongly and positively correlated to richness, this effect remained when the effects of plant functional types were taken into account (Le Roux et al. 2013).
Output: *N_2O-N flux* [g m^{-2} d^{-1}]: measured as short-term potential gross N_2O emission in 2007–2008 (N_2-N losses were not measured). [log] *Herbivory N* [g m^{-2} yr^{-1}]: N stock in plant biomass consumed by invertebrate herbivores in 2012. [log] *N in biomass* [g m^{-2} yr^{-1}]: aboveground N stock calculated from elemental concentration and aboveground plant biomass production in 2007. *NH_4-N leaching* [g m^{-2} yr^{-1}] in 2006, [log] *NO_3-N leaching* [g m^{-2} yr^{-1}] in 2006, [log] *DON leaching* [g^1 m^{-2} yr^{-1}] in 2006 and [log] *TDN* leaching [g m^{-2} yr^{-1}] in 2006, i.e. total annual leaching of the different compounds from the 0–0.3 m soil depth layer obtained from sampling at 0.3 m depth using ceramic plates.

N input

Atmospheric N deposition and N_2 fixation are the two major processes of N input into this grassland ecosystem. Total N atmospheric deposition (wet + dry N deposition) was 2.3 ± 0.1 g N m^{-2} yr^{-1} from April 2003 to March 2004, and similar for all diversity mixtures, although reduced roughness of grass-containing mixtures decreased N dry deposition. With respect to N_2 fixation, the proportion of legume-derived N from the atmosphere in aboveground biomass (%N_{dfa}) differed significantly among the various legume species. On average, it was lower at the beginning of the experiment in 2004 (73 ± 20%) than in later years (2006: 80 ± 16%; 2008: 78 ± 12%), which was mainly due to lower %N_{dfa} in some legumes in the early years of the Jena Experiment, while all legumes showed high %N_{dfa} in the later years. %N_{dfa} increased with increasing species richness in 2004 and 2006,

but not in 2008, when the biomass production of legumes also declined to lower levels. Apart from legume species identity, plant species richness effects on N_2 fixation also depended on the ability of legume species to compete for nutrients and light (Roscher, Thein et al. 2011). Accordingly, the higher N uptake of mixtures with legumes was primarily confined to species-rich mixtures that were dominated by a legume with high N_2-fixation and a non-legume species with high N uptake (Bessler et al. 2012). To analyse the use of N_2 fixed from the air by the plant species in the community, four species, one species from each functional group, were transplanted as 'phytometers' into the plant communities (Temperton et al. 2007). The results showed significantly lower $\delta^{15}N$ values and higher N concentrations and N content per plant in communities with legumes, pointing to a facilitative role of legumes in grassland ecosystems that can be explained by reduced competition for soil nitrate if N_2-fixing legumes are present (Temperton et al. 2007). In a separate study, about 50 species of the Jena Experiment were analysed (Gubsch, Roscher et al. 2011). Grasses had reduced foliar $\delta^{15}N$ and $\Delta\delta^{15}N$ values (= foliar $\delta^{15}N$ − soil $\delta^{15}N$) in communities containing legumes indicating a direct use of legume-derived N depleted in ^{15}N (Gubsch, Roscher et al. 2011). In contrast, increased leaf N concentrations without changes in foliar $\delta^{15}N$ in small and tall herbs suggested that these species made use of additional mineral N not consumed by legumes (Gubsch, Roscher et al. 2011).

Aboveground N stock

Aboveground N stock in plant biomass tended to increase with time and was closely linked to total legume biomass (Oelmann, Wilcke et al. 2007). Maximum aboveground N stock was determined by total productivity and additional N sources associated with legumes (N_2 fixation). Plant species richness positively influenced aboveground N stock and this effect was mainly driven by biomass production (Oelmann, Buchmann et al. 2011). The finding that species richness significantly decreased $\delta^{15}N$ values and N concentrations of transplanted 'phytometer' plants, irrespective of the legume effect (Temperton et al. 2007), suggested a more exhaustive use of the available N pool in soil in line with the findings of Bessler et al. (2012). Analyses correcting for different soil $\delta^{15}N$ background showed that $\Delta\delta^{15}N$ (i.e., $\delta^{15}N_{plant} - \delta^{15}N_{background}$) values decreased with increasing species richness in grasses, while this was not the case in plots containing tall and small herbs. These shifts indicated the utilization of different N sources as well as increasing N partitioning among plant species with increasing species richness (Gubsch, Roscher et al. 2011). The higher N uptake of species-rich mixtures, however, depended mainly on the presence of specific legume-non-legume combinations rather than high species richness, as discussed above (Bessler et al. 2012). The Jena Ecotron Experiment also showed that nitrogen-use efficiency of the canopies was higher in 16-species mixtures compared to 4-species mixtures, the two diversity levels analyzed in the Jena Ecotron Experiment (Milcu et al., unpublished).

Mowing and subsequent removal of the biomass constitutes the main N loss from the system. In the Jena Experiment, different components of soil N were analysed separately, in particular Dissolved Organic Nitrogen (DON, the difference between Total Dissolved Nitrogen (TDN) and Dissolved Inorganic Nitrogen (DIN), comprising N in nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+). DON is not a single compound but a mixture of compounds ranging from simple amino acids to complex humic substances. Legumes increased the removal of N with the harvest and decreased leaching of NH_4–N and DON from the canopy (Oelmann, Kreutziger, Temperton et al. 2007).

Belowground N pools

In the first two years after establishment of our experiment, plant species richness reduced KCl-extractable NO_3-N concentrations in soil in the main experiment (Oelmann, Wilcke et al. 2007; Oelmann, Buchmann et al. 2011) as well as in the dominance experiment (Roscher, Thein et al. 2008). This supports the hypothesis of more efficient resource use by diverse communities resulting in depletion of nutrient concentrations in soil (Tilman et al. 1997; Hooper & Vitousek 1998; Scherer-Lorenzen, Palmborg, Prinz, & Schulze 2003; Palmborg et al. 2005). However, in the following four years (eight sampling campaigns), no relationship between plant species richness and NO_3-N concentrations in soil was observed in the main experiment. Availability of NH_4-N in soil showed the opposite pattern characterized by (i) a positive correlation with plant species richness and (ii) the appearance of this relationship in the later years 2006 and 2007 (Oelmann, Buchmann et al. 2011). Such a relationship had not been published before, probably mainly because of the short duration of most previous experiments focusing on nutrient availability in soil (Tilman et al. 1997; Hooper et al. 1998; Scherer-Lorenzen et al. 2003; Palmborg et al. 2005).

DIN was dominated by nitrate in the grassland plots (Oelmann, Kreutziger, Bol, & Wilcke 2007). In contrast to NO_3-N availability in solid soil, NO_3-N concentrations in soil solution decreased with increasing plant species richness in all analysed years (2003–2006), but this relation reversed if more than ca. 25% of legume species were included in the plant mixture (Leimer, Wirth, Oelmann, & Wilcke 2014). DON and TDN concentrations in soil solution decreased with increasing plant species richness (Oelmann, Wilcke et al. 2007). Plant functional group identity also played an important role for N concentrations in soil solution. The presence of legumes increased and the presence of grasses decreased NO_3-N concentrations compared to mixtures containing only small and tall herbs (Oelmann, Wilcke et al. 2007; Leimer, Wirth et al. 2014). These effects can be related to the symbiotic N_2-fixing ability of legumes and the dense and extensive rooting system of grasses that allows for a more efficient exploitation of N resources (Hooper et al.

1998; Scherer-Lorenzen et al. 2003; Oelmann, Wilcke et al. 2007).

Belowground N dynamics

Plant species richness significantly increased *in situ* net ammonification rates. This species richness effect probably acted indirectly through microclimatic differences among different plant species richness levels and subsequent effects on ammonification rates (Rosenkranz et al. 2012). This is in line with results on increased N mineralization rates under diverse mixtures likely due to increased quality of plant litter and litter input (Zak, Holmes, White, Peacock, & Tilman 2003; Dybzinski, Fargione, Zak, Fornara, & Tilman 2008; Fornara et al. 2011).

Potential nitrification strongly increased with an increasing percentage of legumes in the plant community, with a 60% increase from communities without legumes to communities containing legumes only (Le Roux et al. 2013). Taking into account this positive effect of the percentage of legumes, plant species richness had a negative effect on potential nitrification. In contrast, plant species richness positively affected potential denitrification, 20% higher at highest plant species richness than in monocultures (Le Roux et al. 2013). Potential denitrification increased in the presence of legumes (25% of legumes being enough for a maximal effect) but decreased with an increased percentage of grasses. The different components of plant species richness explained 60% and 44% of the overall variation in potential nitrification and denitrification, respectively, and plant functional group composition explained 13.4 and 1.6 times more variation of nitrification and denitrification, respectively, than plant species richness alone (Le Roux et al. 2013). The effect of plant species richness on potential denitrification was explained by an effect on soil moisture: when moisture was accounted for first, species richness was no longer significant (Le Roux et al. 2013). Furthermore, plant species richness-induced changes in soil moisture and nitrate concentration explained 76% of the observed effects of the different plant species richness components on denitrification, whereas dissolved organic C was not identified as a key driver for denitrification. The abundances of nitrifiers and denitrifiers were also measured in all plots. The effect of plant species richness on potential nitrification was entirely due to the build-up of nitrifying organisms (Le Roux et al. 2013). In contrast, species richness effects on potential denitrification were not related to changes in denitrifier abundances.

Losses of N as N_2O to the atmosphere are expected to be small at our study site ($<0.05\,g\,m^{-2}\,yr^{-1}$), because of predominantly aerobic conditions in soil constraining denitrification. Soil N_2O emissions were independent of plant species richness in the first years of the experiment (Oelmann, Kreutziger, Temperton et al. 2007). When measured later in the experiment (2007–2008), soil N_2O emissions decreased with increasing plant species richness in plots without legumes, and increased in plots with legumes (Niklaus et al. 2016). Structural equation models showed a complex effect of species richness on soil N_2O emissions, with both a direct negative effect, and an indirect positive effect via increased soil moisture stimulating inorganic N cycling (Niklaus et al. 2016).

TDN and NO_3-N leaching from the 0 to 0.3 m soil layer significantly decreased with increasing species richness and was higher in winter with larger differences between species richness treatments than in summer. This stronger effect in winter is probably caused by stronger depletion of available N through plant uptake during the vegetation period, which leaves less N in soil that could be leached during winter (Leimer 2013; Leimer, Oelmann, Wirth, & Wilcke 2015; Leimer et al. 2016). However, Oelmann, Kreutziger, Temperton et al. (2007) did not detect a significant species richness effect on any soil N flux, which is probably related to the smaller sample size used in this early study, which was restricted to only one of the four blocks of the whole experiment. DON leaching contributed most to TDN leaching, particularly in plots without legumes (Oelmann, Kreutziger, Temperton et al. 2007; Oelmann, Wilcke et al. 2007; Leimer et al. 2016). In 2006, species richness decreased DON leaching for the first time (Leimer et al. 2016). This finding coincides with the first detection of a significant positive correlation between species richness and microbial biomass (Eisenhauer, Bessler et al. 2010; Eisenhauer 2012; Strecker et al. 2016). Therefore, the significant species richness effect was attributed to enhanced degradation of DON by microorganisms in species-rich mixtures (Leimer et al. 2016). Legumes increased and grasses decreased NO_3-N, DON, and TDN leaching, which complies with their effects on NO_3-N, DON, and TDN concentrations in soil solution, likely enhanced by the increasing effect of legumes and the decreasing effect of grasses on downward water fluxes from the 0 to 0.3 m soil layer. NH_4-N leaching was not influenced by species richness and only in 2006, a significant positive effect of the presence of legumes on NH_4-N leaching was detected (Leimer 2013; Leimer et al. 2016). NO_3-N concentrations in soil solution and NO_3-N leaching as well as NH_4-N, DON, and TDN leaching were highest at the beginning of the experiment, shortly after conversion from a fertilized agricultural field to the unfertilized grassland, and much lower in the following years (Oelmann, Kreutziger, Temperton et al. 2007; Oelmann, Wilcke et al. 2007; Leimer 2013; Leimer et al. 2015; Leimer et al. 2016).

To summarize, the N cycle has been investigated in the Jena Experiment in much more detail than in other biodiversity experiments, and the results show that some components of the N cycle are significantly affected by plant species richness while others are not. Interestingly, N stocks in the topsoil did not decrease over time despite the continuous harvest, but rather tend to increase at higher species richness. The mechanistic analyses conducted so far point to complex relationships between plant species richness, the soil microbial community, biodiversity effects on the water cycle and plant-plant interactions, all of which may influence N uptake, N storage, and N loss

from the ecosystem. Furthermore, the effects changed over time because of advancing establishment of the plant communities and an ongoing transition from fertilized arable land to the unfertilized experimental grassland, which is accompanied by changes in the soil communities. Progress in understanding the effects of plant species richness on the N cycle will come by analyses bringing together the results of different biodiversity experiments as well as more detailed mechanistic analysis of N cycling.

Phosphorus

Fig. 15 summarizes the effects of plant species richness on phosphorus cycling. Using the installed suction plates, PO_4-P and Dissolved Organic Phosphorus (DOP) concentrations were measured continuously in soil solution [DOP is the difference between Total Dissolved Phosphorus (TDP, also total soluble Phosphorus) and Dissolved Inorganic Phosphorous (DIP). DOP, TDP, and DIP each consist of a mixture of compounds]. Volume-weighted mean (vwm) PO_4-P concentrations decreased from 0.1 mg l^{-1} in spring 2003 to 0.04 mg l^{-1} in winter 2004/05. The high PO_4-P concentrations in soil solution early after the establishment of the Jena Experiment were probably caused by the dissolution of fertilizer-derived P-containing minerals applied to the field before the Jena Experiment was set up. Initial total phosphorus (P_{tot}) concentrations in 2002, assessed by a modified method of Hedley, Stewart, and Chauhan (1982) did not, however, differ from P_{tot} concentrations in 2007 (Oelmann, Richter et al. 2011). Therefore, the possible dissolution of fertilizer-derived P-containing minerals did not affect the P_{tot} concentrations in soil in a way that was detectable with our methods. However, plant-available P concentrations ($NaHCO_3$-extractable P) were significantly higher in 2002 than in 2007. DOP concentrations were highest during winter 2003/04 following the exceptionally dry summer and the increased microbial activity because of rewetting in fall 2003.

