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Research

Nematode communities, plant nutrient economy and life-cycle characteristics jointly determine plant monoculture performance over 12 years

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Knowledge from agriculture and ecological field studies suggests that plant monocultures lose productivity over time, but the drivers underlying the long-term performance of monocultures of grassland species are not completely understood. We examined the performance of 60 grassland species growing in monoculture for 12 years in a biodiversity experiment (Jena Experiment) and studied three groups of biotic drivers potentially affecting plant performance in monocultures over time: 1) soil biota (nematode communities, arbuscular mycorrhizal fungi), 2) leaf traits related to leaf economics spectrum, and 3) plant life-cycle characteristics related to buffered population growth (viable seeds in topsoil, seedling density, seed survival). Monocultures of 15 out of 60 species increased productivity, while monocultures of the remaining 45 species showed slight to strong losses of productivity over time, resulting in zero biomass in 15 species. All three biotic drivers were related to the varying long-term performance of monocultures. Their combined influence on monoculture performance could be interpreted as a tradeoff between 'fast' versus 'slow' life strategies. 'Fast' species showed rapid resource use and little buffering of population growth through a viable seed bank, which led to high biomass production in young monocultures but a consecutive loss of biomass production over time. 'Slow' species were characterized by positive nematode effects (high abundance of predatory nematodes), conservative use of resources, and a viable seed bank with high recruitment success resulting in gradually increasing productivity over time. In summary, our study highlights the importance of studying long-term field monocultures to investigate the complex role of different biotic drivers responsible for productivity changes over time. These insights provide an essential baseline for estimating biodiversity effects on productivity as well as to understand and predict long-term performance of plant populations.

Keywords: leaf nitrogen, performance change, predators, slow versus fast life strategy, specific leaf area, viable seed bank

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Introduction

Many biodiversity experiments have shown that plant communities are more productive with higher plant species and functional group richness (Hector et al. 1999, Cardinale et al. 2007, Marquard et al. 2009). Comparisons of highly diverse mixtures with monocultures showed substantially higher biomass production in mixtures than expected from the monocultures (Tilman et al. 2001, Marquard et al. 2009, Reich et al. 2012), which is commonly referred to as 'overyielding' in biodiversity experiments. In long-term biodiversity experiments, species richness–productivity relationships strengthen over time (Reich et al. 2012, Guerrero-Ramírez et al. 2017). This may result from a deterioration of monoculture performance or an increase in mixture performance over time (Meyer et al. 2016, Guerrero-Ramírez et al. 2017). Analysis of monoculture performance over nine years in a grassland biodiversity experiment, however, did not provide evidence for increasing overyielding due to reduced performance of species in monocultures. Instead, monoculture and mixture biomass production generally declined over time, but temporal changes varied considerable among species (Marquard et al. 2013). The mechanisms underlying these varying performances of plant species in monoculture are not well understood.

Therefore, the aim of the present work was to investigate the role of different biotic drivers that potentially cause differences among plant species in their change in productivity over time when growing in monoculture. We focus on: 1) soil biota (here represented by nematode communities and arbuscular mycorrhizal fungi), 2) nutrient economy, and 3) life-cycle characteristics. We chose these non-mutually exclusive drivers for the following reasons.

First, there is a plethora of studies (Klironomos 2002, Ehrenfeld et al. 2005, Bezemer et al. 2006) providing evidence that the change in monoculture yield is due to effects that a plant species can have on the abiotic and biotic conditions of its own soil, a phenomenon referred to as plant–soil feedback (PSF) (Bever et al. 1997, van der Putten et al. 2013). One of the most influential soil biota are nematodes, because they are the most abundant metazoa in soil and play a key role in the soil food web with considerable effects on soil ecosystem processes and plant community structure (Bongers and Bongers 1998, van der Putten and van der Stoel 1998, de Deyn et al. 2003). They occupy all trophic levels by feeding on most soil organisms, such as bacteria and fungi, but also on higher plants and higher trophic levels (Bongers and Bongers 1998). Plant species, in turn, affect the diversity and abundance of nematodes in the soil food web (de Deyn et al. 2004, Viketoft et al. 2005, Eisenhauer et al. 2011), which can have a significant effect on biomass production of the plant itself (Eisenhauer et al. 2010). Another important group of soil biota are arbuscular mycorrhizal fungi (AMF) that form symbiotic associations with the majority of vascular plant species present in grassland ecosystems (Wang and Qiu 2006). This plant–mycorrhizal relationship can facilitate plant nutrient uptake, defense and growth (Smith and Read 2010). Numerous studies showed an overall positive effect of AMF on plant community productivity (van der Heijden et al. 1998, Klironomos et al. 2000); however, the dependency of plants on AMF associations varies among plant species from beneficial to antagonistic (van der Heijden and Horton 2009, Johnson 2010).

Second, plant productivity may also depend on species-specific abilities of plants to use soil resources. In most terrestrial ecosystems, including temperate grasslands, nitrogen is one of the most important resources limiting plant growth (Vitousek and Howarth 1991). Positive biodiversity–productivity relationships have often been related to complementarity in the use of soil resources (Roscher et al. 2008, Beßler et al. 2012), while high competition for similar resources could increase the risk of resource depletion and productivity loss over time. According to the leaf economics spectrum, plants fall along a gradient ranging from 'fast' plant species that produce leaves with an acquisitive use of resources and fast carbon capture (i.e. high specific leaf area (SLA), high leaf nitrogen concentrations, high rates of photosynthesis, but a short leaf life span) to 'slow' plant species producing leaves with a 'conservative' use of resources and slow carbon capture (i.e. low SLA, low leaf nitrogen, low rates of photosynthesis, but a long leaf life span) (Reich et al. 1998, Wright et al. 2004).

Third, life-history traits may affect the long-term persistence of plant populations. Such persistence mechanisms include the buffering of population growth (e.g. formation of a persistent seed bank, long-lived adult plant individuals) and vital rates (e.g. sexual reproduction and establishment from seeds) (Chesson 2000).

So far, little is known about the interplay of soil biota (nematodes and AMF), plant nutrient economy and life-cycle characteristics, leading to productivity changes over long time in monocultures (de Deyn 2017). To test how these potential drivers are related to different performances of plant species growing in monoculture, we studied the changes in aboveground biomass production over 12 years of 60 grassland species in monocultures in a large biodiversity experiment ('Monoculture experiment' as part of the Jena Experiment). We hypothesized, that performance declines of monocultures in the long term are caused by accumulation of plant-feeding nematodes and lower colonization by AMF, fast resource depletion and low buffering of population growth through a viable seed bank, while more stable or increasing performances over time are related to lower number of plant feeders and higher colonization by AMF, slow resource depletion and a viable seed bank with high recruitment success.

Material and methods

Study site – The Jena Experiment

The Jena Experiment is a large field experiment for investigating biodiversity effects on element cycling and trophic interactions in grassland communities (Roscher et al. 2004).

The study site is located in the floodplain of the Saale River near Jena (Thuringia, Germany, 50°55′N, 11°35′E, 130 m a.s.l.). The experimental site was converted from grassland to an arable field around 1960 and received high fertilizer inputs for the last decades prior to the establishment of the biodiversity experiment in 2002. The mean annual air temperature is 9.9°C, and annual precipitation is 660 mm in the region (1980–2010) (Hoffmann et al. 2014). A pool of 60 plant species typical for central European mesophilic grasslands of the Arrhenatherion type (Ellenberg 1988) was selected and divided into four functional groups: grasses (16 species), legumes (12 species), small herbs (12 species) and tall herbs (20 species) using above- and belowground morphological and phenological traits and N_2 fixation ability derived from the literature (Roscher et al. 2004). A full list of species and assignment to functional groups is given in the Supplementary material Appendix 1 Table A1. In the Monoculture Experiment, all these species were grown on plots of 3.5×3.5 m from 2002 to 2009 reduced to 1×1 m plots from 2010 to 2014. Seeds were sown in early May 2002 with a density of 1000 viable seeds per m^2 (adjusted for germination rates from laboratory tests). In November 2002, not or sparsely established species (Heiße et al. 2007) were re-sown (in total 10 out of 60 species; for details see Supplementary material Appendix 1 Table A1 and Roscher et al. 2004). Further sowings did not happen. There were two replicate monoculture plots per species until 2008 and one plot per species from 2008 to 2014. The plots were mown every year in June and September, and mown plant material was removed as it is typical for management of hay meadows in the study region. Additionally, plots were weeded (weeds were cut out with knifes) two to three times per year (April, July, October) to keep pure monocultures. Plots did not receive any fertilizer.