The presence of legumes increased aboveground P storage in plants and decreased labile P_i concentrations in soil (Oelmann, Richter et al. 2011). During cold periods, vwm PO_4-P concentrations in soil solution increased in legume-containing mixtures, caused presumably by leaching from P-rich residues. Plant growth in most legume-containing mixtures was, however, not limited by P in the first five years of the experiment as N:P ratios above 16 were found in only 0–5% of mixtures containing legumes during the 2003–2007 study period (Oelmann, Richter et al. 2011). Nevertheless, we cannot exclude that legumes themselves, which require larger amounts of P for the energy-demanding N_2 fixation, became P-limited over time. Indeed, leaf P concentrations and foliar N:P ratios of legumes declined over time. In 2008, all legumes (with the exception of *Trifolium repens*) showed foliar N:P ratios >16 (Roscher, Thein et al. 2011).

Plant species richness did not significantly influence vwm PO_4-P concentrations in any season or depth ($0.06 < p < 0.6$) (Oelmann, Richter et al. 2011). There was a positive effect of plant species richness on P storage in aboveground biomass (Fig. 15) which was also true for P exploitation (i.e. stock of P in aboveground biomass divided by the sum of stock of bioavailable P in soil and stock of P in aboveground biomass) (Oelmann, Richter et al. 2011). vwm DOP concentrations in soil solution were not affected by plant species or functional group richness (always between not detected and 0.2 mg l^{-1}). Total dissolved P leaching from the canopy increased in the presence of grasses, which is attributable to the decreased P demand of grass-containing mixtures (Oelmann, Kreutziger, Temperton et al. 2007).

Budgeting 20 plots from the Jena Experiment showed that P input by bulk and dry deposition was 0.2 ± 0.01 g m^{-2} yr^{-1} (Oelmann, Kreutziger, Temperton et al. 2007). As there was no grazing, P cycling was largely constrained to the plots themselves (i.e., no excrement input or output). Accordingly, removal of biomass by mowing was the main P loss in the system, resulting in an overall negative P budget (-1.9 ± 0.2 g P m^{-2} yr^{-1}). Negative P budgets indicate that strategies to efficiently acquire P by plants and microorganisms in soil become increasingly important in the grasslands under study. This is corroborated by a positive relationship between plant species richness and alkaline phosphatases (organic-matter degrading enzymes) in later years of the experiment (Hacker et al. 2015). Reduced roughness of grass-containing mixtures also decreased dry deposition of P (Oelmann, Kreutziger, Temperton et al. 2007).

Overall, the results show that plant species richness affects some components of the P cycle. The effect on P storage in aboveground biomass (Fig. 15) is likely to be driven by the increased N uptake in more diverse plant communities, because the study system was N- and not P-limited, at least in the first years. It will be interesting to see if this also holds true in the longer run, when the grassland is likely to shift to (co-)limitation by P. This is indicated, e.g., by the decline in legume contribution to overall biomass and an increasingly negative correlation between N_2-fixation rates and leaf P concentrations in legumes (Roscher, Thein et al. 2011), which may be due to their higher demands for P associated with N_2 fixation.

Carbon

Soil C storage is a slow process. In the Jena Experiment, it took four years before renewed C storage was detected after the C loss due to the conversion of the site from a cropland to an experimental grassland (Steinbeiss, Bessler et al. 2008). C storage significantly increased with increasing plant species richness in the topsoil (0–0.1 m), while in deeper soil segments (0.1–0.3 m) C losses were significantly smaller with higher species richness (Fig. 16). Although increasing species diversity increased root biomass (see Section "The effects of plant species richness on plant community biomass"),

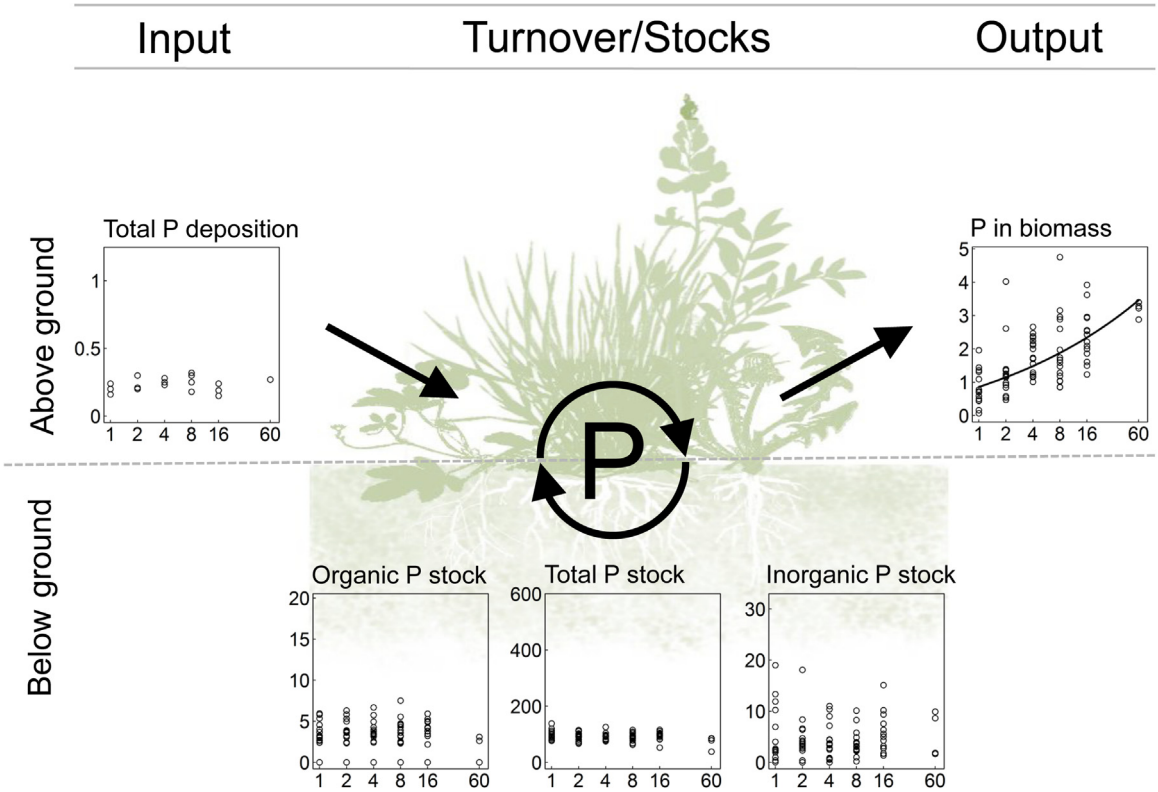

Fig. 15. Effects of plant species richness on different components of the phosphorus cycle. See Fig. 14 for explanations of how results are displayed.
Input: *Total P deposition* [g m^{-2} yr^{-1}] from continuous collection of incident precipitation and throughfall in Block 2 in 2003 (dry deposition estimated with the canopy budget model of Ulrich (1983), using chloride as tracer).
Turnover/Stocks: bioavailable *Organic P stock* [g m^{-2}] in the 0–20 cm soil depth layer, Total *P stock* [g m^{-2}] in the 0–15 cm soil depth layer. Species richness had a significant effect on P stocks in the 0-0.1 m depth layer of soil. However, this effect was attributable to the faster soil compaction with increasing species richness after conversion from cropping to grassland. If the reference soil depth was corrected for the compaction effect (i.e. proportionally reduced), there was no relationship between species richness and compaction-corrected P stocks. Therefore, we do not show a regression line. Bioavailable *Inorganic P stock* [g m^{-2}] in the 0–15 cm soil depth layer. .
Output: [log] *P in biomass* [g m^{-2} yr^{-1}]: aboveground P stock calculated from elemental concentrations and aboveground plant biomass production in 2007.

the increase in soil organic C concentration remained to be positively affected by plant species richness even when biomass was fitted first in sequential General Linear Models (Steinbeiss, Bessler et al. 2008). This indicates that plant species richness drives C storage not only by root biomass inputs; plant species richness also appears to influence the transfer of labile, plant-derived C (root exudates) to soil organic matter. A key role in this transfer process holds the microbial community (Gleixner, Poirier, Bol, & Balesdent 2002).

A recent study from the Jena Experiment described a mechanism for the positive plant species richness–soil C storage relationship (Lange et al. 2015). Statistical modelling showed that elevated C storage at high plant species richness is a direct function of elevated soil microbial activity in the high diversity plots. At higher plant species richness, rhizosphere C inputs into the microbial community are increased, resulting in both increased microbial activity and increased C storage. Thus, with increasing root biomass, soil C storage benefits not only from the direct input of (decaying) roots, but also from the increased access of the root-associated microbial community to plant root exudates (Mellado-Vazquez et al. 2016). In addition, increasing plant diversity strongly increased the diversity of organic compounds with low molecular weight, while this effect was weak for organic acids and phenolics (El Moujahid et al. 2017). Organic acid richness in bare ground plots was lower than the richness of organic acids in plots with plants, and the richness of the soil compounds increased twofold when plant species richness increased from 1 to 16 (El Moujahid et al. 2017). Plant species richness increased the richness of these compounds mainly through complementarity effects among plant species associated with contrasting spectra of soil compounds (El Moujahid et al. 2017). This could also explain previously

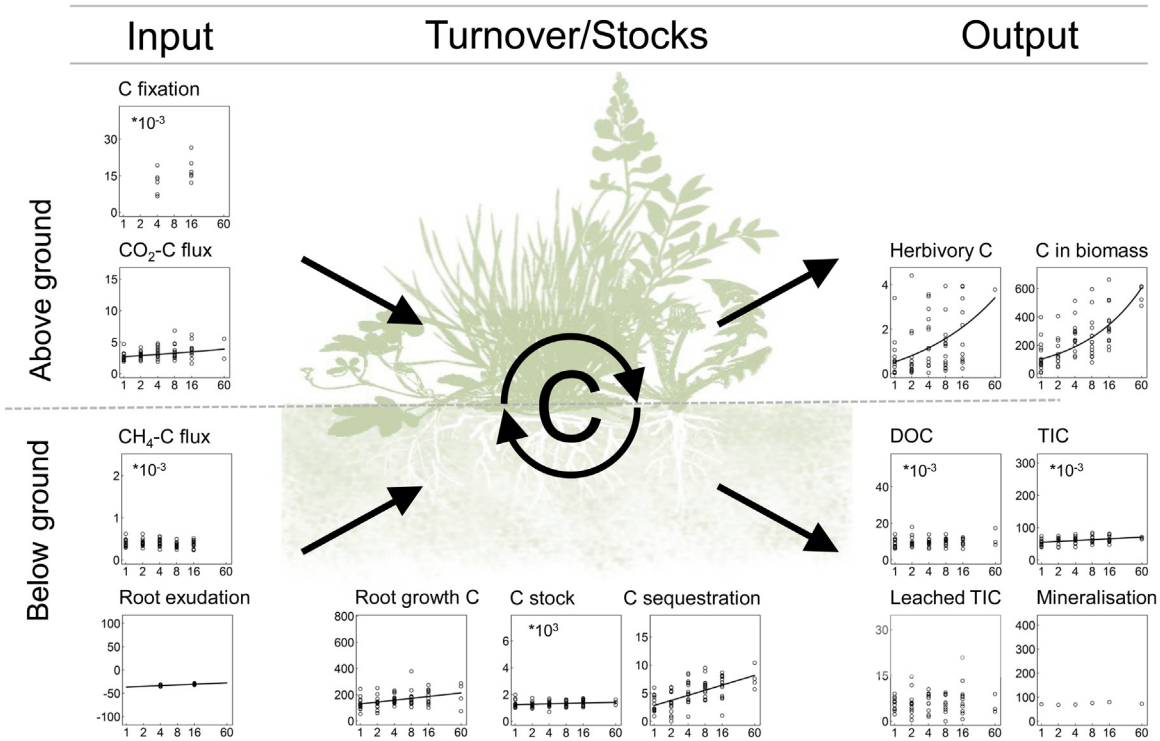

Fig. 16. Effects of plant species richness on different components of the carbon cycle. See Fig. 14 for explanations of how results are displayed. Input: *C fixation*, approximated by quantifying the ^{13}C excess of biomass in an Ecotron experiment [g m^{-2}]. [log] *CO_2-C flux* [g m^{-2} d^{-1}] in 2007–2008 and *CH_4–C flux* [g m^{-2} d^{-1}], each corrected for block effect. *Root exudation* [‰]: microbial uptake of recently photo-synthesized C measured as isotopic composition ($\delta^{13}C$) of phospholipid fatty acids by root-associated microbial community, measured in Ecotron experiment in 0–0.05 m topsoil.
Turnover/Stocks: *Root growth C* [g m^{-2} yr^{-1}] annual root biomass production as a measure of annual C input by root turnover. Total *C stock* [g m^{-2}]: total carbon in topsoil 0–0.05 m soil 2011. *C sequestration* [g kg^{-1}]: total increase in C between 2002–2011 in the 0–0.05 m topsoil in which C storage was strongest.
Output: [log] *Herbivory C* [g m^{-2} yr^{-1}]: C removed by invertebrate herbivory measured from leaf area loss in 2012 (Loranger et al. 2014). [log] *C in biomass* [g m^{-2} yr^{-1}]: total C in aboveground plant biomass calculated from elemental concentration and above ground plant biomass production (sum of summer and spring harvests in 2007). Concentrations of dissolved organic carbon *DOC* [g L^{-1}] and total inorganic carbon *TIC* [g L^{-1}] in soil solution in 0.3 m depth in 2012. Amounts of *Leached TIC* [g m^{-2} yr^{-1}] in 0.3 m soil depth in 2012. *Mineralisation* [g m^{-2}]: modelled decomposition of pre-experimental carbon in topsoil (0–0.05 m), until 2011.

reported effects of plant diversity on the diversity of soil heterotrophic microorganisms.