Biomass changes over time

Aboveground biomass was harvested twice a year in late May and late August from 2003 to 2014. Two 0.2×0.5 m sample areas were randomly chosen within the monoculture plots, excluding the outer margin (0.5 m) between 2003 and 2010. One sample in the plot centre was taken after reduction of plot size to 1×1 m (from 2010 onwards). If no biomass was available in the 0.2×0.5 m of the harvest frame, the sample area was doubled to increase the probability to sample biomass of sparsely or patchily distributed species. Plants were cut 3 cm above ground level. Samples were dried at 70°C for 48 h and weighed. The annual aboveground biomass production (2003–2014) per plot was calculated as the sum of the two biomass harvests per year (biomass of replicated samples per plot and harvest were averaged) and extrapolated to 1 m^2. In order to get a measure of species performance over time, the biomass slope over the 12 measurement years was calculated as follows. First, biomass of replicated monocultures was averaged (2003–2007) to get a mean value of monoculture biomass production per species and year. Second, the annual aboveground biomass production of each monoculture was divided by the mean of the annual aboveground community biomass across all mixture plots of the Jena Experiment (n = 66 plots) (Weigelt et al. 2010) to correct for differences between years and a general decline in productivity over time (Roscher et al. 2013). Third, the corrected yearly monoculture values were fitted against time as a linear variable (years 2003–2014) in regression models. A negative slope of the regression line indicated a decline of biomass production over the years, while a positive slope indicated an increase. We also calculated regression slopes over time for uncorrected biomass data to assess the effects of the applied correction, but corrected and uncorrected slopes were strongly correlated (r = 0.70, p < 0.01). In addition to the biomass slope, annual biomass production of the final study year 2014 (= biomass production in 2014) was used as second response variable in the analyses.

For a more detailed investigation of monoculture performance over time, species were categorized into three monoculture performance categories: (A) $zero\_BM_{2014}$ = species, without biomass in the sample area in 2014 (either species were extinct or had few individuals outside the sample area; 15 species), (B) neg_BM_{slope} = species with biomass production $> 0\ g_{dw}\ m^{-2}$ in 2014 and negative biomass slope (30 species), and (C) pos_BM_{slope} = species with biomass production $> 0\ g_{dw}\ m^{-2}$ in 2014 and positive slope (15 species).

Nematode communities

On 19 and 20 May 2014, i.e. shortly before peak plant biomass, three soil cores (1 cm diameter, 15 cm depth) in the root zone of target species were taken in each of the 60 monocultures. Samples were pooled per plot, sieved at 2 mm to remove stones and roots and then stored in a refrigerator (4°C) for a maximum of four days. For free-living nematode extraction, a modified Baermann method was used (Ruess 1995): 70 g soil of each sample were weighed and filled into plastic pots with a bottom consisting of gauze and coated with a milk filter. Pots were placed in funnels, which were extended by a plastic tube closed with a clamp at the bottom. Funnels were then filled with water up to the bottom of the pot and kept for 72 h at room temperature (~20°C) for nematode migration from soil to water. Thereafter, Baermann funnels were drained and the extracted nematodes were killed and fixed in formaldehyde solution (4%). After extraction, soil samples were dried at 50°C for 48 h and weighed. Animals were counted per sample, and 100 individuals (or all if less than 100 occurred in a sample) were identified to genus level (Bongers 1988) using 1000× magnification. Total number of nematodes per gram dry soil, genus richness and Shannon Wiener index based on genera (genus diversity) were calculated (Neher and Darby 2006). Afterwards, nematodes were divided into trophic groups: plant feeders, fungal feeders, bacterial feeders, predators and omnivores (we used main food source for division) (Yeates et al. 1993). Within the trophic group of plant feeders, the Shannon Wiener index based on plant-feeding types (migratory endoparasites, semi-endoparasites, ectoparasites, epidermal cell and root hair feeders) was calculated, because of a large range of different strategies

of plant-feeding nematodes (= plant feeder type diversity) (Yeates et al. 1993, Bongers and Bongers 1998, Neher and Darby 2006). Furthermore, the predator–prey ratio (abundance of predatory nematodes divided by abundance of plant-feeding nematodes) was derived, which indicates the ability of the nematode community to prevent accumulation of plant feeders (top–down control) (Neher and Darby 2006).