Another key aspect of the plant diversity effect on C cycling was that soil CH_4 uptake, a process driven by methanotrophic bacteria, tended to decrease with increasing plant species richness (effect becomes significant when multiple measurements are used and spatial autocorrelations are corrected for, Niklaus et al. 2016). Structural equation modelling revealed a concomitant indirect positive effect of species richness on CH_4 uptake, through increased soil moisture that stimulated nitrification (Niklaus et al. 2016).

Thus, plant species richness affected various components of the C-cycle, in particular C storage. Open questions that arise from these results are: (1) Will C storage continue over the next years until soil C profiles typical for grasslands are reached? (2) Will this storage process be faster in more diverse plots? (3) Does higher plant species richness increase the capability of soils to store C?

Stoichiometry

The Jena Experiment allowed for testing how plant species richness alters the integration of the elemental cycles. While stoichiometric consequences of altered producer diversity have been postulated (Hillebrand, Cowles, Lewandowska, Van de Waal, & Plum 2014), empirical evidence for such biodiversity effects remains rare.

Community stoichiometry was significantly influenced by species richness and functional group richness (Abbas et al. 2013). Increased C:P and N:P ratios with increasing species richness were observed in most years. Legumes and grasses had antagonistic effects on C:N ratios, with 27.7% lower C:N ratios in the presence of legumes and 32.7% higher C:N ratios in the presence of grasses. The reduction in variance of the chemical composition for all investigated element ratios with increasing species richness could possibly reflect an optimization of nutrient uptake at

high diversity (Abbas et al. 2013). The N:P ratios in plants generally reflected the ratios of available N:P in the soil (Abbas 2015). This correlation was significantly affected by plant species richness, more diverse plant communities had on average lower N:P ratios than predicted by the available N:P ratios.

Over time, the C:N ratios of plant material increased in the Jena Experiment, from an overall mean of 24 in 2003 to 37 in 2011, and this trend was significantly faster with increasing species richness (Guiz et al. 2016). The temporal trend in C:N was driven by changes in realized contributions of plant functional groups: at high species richness, legumes were replaced by forbs much faster than at low species richness, leading to a reduction in the N-concentration of the average plant matter and a more rapid increase in C:N. The temporal analyses also revealed a decrease in C:N-variability over time at highest diversity, while low-diversity plots showed an increase in variability over time. Thus, plant species richness stabilized the temporal variation in plant chemical composition.

These plant species richness effects on stoichiometry propagate to other trophic levels (Abbas et al. 2014). For representatives of herbivores (the grasshopper *Chorthippus parallelus*) and pollinators (the bee species *Chelostoma distinctum*), animal stoichiometry was positively related to plant stoichiometry (male bees: C:P and N:P; grasshoppers: C:N and N:P). Additionally, plant species richness was positively related to the C:N ratios of male bees and female grasshoppers. The role of plant C:N for the aboveground herbivore community was also shown in a separate analysis using structural equation modelling to explain decomposer and herbivore abundances. The analysis found that the overall abundance of herbivore insects and the transfer of plant material to the herbivores (% herbivory) were mediated via altered plant C:N ratios (Ebeling, Meyer et al. 2014).

Animal-mediated ecosystem processes

Many processes in an ecosystem are mediated by the animal community, which itself responds to changes in plant species richness. In general, a strong effect of plant species richness on the process rate of animal-mediated processes was found (Scherber, Eisenhauer et al. 2010). In this section, we highlight some of these effects in more detail focusing on processes mediated by above- and belowground invertebrates.

Aboveground herbivory

While plant species richness has been shown to consistently increase the abundance and diversity of herbivores (Haddad et al. 2009; Scherber, Eisenhauer et al. 2010; Borer, Seabloom, & Tilman 2012), effects of plant species richness on herbivory rates remain less clear. There are several hypotheses predicting that herbivore effects on plant communities should be affected by plant species richness (Loranger et al. 2014). While earlier theory predicted a decrease in herbivory with increasing plant species richness (Root 1973), later theory showed that rates of herbivory may increase or decrease with diversity, depending on herbivore behavior and the details of plant-herbivore interactions (Hambäck & Englund 2005). Empirical results are mixed; about half of the studies conducted so far found an increase of herbivory with plant species richness, while the other half showed negative or non-significant effects of plant species richness on herbivory (Meyer et al. 2017). Importantly, many of the studies were conducted only in a single year or over two years.

In the Jena Experiment, rates of herbivory were assessed repeatedly in the main experiment, both by measuring herbivory of the constituent members of the plant communities, and by using a phytometer approach for individual species. At the beginning of the experiment (2003-2005), community level herbivory rates tended to increase with plant species richness but effects were only weak or non-significant (Scherber, Mwangi et al. 2006; Scherber, Heimann, Kohler, Mitschunas, & Weisser 2010). In 2010, the percentage of standing leaf herbivory damage increased significantly with plant species richness in August, but not in May, while consumed leaf biomass increased significantly both in May and August (Loranger et al. 2014). In addition to plant species richness, the presence of individual functional groups affected levels of herbivory. Legume presence strongly increased, while grass presence decreased, herbivory at the community level (Scherber, Mwangi et al. 2006; Loranger et al. 2014). For phytometers, the effect of plant species richness on herbivory partly depended on the species used. In early years, herbivory on phytometers (*Rumex acetosa*, *Trifolium pratense*, *Plantago lanceolata*) showed negative, positive or neutral responses to plant species richness (Scherber, Milcu et al. 2006; Scherber, Mwangi et al. 2006). Later in the experiment (2008), four (*Cirsium oleraceum*, *Crepis biennis*, *Plantago media*, and *R. acetosa)* out of five tested phytometer species showed increased herbivory in mixtures compared to monocultures (Lipowsky et al. 2011). Thus, results from the Jena Experiment support the notion that rates of herbivory increase rather than decrease with increasing plant species richness, consistent with the overall increase in herbivore abundance and diversity. It was again important that the study was conducted over a longer time period, so that communities of herbivores could assemble. Herbivory is currently measured every year in the main or the trait-based experiment, and while there are differences between years, and between seasons, the positive effect of plant species richness on herbivory is now consistent in both experiments (Meyer et al. 2017).

A range of mechanisms has been identified to explain the levels of herbivory observed in the Jena Experiment. First, individual species and plant functional groups differed

in herbivory suffered in monocultures (Loranger et al. 2014). These differences among plant species could be largely explained by chemical traits of the plant species, most importantly leaf N and lignin concentrations. Other important plant traits were the number of coleopteran and hemipteran herbivores potentially feeding on the plants, leaf life span, stem growth form, and root architecture (Loranger et al. 2012). Second, interspecific interactions appear to change along the diversity gradient, affecting herbivory rates (Loranger et al. 2013). When the traits predicting herbivory in monocultures were used to predict herbivory in mixtures (additive models, Loranger et al. 2013), community level herbivory was higher in polycultures than what was expected from the monoculture data. In addition, traits identified to predict herbivory in mixtures also differed from those previously identified for monocultures (Loranger et al. 2013). The observed strong non-additive effects increased with increasing plant species richness. This result suggests that traits measured in monocultures may not be suitable to predict ecosystem variables in mixtures, due to changes in the interactions between plants, and between plants and herbivores (see also Section "Responses of individual plant species to plant species richness"). Plasticity of defense traits along the diversity gradient also contributed to altered herbivore-plant interactions along the diversity gradient, as exemplified by changes in leaf chemistry and herbivory of *P. lanceolata* (Mraja, Unsicker, Reichelt, Gershenzon, & Roscher 2011). Third, there are changes in the community of arthropod herbivores along the diversity gradient, in particular an increasing abundance of herbivores in plots of higher diversity (Ebeling, Meyer et al. 2014).

Despite the relatively low intensity of herbivory measured in the Jena Experiment (<2% of standing herbivory damage on the community level; Loranger et al. 2014), it had measurable effects on the plant community. The removal of herbivores by insecticide application increased both phytometer biomass (Scherber, Milcu et al. 2006) and overall plant community biomass (unpubl. data). These effects did not interact with but were additive to the effects of plant diversity which reduced plant height and inflorescence size of phytometer individuals, and increased community level biomass. Further, herbivores induced changes in plant community composition as demonstrated in an experiment with caged grasshoppers where herb cover increased consistently with increasing intensity of grasshopper herbivory (Scherber, Heimann et al. 2010). There was also some evidence for a selective effect of herbivores on genotype selection in the plant communities. In a study that compared, for several species, the performance of plants derived from different seed origins (monocultures vs. mixtures), the results were consistent with the hypothesis that herbivory counteracts rapid local adaptation of plants, potentially because of trade-offs between growth and defense traits (Lipowsky et al. 2011). A greenhouse study using seed families of *P. lanceolata* collected in the 6-year-old plant communities of the Jena Experiment, however, indicated that changes in leaf defence chemistry in response to higher plant diversity (Mraja et al. 2011) were caused by phenotypic plasticity and not due to a genetic differentiation with respect to defence compounds (Miehe-Steier, Roscher, Reichelt, Gershenzon, & Unsicker 2015).

To summarize, the Jena Experiment has increased the evidence that community-level herbivory increases with increasing plant diversity, and it has found that herbivory in mixtures is affected by plant interactions, that result in changes in plant defense traits (phenotypic plasticity) and also affect the herbivore (and natural enemy) community. A more recent review of previous studies showed that negative effects of plant species richness on herbivory mostly come from studies that investigated herbivory of individual species rather than at the community level (Meyer et al. 2017). Herbivory can vary drastically between plant species even within the same study site, and in plant species with very low rates of herbivory a diversity effect on herbivory is less likely. Thus, when herbivory of a single plant is investigated, there may be no effect of species richness on herbivory, or even a negative effect, even when overall community herbivory increases with increasing plant species richness.

Belowground fauna effects on plant productivity and invasion

Soil animals had significant effects on aboveground processes, such as plant community productivity, invasibility, and competitive interactions among plant species (Eisenhauer et al. 2008; Eisenhauer, Milcu, Nitschke et al. 2009; Eisenhauer, Milcu, Sabais, & Scheu 2009; Eisenhauer, Ackermann et al. 2010; Eisenhauer et al. 2010). These above-belowground interrelationships were supported by results from laboratory experiments showing that soil organisms not only affect plant communities (Partsch, Milcu, & Scheu 2006; Eisenhauer & Scheu 2008a, 2008b; Eisenhauer, Milcu, Sabais, & Scheu 2009; Eisenhauer, Horsch et al. 2010; Eisenhauer, Sabais, & Scheu 2011; Eisenhauer, Reich, & Isbell 2012) but that these effects also propagate to aboveground food webs (Eisenhauer, Horsch et al. 2010). Importantly, decomposers belonging to different kingdoms (microbial vs. animal decomposers) were found to show synergistic effects on plant and herbivore performance indicating the importance of soil organism diversity and interactions for the aboveground subsystem (Eisenhauer, Horsch et al. 2010).

Field manipulations of earthworm densities on subplots showed that the presence of earthworms increased total plant community productivity (+11%), in particular by enhancing legume shoot biomass (+35%) (Eisenhauer, Milcu, Nitschke et al. 2009). Although single plant functional groups, i.e. legumes, benefited from higher earthworm numbers, earthworm effects did not vary with plant species and functional group richness. As legumes also beneficially affect earthworms (Milcu, Partsch, Scherber, Weisser, & Scheu 2008; Eisenhauer, Milcu, Sabais et al. 2009; Fischer et al.

2014), earthworms and legumes may form a loose mutualistic relationship affecting essential ecosystem variables in temperate grasslands (Milcu et al. 2008; Eisenhauer, Milcu, Sabais et al. 2009), in particular decomposition.

Manipulating the number of plant-feeding nematodes, which are widespread and important herbivores, by applying the nematicide fosthiazate on nematode subplots (Eisenhauer, Ackermann et al. 2010) revealed that the presence of nematodes tended to decrease plant community biomass. However, the effects varied with plant functional group identity: they had a marginally significant effect on the shoot biomass of grasses, significantly affected herb biomass, but had no effect on legumes. This highlights that nematodes likely modify plant community structure of semi-natural plant communities by altering the competition between plant functional groups and by attenuating the diversity–productivity relationship (Eisenhauer, Ackermann et al. 2010; Eisenhauer, Milcu, Sabais et al. 2011).

Plant species richness had strong effects on plant community invasibility (see Section "Invasion into the target communities"), but soil animals were also found to play a role. Two transplanted phytometer plant species (*Centaurea jacea* [tall herb] and *Lolium perenne* [grass]) showed that belowground competition increased with increasing plant species diversity. However, belowground competition was modified by the presence and/or abundance of earthworms (Eisenhauer, Milcu, Nitschke et al. 2009) and Collembola (Eisenhauer et al. 2010), as shown by the increased shoot biomass of the phytometer *C. jacea* (+21%) in earthworm-enriched subplots and increased shoot biomass of both *C. jacea* (+60%) and *L. perenne* (+19%) in subplots with Collembola. Enhanced phytometer performance was likely due to increased nutrient availability in the presence of higher decomposer densities, which changed the competitive interactions among plant species and functional groups.