Arbuscular mycorrhizal fungi colonization

Plant individuals of each species (if possible) were picked out from soil samples taken for seed bank analyses on 7 March 2014 to reduce destructive sampling on the plots. Aboveground plant material was removed and roots were stored in 70% ethanol until further processing. Roots were cleared by heating in 10% KOH at 70°C for 90–180 min (heating times varied depending on species) and then heated for 5 min at 70°C in an ink–vinegar solution (5% black ink: Parker S0037460 Quink Black; 95% vinegar: white household vinegar, 5% acetic acid) following Vierheilig et al. (1998). Roots were rinsed with water several times and stored in tap water with a few drops of vinegar to remove excess stain. Roots were cut into smaller pieces and mounted with 50% glycerin on microscope slides and the %AMF colonization was scored under 200× magnification using the line-intersect method for 100 intersects (McGonigle et al. 1990).

Traits related to nutrient economy

Bulk samples of 10–15 leaves of different plant individuals (if possible) of each species were collected every May in 2003, 2004, 2006, 2007, 2011, 2012 and 2014. Samples were stored in a cool box for transport. In laboratory, total leaf area was measured using a leaf area meter. Leaf samples were dried for 48 h at 70°C and weighed. Then, plant material was ground to fine powder with a mixer mill. Approximately 20 mg of milled plant material was used to determine leaf nitrogen concentrations ($mg\,N\,g^{-1}_{leaf}$) with an elemental analyser. Specific leaf area was calculated as ratio between total leaf area and total leaf mass (SLA; $mm^2_{leaf}g^{-1}_{dw}$).

In order to assess changes in traits related to the leaf economics spectrum over time, leaf nitrogen concentrations and SLA of each species were fitted against year (as a linear variable) in linear regression models, respectively (= leaf nitrogen slope and SLA slope). While a negative slope of a linear regression suggested a decline in trait values over time, a positive slope indicated an increase. In addition, we used leaf nitrogen concentrations and SLA measured in the last study year (2014) as further variable in analyses (= leaf nitrogen concentrations and SLA in 2014).

Life-cycle characteristics

Number of viable seeds in the topsoil

Soil samples were collected using a soil corer with 5.7 cm diameter to 5 cm depth on 7 March 2014. Three cores were taken at 20 cm distance in each of the 60 monoculture plots, pooled to a bulk sample and stored in a cooling chamber until further processing. Samples were concentrated using the bulk reduction method that induces a more rapid and complete germination of seeds in many species (ter Heerdt et al. 1996). To this end, soil samples were wet-sieved with tap water through sieves (2 mm and 0.2 mm) to remove roots and rhizomes (to prevent vegetative propagation) as well as coarse and fine soil particles. Residue of the 0.2 mm sieve was spread out thinly (< 5 mm layer) in trays filled with a heat-sterilized sand–soil mixture (1:1). Trays were placed in an open greenhouse with a roof, which automatically closes at rain and cultivated at ambient temperatures to promote germination by natural daily temperature fluctuations. Trays were regularly watered. They were covered with fine gauze material to prevent contamination by wind-borne seeds. Germinated seedlings were counted every 4–6 weeks until spring 2015 and removed afterwards to prevent double counting. The total number of viable seeds per monoculture was calculated as sum of single counts and extrapolated to $1\,m^2$ (= viable seeds in topsoil).

Seedling density

In each of the 60 monocultures, a study area of $0.2 \times 0.2\,m$ was permanently marked in 2014. Because the experimental species vary in their seasonal patterns of seedling emergence, the number of seedlings (i.e. plants with visible cotyledons) was counted in spring (24–25 April), summer (23–24 June) and autumn (14–21 September) 2014. The sum of the three counts was extrapolated to $1\,m^2$ and used as a measure of seedling density although we could not exclude that additional seedlings germinated between these census dates or that single individuals remained in the seedling stage and were counted again during the subsequent census.

Seed survival in the topsoil

For all 60 monocultures, 100 seeds of the target species (purchased by the same commercial supplier as for the establishment of most monoculture plots) were filled in a nylon bag stabilized with a plastic ring of 5 cm diameter to get an even exposure area (except for *Onobrychis viciifolia* and *Tragopogon pratensis* with 50 seeds per nylon bag because of large seed size). Two bags (or four bags for *O. viciifolia* and *T. pratensis*) of the target species (in total 200 seeds for each species) were buried at a depth of 5 cm in the soil of the corresponding monoculture on 18 October 2014. After six months (2 April 2015), nylon bags were excavated, opened and seeds were rinsed over tap water. Seeds were placed in petri dishes with filter paper and germinated in a climate chamber (12 h day at 24°C, 12 h night at 12°C). Seedlings germinated within four weeks were counted and averaged across replicated samples per species as measure of seed survival (%).