Analyzing natural invasion into soil animal subplots revealed that earthworms modified the diversity–invasibility relationship. While the impacts of earthworms were not significant in low diversity (low earthworm densities) or high diversity plant communities, earthworms increased invasion by plant species in plant communities of intermediate plant species richness (Eisenhauer et al. 2008). Both the number (+10%) and diversity (+12%) of plant invaders was significantly higher in earthworm subplots than in control subplots. Earthworms reduce aboveground seed predation and create nutrient-rich regeneration niches (earthworm middens), thereby enhancing invader success, particularly that of grasses (Eisenhauer et al. 2008; Eisenhauer & Scheu 2008b).

Grassland management, productivity and bioenergy production

The grasslands that serve as a model system for the Jena Experiment are managed extensively, with two cuts for haymaking and no fertilisation. This management is at the lower end of the grassland management intensity gradient in Central Europe. While these species-rich meadows are considered to be 'High Nature Value Grassland' (Paracchini et al. 2008), they are under increasing pressure of conversion into species-poor, highly fertilized leys, i.e. non-permanent grasslands sown with a seed mixture of commercial grassland plants, often after the field is ploughed. In permanent grasslands, intensification of grassland management by increasing fertilizer input, increasing the frequency of mowing, or, in the case of pastures, increasing stocking rates of grazing animals, results in strong decrease in diversity of many taxa, both above- and belowground, also at the landscape scale (e.g., Allan et al. 2014; Gossner et al. 2016). To hold the loss of biodiversity in grassland, low-intensive management is supported by agri-environmental schemes of the European Union, but the future of these grasslands is still uncertain.

Farmers and land managers have been interested in the results of the Jena Experiment and in the question whether results from a low-intensive management model system, in particular the effect of plant species richness on productivity, can be extrapolated to grasslands in general, especially to grasslands that are fertilized or that are mown more frequently. The Jena Experiment was thus used to answer the following questions, that have fundamental as well as applied relevance:

(1) Is the relationship between plant species richness and ecosystem functioning equally valid in high-input grassland systems?
(2) How large is the diversity effect on productivity compared to the 'management effect', i.e. the increase in productivity due to higher fertilizer input and more frequent mowing?

Low-input high-diversity grasslands have recently also been suggested to provide a solution for reducing the negative environmental consequences of biofuel production, because they do not require land conversion or the input of fertilizer or energy that strongly reduce the carbon and energy yield of conventional bioenergy production (Tilman, Hill, & Lehman 2006). Grassland biomass from 'High Nature Value Grassland' is rarely used for bioenergy production in Europe. This led to the following questions:

(3) How do grasslands of different diversity levels differ in properties relevant for bioenergy production?
(4) What is the relationship between plant species richness and bioenergy yield in European grasslands managed at low intensity?

Grassland management and the biodiversity effect on productivity

The interactions between management intensity and plant species richness were tested on five subplots within the

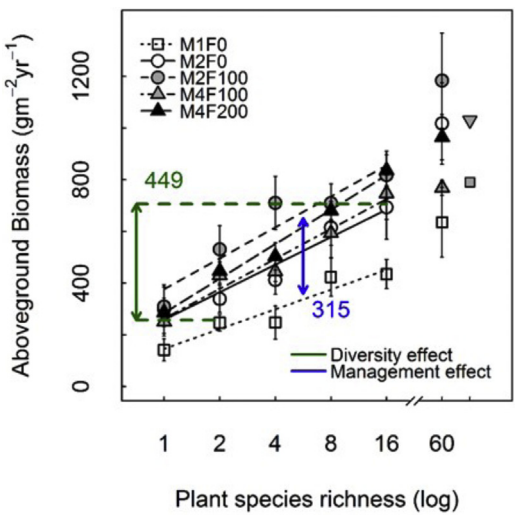

Fig. 17. Effect of grassland management on the plant species richness–productivity relationship (management experiment, modified from Weigelt et al. 2009). Values are mean annual forage yields as sum of the total yield production per year. Management treatments: Mowing frequency (*M*) is given in cuts per year (*M*1, *M*2, *M*4), all fertilisation values (*F*) are given in kg ha^{-1} yr^{-1}. Nitrogen was applied as NO_3-N and NH_4-N in equal proportions (0, 100, 200), phosphorus as P_2O_5-P (0, 43.6, 87.2) and potassium as K_2O-K (0, 83, 166). *M2F*0 represents the standard management intensity of the Jena Experiment. The blue arrow indicates the mean management effect, i.e. increasing management intensity from M1F0 to M4 F200 (315 g/m^{-2}), while the green lines and the green arrow indicate the mean biodiversity effect, i.e. the mean difference between monocultures and the 16-species mixtures (449 g/m^{-2}). Species poor grasslands which are agriculturally optimized for the single function of hay production (e.g., clover-grass mixtures using particular varieties) with fertiliser input (ca. 200 kg N ha^{-1} yr^{-1} and other nutrients) and up to six cuts per year can generally achieve forage yields between 1000 and 1400 g m^{-2} yr^{-1}. For the German state Thuringia, where the study site is located, mean forage yields are 790 g m^{-2} yr^{-1} for conventionally managed permanent grassland with fertilisation and 3–4 cuts per year (grey square on the right), and 1030 g m^{-2} yr^{-1} for clover-grass mixtures without fertilization (grey triangle on the right), according to the Thuringian Agricultural Institute (TLL).

large plots of the main experiment, by varying mowing frequency (1, 2 or 4 cuts per growing season) and NPK-fertilisation, characteristic of grassland intensification in Central Europe (see Section "The Jena Experiment, its origin and design": Management experiment). Aboveground community biomass along the plant species richness gradient was evaluated in two successive years, before management itself induced changes in the plant species communities. In both years, management intensification increased biomass production (Weigelt et al. 2009, Fig. 17). However, the positive species richness–biomass relationship was maintained, i.e. intensification increased productivity along the diversity gradient. Importantly, high-diversity low-input grassland communities (16 species, two cuts, no fertilisation) had a similar productivity as the average of the low-diversity highest-input communities (monoculture, 4 cuts, 200 kg N ha^{-1} year^{-1}). The overall biodiversity effect, i.e. the average difference between monocultures and 16-species mixtures was 449 g m^{-2} year^{-1}, higher than the average intensification effect (315 g m^{-2} year^{-1}, average difference between the lowest and highest intensification treatment) (Fig. 17, Weigelt et al. 2009). Thus, the biological mechanisms leading to enhanced biomass production in diverse grassland communities are as effective for productivity as a combination of several agricultural measures, in particular increased fertilization and mowing frequency. Unpublished results from the management experiment have additionally shown that forage quality, quantified using six forage quality indicators (organic matter, crude protein, usable raw protein, raw fat, neutral detergent fibre, metabolisable energy) was independent of mean plant species richness, and that forage quality yield (i.e. quality concentrations × biomass) increased with diversity due to the positive effect on biomass production (Scherer-Lorenzen et al, unpubl.).

In addition, soil N_2O emissions decreased with increasing plant species richness in the extensively managed plots, but were increased by fertilizer addition, while soil CH_4 uptake was not affected by fertilization (Niklaus et al. 2016). Thus, extensive management of species-rich measures may also contribute to climate change mitigation.

The management experiment also affected herbivore-plant interactions in the Jena Experiment. Fertilization increased community herbivory independent of the plant species richness gradient (Hudewenz et al. 2012). At the same time, fertilization increased generalist herbivore reproductive success and therefore ultimately abundances with resulting effects on herbivory (Ebeling et al. 2013).

To summarize, the Jena Experiment showed for the first time that the biodiversity effect on productivity is at least of the same magnitude, in absolute terms, as the average effect of land-use intensification on productivity. It also showed that the positive species richness — productivity relationship is maintained when grasslands are managed more intensively, at least in the short term and as long as this management does not decrease plant species richness in the more diverse plots. Both of these results are important as they show that the biodiversity effect on productivity is not restricted to conservation grasslands where productivity is generally not a management aim, but also to 'real-world' scenarios for multifunctional grasslands where productivity is one of the ecosystem services grasslands are expected to deliver.

Bioenergy production

We assessed how plant species richness affects the bioenergy potential of the Jena Experiment grasslands focusing on three major energetic conversion techniques. Overall, there was a positive effect of plant species richness on the bioenergy potential of the grasslands, because potential

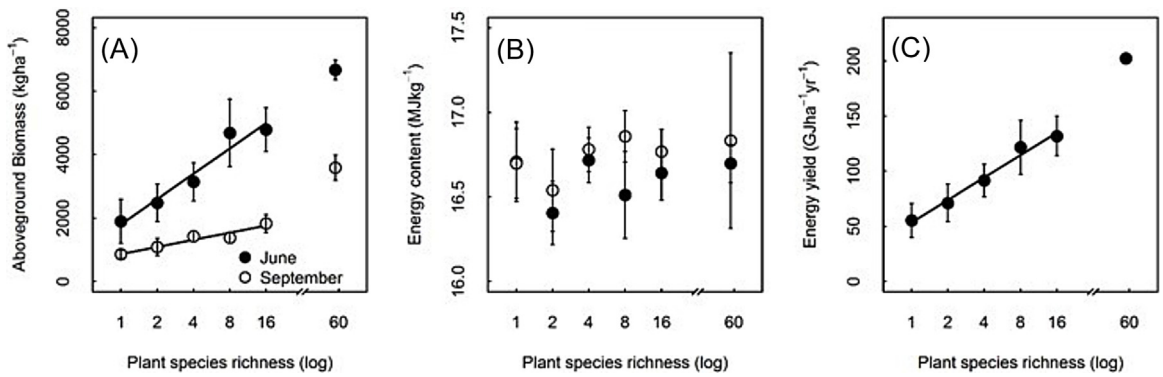

Fig. 18. The potential for bioenergy production from plant communities in the Jena Experiment. (A) Relationship between plant species richness and aboveground biomass for the spring and autumn harvest in 2008 (Weigelt et al. 2010). (B) Energy content calculated for plant biomass for spring (closed circles) and autumn (open circles) samples, (C) Total potential energy yield as the product of biomass * energy content summed over the year.

energy yield was independent of biodiversity while total biomass increased with species richness (Fig. 18, Khalsa 2012; Khalsa, Fricke, Weisser, Weigelt, & Wachendorf 2012).

Grassland biomass conversion via anaerobic methane production

Crude fibre (CF) increased, and crude protein (CP, important chemical component in biogas production) decreased, with increasing species richness, and increased in the presence of grasses (Khalsa 2012). This led to a negative effect of species richness on substrate-specific methane yield ($CH_{4\,sub}$), while area-specific methane yield ($CH_{4\,area} = CH_{sub} \times$ biomass) increased, due to the strong increase in aboveground plant biomass with increasing plant species richness. Decrease of CH_{4sub} was more pronounced in the first than in the second cut. The presence of legumes increased, whereas the presence of grasses decreased $CH_{4\,sub}$, in both cuts. This was also reflected in the monocultures, where grasses had the lowest mean values in both cuts, while legumes had the highest. Overall, for an unfertilized two-cut grassland, the level of $CH_{4\,sub}$ was relatively high. A major reason may have been the early cutting date in mid-May, compared to cutting dates in conservation grasslands at the end of June/start of July, when $CH_{4\,sub}$ is frequently well below 150 L_N kg^{-1} VS, where L_N is normal litre (Hensgen, Buehle, Donnison, Heinsoo, & Wachendorf 2014; Richter, Fricke, & Wachendorf 2011).

Grassland biomass conversion via hay combustion

Plant species richness and in particular legume presence increased energy output (Khalsa et al. 2012). One major problem for hay combustion is the presence of ash, as ash content negatively correlates with energy content (in this study, $R^2 = 0.54$), i.e. ash reduces the energy output. Mean ash content for the plant communities of the Jena Experiment was slightly higher than ash contents found in other grassland studies (Florine, Moore, Fales, White, & Burras 2006; Richter, Fricke, & Wachendorf 2010; Tonn, Thumm, & Claupein 2010). This may be partly explained by the bare ground found in low diversity plots. Higher proportions of bare ground may have led to an increasing amount of soil particles stuck to plant biomass, caused e.g. by heavy rainfall. Considering ash composition, plants differed in their effects on fuel quality. For example, legumes increased Ca concentrations (resulting in an increasing ash melting temperature, AST) and, at the same time, increased N concentrations (promoting NO_x emissions), whilst grasses reduced N concentrations (reducing NO_x emissions) but increased K concentrations (decreasing AST). Thus, in order to facilitate a high energy output in extensive grassland systems, the defoliation and nutrient management should focus on the maintenance of highly diverse, legume-based grassland communities. Given the high concentrations of corrosion and emission-related constituents, it can be concluded that the biomass from highly diverse grasslands is not suitable for commercial biomass combustion. Instead, a treatment of the fuel through nutrient leaching as well as technical adaptation of the combustion plants is recommended for grassland biomass at all levels of diversity.