Statistical analysis

To test for linear relationships between species performances and biotic drivers, simple regression models were fitted with

Table 1. List of variables derived from measurements in monocultures of the Jena Experiment.

Variable	Abbreviation	Unit	Transformation
Biomass production in 2014	BM_{2014}	dry mass per unit area ($g\,m^{-2}$)	log
Biomass slope	BM_{slope}	–	–
Monoculture performance categories	**Performance categories**	–	–
Biomass absent in 2014	$Zero\_BM_{2014}$	$BM_{2014} = 0\,g\,m^{-2}$	–
Negative biomass slope	Neg_BM_{slope}	$BM_{2014} > 0\,g\,m^{-2}$; $BM_{slope} < 0$	–
Positive biomass slope	Pos_BM_{slope}	$BM_{2014} > 0\,g\,m^{-2}$; $BM_{slope} > 0$	–
Soil biota	**Soil biota**	–	–
Nematode communities	Nematodes	–	–
Total number of nematodes	Total number	individuals per unit dry soil (individuals g^{-1}_{soil})	–
Genus richness	Genus richness	–	–
Genus diversity (Shannon–Wiener index)	Genus diversity	–	–
Plant-feeding nematodes	Plant feeders	individuals per unit dry soil (individuals g^{-1}_{soil})	log
Plant feeder type diversity (S.–W. index)	PFT diversity	–	–
Fungal-feeding nematodes	Fungal feeders	individuals per unit dry soil (individuals g^{-1}_{soil})	log + 1
Bacterial-feeding nematodes	Bacterial feeders	individuals per unit dry soil (individuals g^{-1}_{soil})	log + 1
Predatory nematodes	Predators	individuals per unit dry soil (individuals g^{-1}_{soil})	log+0.1
Predator versus Plant feeder ratio	Predator–prey ratio	–	log+0.1
Omnivorous nematodes	Omnivores	individuals per unit dry soil (individuals g^{-1}_{soil})	log+0.1
Arbuscular mycorrhizal fungi colonization	AMF colonization	percentage of fungi colonizing total root system	–
Plant traits related to nutrient economy	**Nutrient economy**	–	–
Leaf nitrogen concentrations in 2014	Leaf nitrogen concentrations	nitrogen per leaf dry mass ($mg\,N\,g^{-1}_{leaf}$)	log
Leaf nitrogen slope	Leaf nitrogen slope	–	–
Specific leaf area in 2014	SLA	leaf area per leaf dry mass ($mm^2_{leaf}\,mg^{-1}_{leaf}$)	–
Specific leaf area slope	SLA slope	–	–
Life-cycle characteristics	**Life-cycle characteristics**	–	–
Number of viable seeds in topsoil	Seed bank	viable seeds per unit soil surface (seeds m^{-2})	log + 1
Number of emerged seedlings	Seedling density	seedlings per unit soil surface (seedlings m^{-2})	log + 1
Number of survived seeds (seed burial experiment)	Seed survival	number of viable seeds after burial	log + 1

biomass production in 2014 or biomass slope as response variable and each continuous predictor variable (see Table 1: soil biota, nutrient economy, life cycle characteristics) as single explanatory term. Biomass production of the 15 species without biomass in 2014 (zero_BM$_{2014}$) as well as biomass slope of *Onobrychis viciifolia* (extremely large value, was considered as outlier, Fig. 1) were excluded from the regression analysis. To test for differences in the extent of biotic drivers between poorly- and well-performing species, we determined how the continuous predictor variables (see Table 1: biomass production in 2014, biomass slope, soil biota, nutrient economy, life cycle characteristics) differed among the following explanatory terms: plots with and without biomass (factor with two levels: biomass present (45 species), biomass absent (15 species) in 2014) and categories of monoculture performances (factor with three levels: zero_BM$_{2014}$ (15 species), neg_BM$_{slope}$ (30 species), pos_BM$_{slope}$ (15 species)). We used general linear models with subsequent analysis of variance (ANOVA) or a hurdle model (viable seeds in topsoil, seedling density).