Grassland biomass conversion by the integrated generation of solid fuels and biogas from biomass

To overcome the difficulties with unfavourable constituents and to increase energy efficiency of biomass conversion, it has been suggested that energy should be produced from grassland biomass by the integrated generation of both, solid fuels and biogas (known as 'IFBB', Wachendorf, Richter, Fricke, Grass, & Neff 2009; Buhle, Hensgen, Donnison, Heinsoo, & Wachendorf 2012). The basic process includes silage making of the raw biomass followed by soaking the material in warm water and mechanically squeezing the material using a screw extruder. The resulting liquid can be used in a biogas plant whereby the digestate can be returned to the field, to avoid depletion in mineral nutrients. The press cake can be used as solid fuel. Based on data from the main experiment, Khalsa (2012) showed the potential of the IFBB procedure to enhance solid

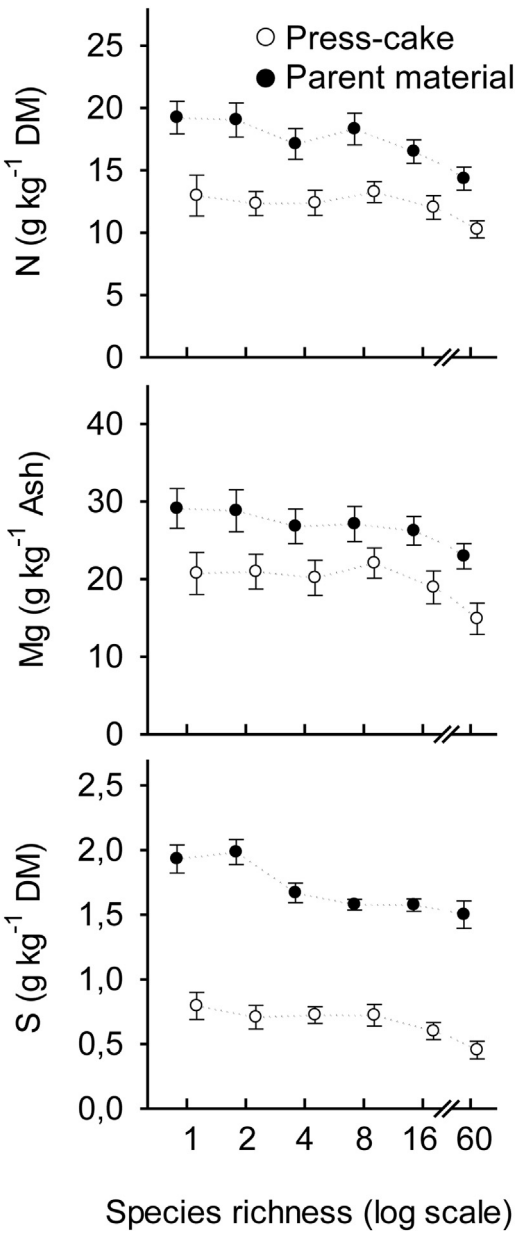

Fig. 19. S, Mg and N-concentrations in the biomass of the different diversity mixtures (redrawn from Khalsa 2012; Khalsa et al. 2012). Filled circles: parent material, open circles: press cake.

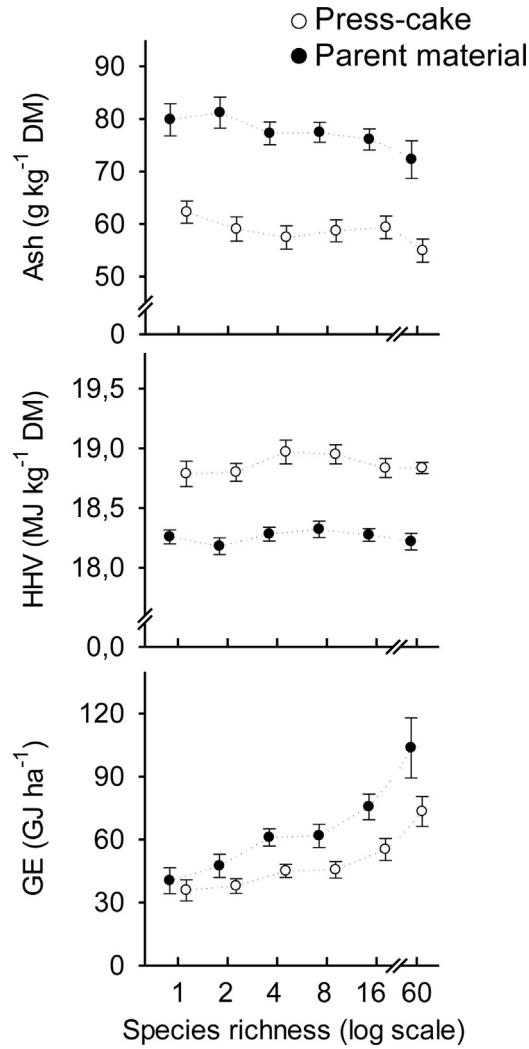

Fig. 20. Ash content, higher heating value (HHV) and gross energy production (GE) of press material and original biomass (parent material) as affected by plant species richness (redrawn from Khalsa 2012; Khalsa et al. 2012).

fuel quality through this hydro-thermal conditioning and mechanical dewatering of extensively managed grassland biomass. With increasing species richness, concentrations of Mg, N and S significantly declined in the parent material (Khalsa 2012). These biodiversity effects vanished after application of IFBB, i.e. the IFBB procedure led to equally low levels of concentrations across all species richness levels (Fig. 19). Further, the reduction of ash by 23% from the biomass to the presscake resulted in an increased energy content (expressed as higher heating value, HHV) by about 3.5%. However, as approximately 30% of the mass flow of dry matter is directed into the press-fluid and thus not available for combustion, total gross energy yield (GE) decreased by 27% (Fig. 20). Those 30% will be used for anaerobic digestion as part of the IFBB procedure with a mean CH_{4sub} of 299 L_N kg^{-1} VS. The biogas can then be used in a combined heat and power plant to produce electricity and heat, which is available for drying of the presscake to produce a store- and marketable solid fuel. Thus, the reduction in GE is in fact a partial relocation of the energy from the combustion path to the complementary biogas path within the same system. Alternatively, the press fluid can also be used as a substrate in conventional biogas plants, as it provides an easily fermentable C source allowing a demand-adapted, flexible biogas production with superior economic benefit and energy efficiency compared to conventional biogas plant configurations solely based on whole crop silages from arable crops (Hahn, Ganagin, Hartmann, & Wachendorf 2014; Hahn, Krautkremer, Hartmann, &

Wachendorf 2014). To summarize, there are positive effects of plant species richness on bioenergy production potential in the extensively used 'High Nature Value grasslands'. Given that extensive grassland management is desirable to conserve biodiversity and many ecosystem services, energetic utilization can provide an interesting alternative to feeding of livestock, to make use of the biomass produced. IFBB proved to be a feasible conversion concept with superior efficiency compared to biogas production from whole-crop silages (Buhle et al. 2012).

The results of this section clearly emphasize that the overall biodiversity effect is significant when put into a real-world perspective, suggesting that high-diversity, low-input systems offer a realistic management option that is able to combine nature conservation goals with production-orientated goals, while also reducing detrimental effects of agrochemical use and greenhouse gas emissions in bioenergy production (Tilman, Hill et al. 2006). Because the Jena Experiment grasslands grow on fertile alluvial loam, the productivity of the 60 species mixture without fertilisation and with a classical hay-making management is equally productive as a medium-intensive low-diversity 'classic' agricultural grassland in the region (Weigelt et al. 2009). Thus, at least for this region, these diverse grasslands represent multifunctional grasslands, that are also competitive with respect to productivity. A caveat is that in real-world bioenergy systems, there will be no weeding of the communities, so that species composition will change (see Section "Invasion into the target communities"). Tests are necessary to understand which mixtures of several species can be maintained under real-world conditions (cf. Dickson & Gross 2015; Jungers et al. 2015).

Weeding issues and monoculture performance

From the beginning of the experiment, plant mixtures had to be weeded in order to maintain the species composition of the plots. Biodiversity experiments have been criticized for the continuous manipulation needed to maintain the designed species compositions, which may be seen as an undesirable disturbance or an unusual, time-consuming management practice (Wardle 2001; Lepš 2004). In addition, there is the important criticism that weeding itself, which is often most intense in monocultures, is a major cause of the poor performance of monocultures observed in many biodiversity experiments. The poor performance of monocultures is important, because early studies, that focussed on individual ecosystem variables such as plant community biomass, often showed strong diversity effects only at the low end of the plant species richness gradient (but see more recent results, e.g., Reich et al. 2012; Meyer et al. 2016). This issue, whether the poor performance of monocultures is a true biodiversity effect with a biological basis, or an experimental artefact, has been taken seriously by the Jena Experiment consortium since the beginning of experiment.

We addressed the following questions:

1) Is the performance of the monocultures indeed lower than expected?
2) Are there biological mechanisms that can lead to poor monoculture performance?
3) How does weeding affect the development of monocultures, in particular recruitment in monoculture plots?
4) Does weeding change abundance distributions and evenness of the target communities in more diverse plots?
5) How strongly is weeding effort correlated with plant species richness and is plant species richness still a significant predictor of ecosystem variables when weeding effort is accounted for?

The first question, whether monocultures are indeed performing worse than expected by chance, was addressed by Marquard et al. (2013) who studied productivity of the monocultures over nine years. There are several potential ways to assess whether performance of a species in a monoculture indeed differs from expected values. We calculated the expected biomass production of species in monocultures as their average biomass in mixtures scaled to a theoretical monoculture. Scaling was done by multiplying biomass in mixture by the number of species present. The rationale underlying this is that a generally low-productive species will not become highly productive just because it occurs in a monoculture and vice versa. Monocultures produced less biomass than expected from the mixtures but this effect took some time to develop (Marquard et al. 2013). The productivity of the 60 monocultures decreased over time, and this decrease was stronger than the decrease in biomass of the same species in mixtures. As a consequence, the net biodiversity effect increased over time. Monoculture performance decreased over time also for ecosystem variables other than plant biomass (Meyer et al. 2016). Thus, monocultures perform worse than expected but this pattern takes time to develop.

The second question was addressed by soil-feedback experiments, testing in particular whether a build-up of pathogens in monocultures decreases monoculture performance over time. Such effects are well-known in agriculture, where they are termed 'soil fatigue' and often require adequate crop rotations. Early on in the experiment, there was already some evidence for (aboveground) pathogen effects on monocultures, as the monoculture of *Bellis perennis* was strongly infected by the rust fungus *Puccinia distincta*. This rust fungus is an invasive species causing epidemics on *B. perennis* in Europe (Weber, Webster, & Engel 2003). The well-established large monoculture of *B. perennis* had to be abandoned in later years, owing to heavy infestation and subsequent plant death caused by the fungus. In a study of fungal infections of all plant species in the Jena Experiment, fungal infections were shown to generally

decrease with increasing plant species richness (Rottstock et al. 2014), and for *B. perennis* this effect seemed particularly strong because it survived well in mixture plots.

Effects of pathogens on plant performance in monocultures were also assessed in a large plant-soil feedback greenhouse experiment (Cortois et al. 2016): 48 plants from the species pool were grown in sterilized soil, sterilized soil with soil inoculum of the same species ('home soil'), and sterilized soil with soil inoculum from all species ('all species soil'). Plant species from all plant functional groups grew better in all species soil, but the mechanism differed among species. Grasses benefited from a reduced net negative effect of soil biota whereas tall herbs benefited from a higher net positive effect of soil biota (Cortois et al. 2016). Legumes responded more neutrally to their own soil biota. In general, species that showed a high specific root length (SRL) and low rates of arbuscular mycorrhizal fungi colonization (AMF) experienced the most negative soil feedback (Cortois et al. 2016). In contrast, resource conservative species (low SRL, high %AMF colonization) benefitted from soil feedback of all species soil biota. Thus, the responses among species varied, but overall, the results indicate that plant-soil feedbacks contribute to the deterioration of monoculture performance (cf. Zuppinger-Dingley et al. 2014), similar to what has been observed in other biodiversity experiments (e.g. Petermann, Fergus, Turnbull, & Schmid 2008).

Soil feedback effects develop over time, as the soil microbial community changes over time, probably with an increasing number of pathogens accumulating in plant monocultures (Eisenhauer, Ackermann et al. 2010; Eisenhauer, Reich, & Scheu 2012). On the other hand, higher densities and activities of biocontrol bacteria in the soil of high-diversity plant communities are likely to protect plants against soil-borne pathogens (Latz et al. 2012). These mechanisms are likely to contribute significantly to the increase in the biodiversity effect on ecosystem variables over time (Meyer et al. 2016). Overall, the plant soil-feedback experiments suggest that the poor performance of monocultures is caused by biological mechanisms and hence a 'true' biodiversity effect. More recent results suggest that monocultures of a species may over time accommodate to soil conditions such that positive plant-soil feedbacks develop (Zuppinger-Dingley et al. 2016). This emphasizes again the need to run biodiversity experiment long enough to detect changes in the interactions between plants and other organisms.

The third and fourth questions on direct weeding effects on the plant communities were studied by comparing weeded with never weeded subplots, established in each plot of the main experiment and by quantifying plant recruitment (Roscher, Temperton et al. 2009). Weeding did not affect the early establishment of the sown (target) species: both the number, productivities and the abundance distributions of established species were similar in both subplots in the first two years after sowing (Roscher, Temperton et al. 2009). Expanding this comparison to a 5-year time span (2003–2007) revealed that the invasion of new species had increasingly negative effects on the cover and biomass production of the sown species in the never-weeded subplots. No decrease in recruitment was observed in the weeded subplots. As a consequence, temporal stability of total target species cover and biomass production, as well as the recruitment of new individuals of the target species from seeds, were higher in regularly weeded subplots (Roscher, Fergus et al. 2013). Changes in target species composition were also lower in regularly weeded than in never-weeded subplots (Roscher, Fergus et al. 2013). In the never-weeded subplots, as well as in subplots where weeding was stopped after several years, there was rapid convergence in total species richness (i.e. sown species plus invading species, Roscher et al. 2014; Steinauer et al. 2016), functional group composition, and functional and phylogenetic diversity (Roscher, Fergus et al. 2013; Roscher et al. 2016). Weeding was therefore essential for maintaining the experimental species richness gradient and functional group compositions: species poor communities or those with few functional groups were rapidly invaded and did not persist in the absence of weeding. Species evenness and functional trait diversity among the target species (i.e. sown species) were not different between weeded and never-weeded subplots (Roscher, Fergus et al. 2013). Thus, there was little evidence that weeding prevented species recruitment in monocultures and hence causes poor monoculture performance.