To test for relationships between different biotic drivers and to monoculture performance categories, we used standardized principal component analysis (PCA) and a correlation matrix (type = pearson). For the PCA we used all predictor variables except for AMF (missing data for 33 species), leaf

nitrogen concentrations and SLA in 2014 (missing data for 13 species) and excluded three species (*Cardamine pratensis*, *Bromus erectus* and *Bromus hordeaceus*) due to missing values for leaf nitrogen slope and SLA slope or seed survival (n = 57).

All calculations were done using the statistical software R (ver. 3.1.2, <www.r-project.org>) including the packages 'pscl' (Zeileis et al. 2008) for hurdle models, 'vegan' (Oksanen et al. 2018) for PCA and 'Hmisc' for correlation matrix (Harrel and Dupont 2017).

Results

Monoculture performance

On average, biomass production of species declined over time (average biomass slope = -0.021 ± 0.006 SE), but changes in biomass production of the 60 species varied greatly and ranged from strong declines to slight increases (Fig. 1). Biomass slope and biomass production in 2014 were positively correlated (r = 0.47, p < 0.001; Fig. 1, Supplementary material Appendix 1 Fig. A1). Species that did not produce any biomass in 2014 (zero_BM$_{2014}$) had a negative biomass slope (-0.049 ± 0.017), i.e. decreasing productivity over time. Species, which produced biomass in 2014, but showed
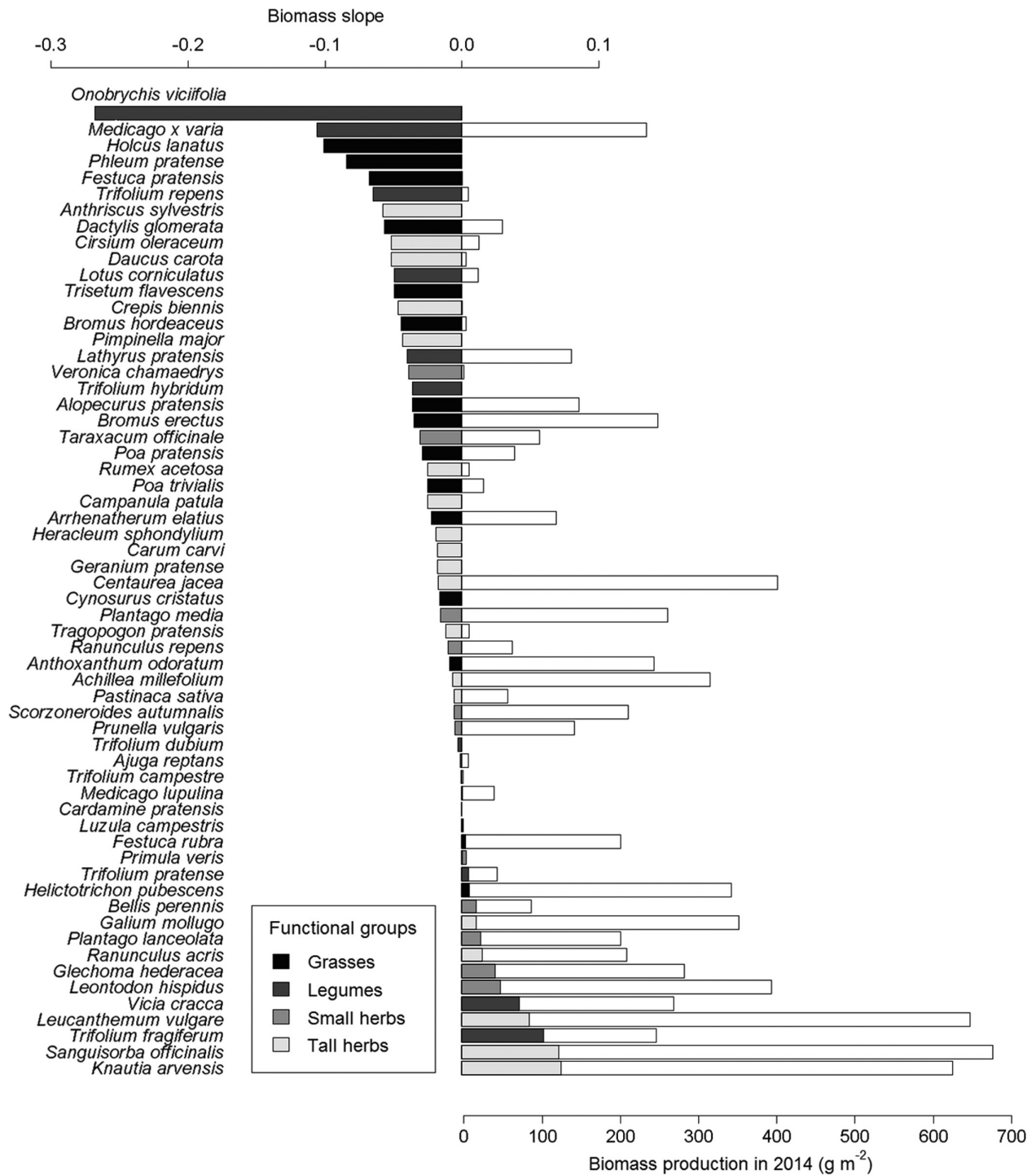
Figure 1. Biomass slope (grey bars) and biomass production in 2014 (white bars) for 60 grassland species studied in 12-year-old monocultures. Bright to dark grey bars indicate different plant functional groups of the Jena Experiment.