To address the fifth question, the number of hours that PhD students, gardeners, and student helpers spent weeding the plots was recorded in several years and 'weeding hours' was analysed as a measure of weeding intensity. Weeding hours generally decreased with increasing plant species richness (2005: $p = 0.08$, 2006: $p < 0.01$, 2007: $p < 0.011$, excluding 60-species plots, which did not require weeding) such that, on average, weeding hours in 16-species mixtures were only 33% (23–39%) of those in monocultures. However, the correlation between diversity and weeding hours was relatively low (R^2 between 0.04 to 0.19 for models with only species richness), because several of the low-diversity plots established well and required little weeding, while some of the higher diversity plots did require substantial weeding. To test whether the extra weeding effort in monocultures could drive positive effects of species richness on ecosystem functioning, we did a series of analyses fitting weeding hours before species richness in an analysis of 242 ecosystem variables, from 2005 to 2007. The sequential General Linear Models contained terms for block and species richness and were tested with or without an additional term for weeding hours. The effect size of species richness was then calculated as a *Zr* value (based on type I sums of squares, Allan, Weisser et al. 2013), and the significance of the species richness term was recorded. The 242 measures were then grouped according to broad ecosystem process categories and the average effect size, and proportion of significant effects ($p < 0.05$), of species richness with and without weeding hours were calculated for variables in each

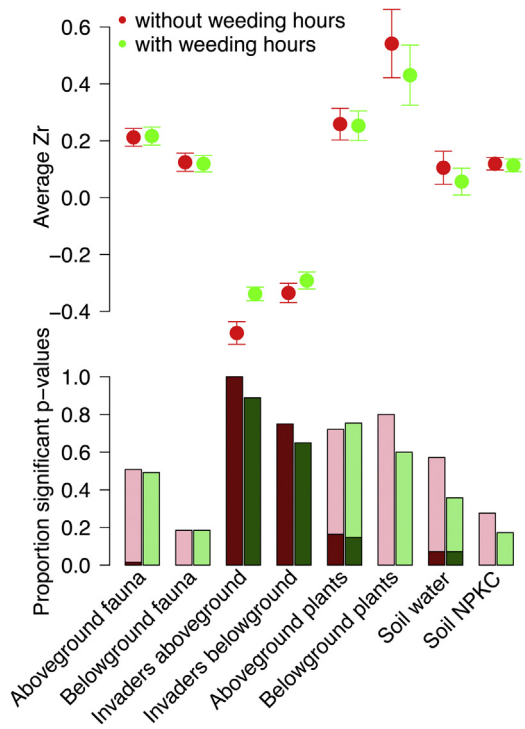

Fig. 21. The effect of weeding on the relationship between species richness and ecosystem processes as a function of ecosystem process category. Weeding effort was measured as the number of hours student helpers and gardeners weeded in a particular plot. Top panel: effect sizes (Zr — values, see Fig. 9) from an ANOVA model where weeding hours were not included in the model (red dots) or fitted first before plant species richness (green dots/bars). Bottom panel: bars present the fraction of ANOVA analyses that showed a significant effect of plant species richness. The darker portion of each bar indicates variables that are negatively affected, the lighter part those that are positively affected by plant species richness. For methods see Fig. 9 and Allan, Weisser et al. (2013).

of those groups (Fig. 21). Fitting weeding hours before species richness did not decrease overall effect sizes for any of the ecosystem process groups, except for 'plants non-target aboveground', i.e. variables related to the number, biomass and species richness of the weeds themselves. Unsurprisingly, the species richness effects were larger (more strongly negative) when weeding hours were not included in the model. Species richness had more significant effects when weeding hours were not included in the model for the invasion-related variables, but also for belowground plant-related variables (e.g. roots) as well as for soil nutrient and other abiotic variables. In these cases, the number of significant species richness effects decreased by about one third. Overall, the analysis suggests that the majority of plant species richness effects are not driven by variation in weeding effort among plots. As an example, for aboveground plant community biomass, there was no significant correlation between weeding hours per plot and biomass (Fig. 22, 2005: $R^2 = 0.007$, 2006: $R^2 = 0.008$, 2007: $R^2 = 0.03$). Note, however, that this analysis is conservative because it cannot answer the question of whether the increased weeding effort in low-diversity communities is the cause or consequence of the low performance of monocultures. If weeding effort is the consequence of poor monoculture performance then even the effects of weeding pressure on ecosystem functioning, could be considered indirect effects of biodiversity.

To summarize, weeding undoubtedly represents a disturbance and there is the danger that weeding negatively affects the communities of plants and other organisms. To reduce these effects, plots in the Jena Experiment were only weeded when plants were very small, i.e. as early as possible after winter, after the mowing in June, and in late autumn, after the second mowing event. Our results indicate that recruitment of plants that belong to the sown mixture of a particular plot was not systematically reduced by weeding. Thus, the low cover and biomass of species in monocultures is not caused by weeding. Instead, there is ample evidence from the Jena Experiment, and also from other studies (Mitchell, Tilman, & Groth 2002; Maron et al. 2011; Schnitzer et al. 2011), that plants in monocultures suffer from a variety of pathogens. This is likely to be one of the main underlying mechanisms

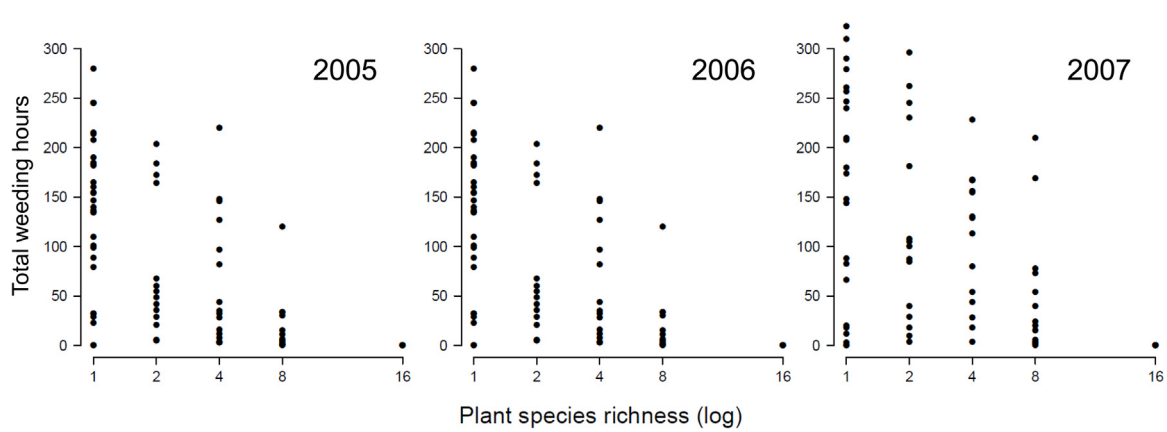

Fig. 22. Relationship between annual weeding effort, measured in 'weeding hours', i.e. the number of hours a particular plot was weeded by student helpers, PhD-students or gardeners, and target plant species richness, in three years.

of the increasingly bad performance of monocultures over time (see also Section "Mechanisms underlying the biodiversity–ecosystem functioning relationships"). The increased weeding effort in monocultures is therefore largely, if not exclusively, the consequence, and not the cause, of low monoculture performance.

Mechanisms underlying the biodiversity–ecosystem functioning relationships

In the first generation of biodiversity studies, the emphasis was on searching for patterns related to the question if plant species richness affects processes at the ecosystem level. The underlying mechanisms were not the main focus of the early studies, even though several hypotheses were formulated, for example that complementarity in rooting depths among plant species could lead to a more efficient soil nutrient uptake in more diverse communities. Biodiversity experiments thereafter became more mechanistic and the analyses increasingly focussed on the question whether complementarity or selection drive an observed biodiversity effect (see Section "The Jena Experiment, its origin and design"). However, most of these analyses concentrated on using statistical methods, such as the additive partitioning method proposed by Loreau & Hector (2001), to find out whether there are positive interactions between species affecting an ecosystem variable when diversity is increased. Testing the mechanisms underlying such positive interactions, such as niche complementarity between plants, have become more and more common only recently, and such studies often use additional experiments specifically designed for testing particular hypotheses (e.g., Dimitrakopoulos et al. 2004). The Jena Experiment has, from the beginning, aimed to unravel mechanisms underlying biodiversity effects on ecosystem variables. Here we give examples of two lines of such research in the Jena Experiment. The first one involves the use of functional plant traits. The underlying rationale is that organisms interact with their environment through their phenotypes, i.e. their traits. Identifying the changes in the trait distribution between plant communities at different levels of diversity, and linking this to changes in ecosystem variables, therefore promises to unravel the mechanisms causing these changes. For example, if there is an increase in the diversity of rooting depths with increasing plant species richness and if this is correlated with elevated nutrient uptake by the plant community, then it is likely that nutrient uptake is limited in low-diversity communities because these communities obtain nutrients from only a part of the soil profile. We review the progress that has been made in the Jena Experiment using plant functional traits, but also outline potential limitations of the approach that have been found in the studies.

A second line of research concerns the various mechanisms that have been proposed to underlie the increase in aboveground plant biomass with increasing plant species richness. The Jena Experiment has tested several of the proposed mechanisms — from complementarity in resource uptake to changes in plant-microbe interactions — to understand whether they contribute to the observed biodiversity effect.

Functional traits as predictors of ecosystem functioning

In the Jena Experiment, trait-based analyses have focussed so far on a number of processes, in particular biomass production (Roscher et al. 2012; Roscher, Schumacher et al. 2013), herbivory (Loranger et al. 2012; Loranger et al. 2013), the abundance and diversity of soil biota (Milcu et al. 2013) and carbon and water fluxes (Milcu et al. 2014). For analysing biomass production, Roscher et al. (2012) used two community-wide measures of functional trait composition, namely (1) community-weighted means of trait values (CWM), and (2) functional trait diversity calculated as Rao's quadratic diversity (FD_Q), based on 18 functional traits measured for plant individuals in the Jena Experiment. Functional composition explained a larger proportion of variation in community biomass and measures of biodiversity effects (40–82%) than sown species richness (<1–13% of explained variation). Community-weighted mean traits (CWM), i.e. the dominant trait values in a community, were more important than FD_Q in explaining community biomass production and net diversity effects, while FD_Q explained a greater proportion of variation in complementarity and selection effects than CWM. However, in all analyses, CWM and FD_Q in combination best explained community biomass production and measures of diversity effects, providing evidence that both dominant trait values and trait diversity are important for primary productivity (Roscher et al. 2012). In these analyses based on a single year, traits characterising plant light and nutrient acquisition as well as life history formed the best statistical models for community biomass production. The extension to several years (2003–2009) revealed that traits related to N-acquisition strategies became more important in explaining high community biomass production over time (Roscher, Schumacher et al. 2013). In line with the observation that diversity in traits associated with N acquisition may explain high community biomass production, the results of the Ecotron experiment with twelve lysimeters from Jena Experiment communities showed that a high diversity in leaf N concentrations best explained diversity effects on ecosystem C fluxes (Milcu et al. 2014).

Using plant functional traits, Loranger et al. (2013) also showed that multiple plant traits — related to plant morphology, physiology, phenology and the number of natural enemies– are necessary to successfully predict invertebrate herbivory in plant monocultures. The application of the same trait-based approach on invertebrate herbivory in plant mixtures showed, however, strong non-additive effects,

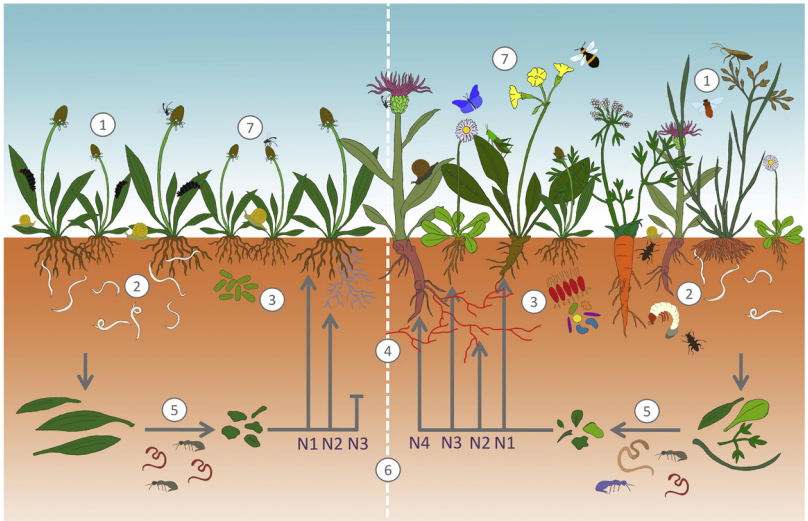

Fig. 23. Illustration of various mechanisms underlying species complementarity as observed in the Jena Experiment. Left: plant monoculture; right: plant polyculture. (1) and (2) above- and belowground herbivory (Eisenhauer, Reich, & Scheu 2012), (3) plant protection against soil-borne pathogens by soil bacteria (including plant growth promoting rhizobacteria) (Latz et al. 2012) and effects of microbial pathogens (Maron et al. 2011; Schnitzer et al. 2011); (4) soil nutrient uptake through mycorrhizal fungi (Wagg et al. 2011); (5) decomposition of organic material by the decomposer community (Eisenhauer, Reich, & Isbell 2012), (6) gradual recycling of nutrients in soil with different nitrogen species (N1–N4, Eisenhauer 2012), (7) pollination. Functional diversity of bee pollinators was shown to increase crop yield (Hoehn, Tscharntke, Tylianakis, & Steffan-Dewenter 2008). Illustration by Manfred Türke.

i.e. proportions of herbivory were likely to be influenced not only by traits of the focal species but also by characteristics of the surrounding vegetation (see Section "Animal-mediated ecosystem processes", Loranger et al. 2013). One possible reason for such non-additive effects is that in these (and in fact almost all) trait analyses, the trait values used derive from monocultures or from databases, and are assumed to be constant along the plant species richness gradient. However, as shown in Section "Responses of individual plant species to plant species richness", many plant functional traits including those related to light or nutrient acquisition show strong diversity-related intraspecific trait variation, which is likely to have consequences for the predictive power of trait approaches.

Overall, trait-based analyses in the Jena Experiment helped to point to mechanisms underlying biodiversity effects on ecosystem variables. A main result was, however, that the plastic response of individual species to plant species richness complicates such analyses. For example, an analysis of seven legume species studied in monocultures and 60-species mixtures showed that diversity-related plasticity in plant height and specific leaf area was positively and negatively related to the increased performance of legumes in the mixtures, respectively (Roscher, Schumacher, Schmid, & Schulze 2015). In addition, intraspecific trait variation did not only occur between individuals of the species in different communities, but also among individuals within the same plant community. Traits may also vary over the growing season and from year to year. It is thus challenging to incorporate these different sources of trait variation in trait-based modelling of ecosystem functioning.

Mechanisms underlying complementarity in biomass production

While the positive relationship between plant species richness and ecosystem functioning is well established (Hooper et al. 2005; Cardinale et al. 2012), current research focuses on the underlying mechanisms, in particular for aboveground plant community biomass (Maron et al. 2011; Schnitzer et al. 2011; Eisenhauer 2012; Reich et al. 2012). One of the most prominent models explaining why high-diversity plant communities perform better than low-diversity ones is that different species complement or facilitate each other in chemical, spatial, and temporal resource use (Loreau, Naeem et al. 2001). The Jena Experiment has provided more insights into the mechanisms of plant complementarity by taking an above-belowground and multitrophic perspective. The suggested mechanisms are not mutually exclusive and likely act in concert (Fig. 23).

Complementarity in resource use

There are a number of different mechanisms by which resource use may differ between high-diversity and low-diversity plant communities, many of which have now been investigated in the Jena Experiment. The first hypothesis considers niche differentiation through spatial differences in rooting depth (Parrish & Bazzaz 1976; Berendse 1982;

Levine & HilleRisLambers 2009); rooting depth activity (Mamolos, Elisseou, & Veresoglou 1995), or differences in N preference (von Felten et al. 2009; von Felten, Niklaus, Scherer-Lorenzen, Hector, & Buchmann 2012). However, contrary to expectation, the recent evidence, also from the Jena Experiment, shows that community root biomass aggregates in the topsoil layer in mixtures compared to monocultures, rather than exhibiting segregation along the depth gradient (Mommer et al. 2010; Ravenek et al. 2014). In the Cedar Creek biodiversity study a larger investment in deeper roots was observed in plots with a higher diversity (Mueller et al. 2013). Other field studies (not biodiversity experiments) also found limited evidence for segregation of roots. Kesanakurti et al. (2011) found in natural grasslands that the degree of aggregation depends on the relatedness of the species: the more related the species, the more root aggregation occurred in the same soil horizons. Frank et al. (2010) found that roots of different species hardly segregated over the soil profile. Studies on N partitioning by soil depth also have not found much evidence for such niche differentiation in grasslands (von Felten et al. 2009, 2012).

Second, different plant species may access different nutrient pools via the association with different arbuscular mycorrhizal fungi (Wagg, Jansa, Schmid, & van der Heijden 2011). An early study by van der Heijden et al. (1998) had already shown that the diversity of AMF drives the productivity of plant communities by facilitating P uptake from the soil. In line with those findings, Smith, Jakobsen, and Smith (2000) showed that different AMF species complement each other by acquiring P close to roots (*Scutellospora calospora*) and far from roots (*Glomus caledonium*), suggesting functional complementarity among AMF species, thereby increasing overall P availability to and growth of plants (Koide 2000). Consistent with this mechanism, the diversity of AMF increased with plant species richness in the Jena Experiment (Koenig et al. 2010; Scherber, Eisenhauer et al. 2010; Milcu et al. 2013), and P-mobilisation could be shown to be affected by plant species richness-dependent microbe-rhizophere interactions (see Section "The effects of diversity on biogeochemical cycling", Hacker et al. 2015).

Third, different plant species may also take up different nutrient forms without spatial complementarity. Detritivorous animals fragment litter and incorporate fragments into mineral soil layers, while microbial decomposers account for 90–100% of the mineralization of litter C and the recycling of essential nutrients (van der Heijden, Bardgett, & van Straalen 2008). The density and diversity of both microbial and animal decomposers increased with plant species richness in the Jena Experiment (Eisenhauer, Bessler et al. 2010; Eisenhauer, Milcu, Sabais et al. 2011; Ebeling, Meyer et al. 2014; Lange et al. 2015). The breakdown of organic matter by the decomposer community is a gradual process with many intermediate steps, resulting in a large number of chemical compounds. Hence, a higher diversity of decomposers is likely to produce a higher number of compounds (Bardgett 2005; Eisenhauer 2012; Eisenhauer, Reich, & Scheu 2012). Plant species have been shown to vary in timing, depth and chemical form of N uptake (McKane et al., 2002). For plant species in the Jena Experiment, there were differences in the capacity to take up amino acids from soil (Sauheitl 2009). Currently, labelling experiments using stable isotopes are used to shed light on resource partitioning in more diverse communities. These labelling experiments are also aiming at investigating complementarity in water uptake despite the lack of evidence from root distribution patterns (Ravenek et al. 2014). The results are not yet conclusive. Previous labelling studies suggest that there is little differentiation among plants in water uptake and hence no complementarity (Bachmann et al. 2015), at least during times of low water demand. However, analysis based on soil water budgets suggests root water uptake moving to deeper soil in diverse communities during periods of high water demand (Guderle & Hildebrandt 2015).

A fourth mechanism refers to light partitioning. Competition for light has been shown to determine the diversity and stability of grassland plant communities in experimental and natural grasslands (Borer et al. 2014; Hautier et al. 2014). Light is the key resource limiting plant productivity in productive environments supporting the development of dense foliage (e.g. Hautier, Niklaus, & Hector 2009). High diversity plant communities have a more complex and denser vegetation structure than low diversity communities, and may thus be able to use light more efficiently (Spehn, Joshi, Schmid, Diemer, & Körner 2000; Lorentzen et al. 2008; Marquard, Weigelt, Roscher et al. 2009; Wacker, Baudois, Eichenberger-Glinz, & Schmid 2009b; Proulx, Roca, Cuadra, Seiferling, & Wirth 2014). However, niche partitioning along light gradients is only possible if species differ in their light requirements, are able to re-acclimate to changes in the light environment during canopy development, or have a different phenology. For example, smaller-statured species may change their leaf morphology, biochemistry and physiology to adjust to low-light conditions deep in the canopy of high-diversity plant communities (Roscher, Kutsch, Kolle et al. 2011). Temporal development of the plant canopies dependent on plant diversity is currently explored along the gradient of plant species richness in the trait-based experiment, using phenocams (cf. Proulx, Roca, Cuadra, Seiferling, & Wirth 2014) and terrestrial laser scanning (Fig. 24).

Multitrophic interactions

Because plant species richness affects the abundance and diversity of other organisms (Scherber, Eisenhauer et al. 2010), considering trophic interactions in biodiversity experiments is indispensable for understanding the underlying mechanisms of plant complementarity (Eisenhauer 2012). Recent studies accentuated the role of arbuscular mycorrhizal fungi (Klironomos, McCune, Hart, & Neville 2000), soil pathogens (Maron et al. 2011; Schnitzer et al. 2011), plant growth promoting bacteria

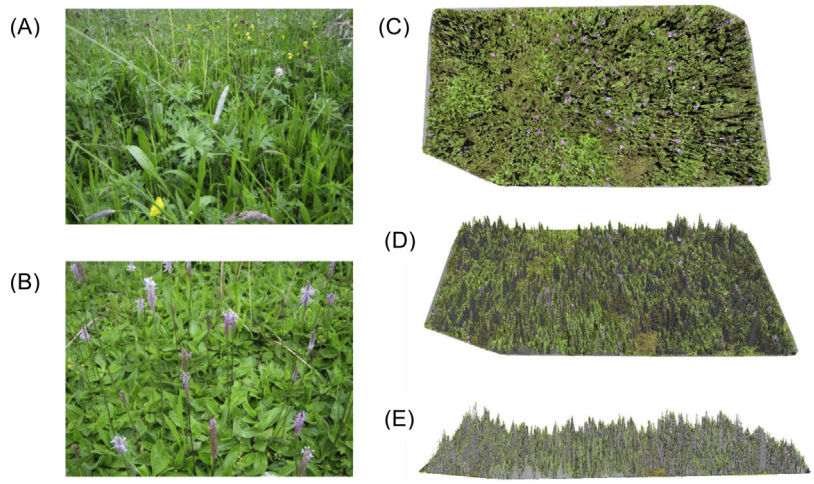

Fig. 24. The temporal dynamics of structural complexity and canopy architechture is recorded with phenocams (hourly intervals) and laser scanning (FARO Focus 3D). Left: Images from phenocam pictures over an eight species mixture (A) and a monoculture of *Plantago media* (B). Right: Three orthographic and triangulated views of a plot of the Trait Based experiment: a scanner view (C), inclination view (D), and horizontal view (E). Laser images yield a high-resolution point cloud reflecting the complexity of the vegetation surface.

(Latz et al. 2012), and decomposers (Eisenhauer, Milcu, Allan et al. 2011; Eisenhauer, Reich, & Isbell 2012) in (co-)determining the positive plant species richness–productivity relationship. Soil biota in particular have been suggested as main drivers of overyielding. In line with evidence from tropical forests, the negative effects of soil biota on plant growth are expected to be species-specific and dependent on the local abundance of a plant species (cf. Janzen–Connell hypothesis; Mordecai 2011; Johnson, Beaulieu, Bever, & Clay 2012). The Janzen-Connell hypothesis was originally developed to explain species coexistence among tropical tree species by low survival of seedlings next to a parent tree, due to pathogens or herbivores, and was subsequently applied to other systems. It implies that soil-borne pathogen effects will reduce root biomass more in monocultures (low diversity, high density of a particular plant species) than in plant mixtures (high diversity, low density), resulting in a positive relationship between productivity and diversity. A first indication that pathogenic soil biota indeed drive this relationship originates from two recent studies where the removal of all soil biota prevented the reduction of biomass in monocultures (Maron et al. 2011; Schnitzer et al. 2011) although the natural 'enemies' themselves were not yet identified. Soil-borne pathogenic fungi (Hersh, Vilgalys, & Clark 2011), plant-parasitic nematodes (van Ruijven, De Deyn, & Berendse 2003), protozoa or bacteria (Westover & Bever 2001; Latz et al. 2012) may, individually or collectively (Philippot, Raaijmakers, Lemanceau, & van der Putten 2013; Raaijmakers, Paulitz, Steinberg, Alabouvette, & Moenne-Loccoz 2009), adversely affect root growth, and thus plant performance in mixtures compared to monocultures. Experimental evidence for the role of negative plant-soil feedback in monocultures vs. mixtures, and for driving overyielding effects is now substantial, also for the Jena Experiment (Petermann et al. 2008; Hendriks et al. 2013; Cortois et al. 2016). The role of selection within the plant communities, i.e. adaptation to soil conditions over time, needs to be explored in more detail (Zuppinger-Dingley et al. 2016).

Results from the Jena Experiment also show that the diversity of foliar fungal pathogens increases, while pathogen infection per plant decreases with increasing plant species richness (Rottstock et al. 2014). Belowground, there are higher densities and activities of biocontrol bacteria in the soil of diverse plant communities, protecting plants against soil-borne pathogens (Latz et al. 2012). Similar results were reported for soil nematodes: species-poor plant assemblages were suggested to suffer from nematode communities detrimental to plants (high proportion of plant feeding nematodes), whereas species-rich plant assemblages supported a higher proportion of fungivorous and bacterivorous nematodes, stimulating nutrient cycling and hence plant performance; i.e. effects of nematodes on plants may switch from negative to positive along the plant species richness gradient (Eisenhauer, Migunova et al. 2011). Thus, in addition to components of traditional niche theory, soil-borne pathogens may represent another essential niche dimension in a multi-dimensional niche space. Petermann et al. (2008) suggested that 'pathogen niches', i.e., pathogen-free space, determine plant coexistence and can explain, at least in part, the positive relationship between plant species richness and productivity. Consistent with this hypothesis, a recent study in the Jena Experiment indicated that plant-soil feedback effects co-determine the selection for niche differentiation along the plant species richness gradient

(Zuppinger-Dingley et al. 2014; Zuppinger-Dingley et al. 2016).

The density and diversity of herbivores also often vary with plant species richness (Haddad et al. 2009; Scherber, Eisenhauer et al. 2010), and herbivory may modulate positive and negative complementarity effects either by stimulating the activity of soil biota (Nitschke et al. 2015) or by promoting nutrient immobilization. However, experimental evidence supporting this view is scarce.

To summarize, results of the Jena Experiment point to a combination of several mechanisms responsible for increased biomass production in more diverse communities. Some of the mechanisms that were proposed to be key drivers of biodiversity effects, e.g. complementarity in rooting depth, may in fact play a smaller role than proposed. In contrast, other mechanisms, such as the role of diversity for protecting plants against pathogens and plant-feeding organisms such as nematodes, which have not been sufficiently studied in previous biodiversity experiments, seem to play an important role. The extent to which other mechanisms contribute to the biodiversity effect is still open to discussion. Here, there is the need for more work on the mechanisms underlying biodiversity effects. Our results also point to the fact that any mesocosm experiment performed in the greenhouse must pay attention to realistic soil conditions, as the belowground interactions are complicated and very important for the outcome of biodiversity effects on ecosystems, but will not take place over a short time period and within standardized greenhouse soil.

Discussion and conclusions

Research on the relationship between biodiversity and ecosystem functioning has revealed a multitude of effects of biodiversity on many ecosystem variables. In this review we have shown that the Jena Experiment has contributed to the overall conclusion that biodiversity *per se* is an important driving factor of ecosystem functioning including important variables such as production, nutrient cycling, C storage, and trophic and non-trophic interactions between organisms. The overall result that biodiversity is important for the functioning of ecosystems allows biodiversity research to now ask more mechanistic questions about the role of various components of diversity for the patterns that are observed. After the first transition from observational approaches to direct manipulations of plant species richness, we now enter a next generation of research. The forthcoming generation of biodiversity experiments will focus less on pattern detection, but more on mechanistic and predictive approaches (Ebeling, Pompe et al. 2014). At the end of our paper we will outline where we see the most pressing needs for further development of theory and empirical research.

First, most studies have been based on the manipulation of species richness, while other measures of diversity, such as trait diversity, have not been studied in similar detail (but see, e.g. Scherer-Lorenzen, Schulze, Don, Schumacher, & Weller 2007). While there have been studies where plant functional richness in addition to species richness was manipulated, i.e. in the main experiment of the Jena Experiment, the *a priori* grouping of species into functional groups does not fully capture functional diversity, because there are many ways in which species can be classified into functional groups and distinguishing broad groups may obscure fine gradients. In addition, the organizational level of diversity relevant to function will depend on the function addressed. Rather than using species traits to construct functional groups it may be more helpful to base measures of the functional diversity of a community directly on traits (Petchey & Gaston 2006; Litchman & Klausmeier 2008; Weigelt, Schumacher, Roscher, & Schmid 2008; Griffin, Mendez, Johnson, Jenkins, & Foggo 2009; Schumacher & Roscher 2009; Wacker, Baudois, Eichenberger-Glinz, & Schmid 2009a; Le Roux et al. 2016). Such an approach would also help to further resolve the ongoing debate on whether species identity (i.e. particular trait values), diversity (trait diversity) or community composition (particular combinations of traits) underlie the observed relationship between diversity and functioning (Ebeling, Pompe et al. 2014). However, one of the striking results of the Jena Experiment is that many plant traits that are functionally important respond themselves to changes in plant species richness, i.e. trait expression is not constant along the diversity gradient (Section "Responses of individual plant species to plant species richness"). Most current trait analyses are, however, based on mean trait data that are taken from databases and that may differ from the actual behaviour of species at a particular site. This may well reduce the predictive power of such trait analyses. Future experiments should make an effort to collect appropriate data on species traits in the experimental plots, and analyses may distinguish between plastic and non-plastic (constitutive) traits. In the Jena Experiment, a large number of plant traits have already been measured, under controlled conditions in the greenhouse (e.g. Schroeder-Georgi et al. 2015), in the monocultures (Heisse et al. 2007; Roscher, Scherer-Lorenzen et al. 2011; Loranger et al. 2013), as well as along the plant species richness gradient (Gubsch, Buchmann et al. 2011; Roscher, Schmid et al. 2011; Lipowsky et al. 2015). In the design of the main experiment, each particular species only occurs in a limited number of diversity mixtures. To distinguish effects of single species from diversity effects more clearly, an experimental design is needed that allows tracking of species along the diversity gradient, as in the dominance experiment or the trait-based experiment; in the latter, trait-diversity of mixtures is designed using a-priori hypotheses concerning functioning, complementarity, and redundancy (Ebeling, Pompe et al. 2014).

Second, most studies have focused on one or very few ecosystem variables, in particular primary production, yet many variables affect ecosystem functioning. Recently, an increasing number of ecosystem variables have been investigated, and an increasing number of studies investigates

the effect of biodiversity on ecosystem multifunctionality (e.g. Hector & Bagchi 2007; Allan et al. 2015; Lefcheck et al. 2015). However, the biodiversity–ecosystem functioning literature still suffers from an imbalance in variables measured. Datasets such as the ones in the Jena Experiment where a large number of variables are measured, now allow addressing the question whether different processes are affected by biodiversity loss in different ways (Allan, Weisser et al. 2013). Importantly, the Jena Experiment makes all data publicly available, through the website of the Jena Experiment, and by publishing the data in cooperation with the public database Pangaea (www.pangaea.de). At the time of writing the manuscript, more than 7000 variables have been published. These include >4000 variables for aboveground plant biomass, e.g. biomass of individual species in individual plots in the spring and summer harvest, >460 for soil C, >570 for soil N, >500 for soil P. Eventually, all data from the Jena Experiment database (Table 4) will be uploaded to Pangaea.

Empirical approaches need to be complemented by the development of appropriate theory. For example, while the stronger effects of plant species richness on herbivore than on carnivore diversity (cf. Scherber, Eisenhauer et al. 2010) can be explained by the close direct trophic dependence of herbivores on plants, it is less clear why plant species richness effects on the C cycle should be stronger than on the N cycle, as suggested by the analysis of Allan, Weisser et al. (2013). Other pending issues are: For what ecosystem variables can stronger complementary effects be expected? When is trait diversity particularly important and when is it a particular trait value that affects an ecosystem process? Can we extend niche theory to not only predict when species should evolve higher niche differentiation but also when this differentiation favours complementary effects? That is, how are mechanisms of coexistence (Chesson 2000) linked to the mechanisms underlying function (Hille Ris Lambers, Harpole, Tilman, Knops, & Reich 2004), and can we predict how traits affecting coexistence are linked to traits affecting process rates (Hillebrand & Matthiessen 2009)? This is closely related to the general discussion on the relationship between response and effect traits (Suding et al. 2008). Coexistence driven by trade-offs in resource use will generally be positively linked to processes depending on overall resource use efficiency, e.g. total productivity. Conversely, if coexistence depends on traits unrelated to biomass production, such as resistance to disturbance or trophic interactions, species diversity may not be expected to affect community productivity. Recently, Ptacnik, Moorthi, and Hillebrand (2010) proposed a conceptual model for addressing these feedbacks between mechanisms in a stoichiometrically explicit framework. They argued that coexistence is enhanced by a stoichiometrically balanced resource supply (an outcome predicted by the resource ratio hypothesis), which at the same time maximizes the efficiency of multi-elemental resource use. The development of theories like this would facilitate designing future biodiversity experiments to test theoretical predictions.

Third, ecological experiments in general, and biodiversity experiments in particular, have often been criticised for generalising results from short-term studies rather than basing conclusions on longer-term data series, because of the transient initial dynamics in a community, and because of the inherent variability in, e.g., weather conditions (Tilman 1989; Grime 1997). Thus, the longer-term effects of biodiversity need to be studied. Only long-term data collection will allow to test if processes such as, e.g., soil C accumulation, will saturate over time, or how temporal dynamics are impacted by diversity (Reich et al. 2012). In these longer-term studies, it is important to include effects of multitrophic interactions above and below the ground in order to consider essential feedback mechanisms (Eisenhauer, Reich, & Scheu 2012). Long-term studies are also the pre-requisite for detecting the insurance effect of biodiversity (Yachi et al. 1999), stabilizing effects of biodiversity (Isbell, Craven et al. 2015), and the importance of temporal fluctuations (Allan et al. 2011). Stable ecosystem service provisioning is of integral importance for human well-being, and biodiversity may represent one important biotic ecosystem component driving the stable delivery of many services (Cardinale et al. 2012). Because biodiversity may have the capacity to buffer ecosystem responses to perturbations (McCann 2000), longer-term studies are necessary to ascertain how the strength of biodiversity effects may also depend on the environmental context (Hautier et al. 2014; Steudel et al. 2012). Future experiments thus should also investigate if the body of diversity–stability theory (McCann 2000) applies to changing environments and increased magnitude and frequency of environmental disturbances.

Finally, there has been a considerable debate about the relevance of experimental results for real-world ecosystems (Duffy 2009), and this debate is still continuing (Eisenhauer et al. 2016; Wardle 2016). A major issue is how much biodiversity contributes to a particular ecosystem variable, such as primary production, compared to the effect of other drivers such as resource availability and local climate (Polis 1999; Huston et al. 2002; Grace et al. 2016). For example, Flombaum and Sala (2008) showed that biodiversity effects on productivity were actually larger in a natural than in artificial communities, thus supporting the patterns found in biodiversity experiments. The importance of biodiversity in the real world is linked to the question of whether global change drivers will have their main effect on ecosystem functioning through direct alteration of the abiotic environment or through indirect effects on species diversity and composition (Lavorel & Garnier 2002; Suding et al. 2008; Allan et al. 2015). Addressing this issue means testing results found in biodiversity experiments in the real world of natural or managed ecosystems, and searching for the importance of the mechanisms identified in biodiversity experiments (Grace et al. 2016). A real-world perspective also implies that a number of different ecosystems need to be considered,

Table 5. Main contributions of the Jena Experiment to functional biodiversity research and key papers marking these advances.

Topic	Key papers
General mechanisms underlying the effects of diversity on ecosystem functioning	
Overyielding	Roscher et al. (2005), Marquard, Weigelt, Temperton et al. (2009), Guenay et al. (2013)
Turnover of complementary species	Allan et al. (2011)
Selection for niche differentiation	Zuppinger-Dingley et al. (2014)
Assembly processes	Roscher et al. (2014), Eisenhauer, Reich, and Scheu (2012)
Effect of plant diversity on diversity on other trophic levels	
Decomposers, soil biota	Milcu, Partsch, Langel, and Scheu (2006), Eisenhauer, Milcu, Sabais et al. (2011), Lange et al. (2015)
Invertebrate herbivores	Scherber, Milcu et al. (2006)
Pollinators	Ebeling et al. (2008)
Food-webs overall effect	Scherber, Eisenhauer et al. (2010)
Protection against soil-borne pathogens	Latz et al. (2012), Latz et al. (2016)
Effect of plant diversity on biogeochemical cycles	
Nitrogen	Oelmann, Wilcke et al. (2007), Oelmann, Buchmann et al. (2011), Leimer et al. (2015), Roscher, Thein et al. (2008)
Phosphorus	Hacker et al. (2015), Oelmann, Kreutziger, Temperton et al. (2007)
Carbon	Steinbeiss, Bessler et al. (2008)
Resource use efficiency, C aquisition	Milcu et al. (2014)
Root decomposition	Chen et al. (2017)
Biodiversity effects on stability	
Spatial stability	Weigelt et al. (2008), Strecker et al. (2016)
Stability across trophic levels	Proulx et al. (2010)
Stability after drought	Vogel et al. (2013)
Stability after flood	Wright et al. (2015)
Stability of soil microbial properties	Strecker et al. (2016)
Biodiversity effects on multiple functions	
Differences in strength of effects among functions	Allan, Weisser et al. (2013), Scherber, Eisenhauer et al. (2010)
Delayed biodiversity effects	Meyer et al. (2016)
Multifunctional agriculture	Weigelt et al. (2009)
Use of traits in analysing functional biodiversity effects	
Plasticity of functional traits	Gubsch, Buchmann et al. (2011)
Exploring role of traits for biodiversity–productivity relationships	Schumacher & Roscher (2009), Roscher et al. (2012)
Hierarchical trait-based prediction of plant performance	Schroeder-Georgi et al. (2015)
Effects of plant traits impact on C acquisition	Milcu et al. (2014)
Mechanisms of community assembly	Roscher, Schumacher et al. (2013)
Predicting insect herbivory	Loranger et al. (2012), Loranger et al. (2013)
Role of plant traits for plant-soil feedbacks	Cortois et al. (2016)

in particular those where the dominant plant species are long-lived, that is, forest ecosystems, so that manipulative experiments will be more difficult (Verheyen et al. 2016). Finally, non-random losses of species with particular traits is likely to be a more realistic scenario for species loss in the real world than the random loss of species simulated in the Jena Experiment (e.g., Lepš 2004). Rare species are more likely to go extinct first, before more dominant species do, yet there is increasing evidence that rare species also affect ecosystem functioning, perhaps disproportionally to their abundance (Soliveres et al. 2016). Exploring the relationship between threat of extinction and importance for ecosystem functioning would help to more precisely predict the consequences of species extinctions for real-world ecosystem functioning.

To summarize, functional biodiversity research has made significant progress in the past 20 years, and the Jena

Experiment has considerably contributed to this progress (Table 5). There is now a broad consensus that biodiversity loss can affect the functioning of ecosystems and the services provided to humankind, and the emphasis has shifted towards identifying why biodiversity affects certain processes and not others. The way ahead lies in the identification of the mechanisms underlying the relationship between biodiversity and functioning, in particular how species interactions change with increasing diversity, above- and belowground. The Jena Experiment has shown that, across almost all taxa investigated, a loss in plant species richness results in a loss of species, even belowground, strongly suggesting that plant diversity effects on element cycling and other processes are linked to changes in the diversity and abundance of these communities, and to changes in organismic interactions. These linkages are only beginning to be unravelled. Experiments should be longer-term, to avoid reliance on transient dynamics, and they should involve interactions with other drivers of global change, such as eutrophication, or climate change. Putting the results from biodiversity experiments into context also requires mechanistic comparisons with natural and managed ecosystems.

The Jena Experiment has had a unique role in biodiversity–ecosystem functioning research by exploring whole-ecosystem responses to changes in biodiversity. The main objectives of the future work in the Jena Experiment are to utilize an interdisciplinary and integrative approach to perform unique within- and across-experiment syntheses and meta-analyses, to continue important time-series in one of the longest-running biodiversity experiments, separate abiotic and biotic drivers of biodiversity–ecosystem functioning relationships, and identify the mechanisms of long-term biodiversity effects on ecosystem functioning, e.g. by performing plant-soil feedback experiments. The interdisciplinary approach will likely yield novel and comprehensive insights regarding the role of biodiversity for the functioning of ecosystems.

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