negative biomass slope (-0.030 ± 0.005; neg_BM_{slope}) were characterized by an intermediate amount of biomass production in 2014 ($99.59 \pm 20.48\,g\,m^{-2}$). Species with positive biomass slope (0.028 ± 0.006; pos_BM_{slope}) had the highest biomass production in 2014 ($307.11 \pm 54.18\,g\,m^{-2}$).

Monoculture performance related to soil biota

Abundance of plant-feeding nematodes ranged between 58 and 723 individuals $100\,g^{-1}_{soil}$ in monoculture soils but had no significant influence on biomass performance. Regression

Table 2. Summary of ANOVA of species with and without monoculture biomass in 2014 and species in different monoculture performance categories for biomass production in 2014, biomass slope (with and without the outlier *O. viciifolia*), soil biota, traits related to nutrient economy and life-cycle characteristics. Listed are degrees of freedom (df), mean sums of squares (MS), F ratios (F) and p-values (p). Significant effects ($p < 0.05$) are given in bold, and marginally significant effects ($p < 0.1$) are given in italics. For list of variables, abbreviations and units see Table 1.

	BM absent versus BM not absent				Monoculture performance categories			
	df	MS	F	p	df	MS	F	p
Biomass production in 2014	–	–	–	–	2	110.77	53.85	**<0.001**
Biomass slope (*O. vic.* deleted)	–	–	–	–	2	0.02	28.02	**<0.001**
Biomass slope	–	–	–	–	2	0.02	15.75	**<0.001**
Soil biota								
Nematode communities								
Total number of nematodes	1	0.01	0.01	0.976	2	0.18	0.02	0.984
Genus richness	1	1.42	0.12	0.727	2	1.80	0.15	0.858
Genus diversity	1	3.66	0.07	0.790	2	2.79	0.05	0.948
Plant-feeding nematodes	1	0.25	0.69	0.410	2	0.25	0.69	0.508
Plant feeder type diversity	1	0.49	9.47	**0.003**	2	0.26	5.06	**0.009**
Fungal-feeding nematodes	1	0.01	0.09	0.762	2	0.04	0.27	0.761
Bacterial-feeding nematodes	1	0.01	0.03	0.869	2	0.01	0.07	0.937
Predatory nematodes	1	2.57	6.31	**0.015**	2	1.39	3.39	**0.041**
Predator–prey ratio	1	0.57	2.69	0.107	2	0.30	1.41	0.254
Omnivorous nematodes	1	8.87	10.16	**0.002**	2	5.71	6.77	**0.002**
AMF colonization	–	–	–	–	1	732.70	1.22	0.280
Nutrient economy								
Leaf nitrogen conc. 2014	1	0.26	2.22	0.143	2	0.13	1.09	0.346
Leaf nitrogen slope	1	1.18	3.27	*0.076*	2	1.08	3.10	*0.053*
Specific leaf area 2014	1	44.28	1.47	0.232	2	22.51	0.73	0.488
Specific leaf area slope	1	0.07	0.36	0.550	2	0.04	0.19	0.826
Life-cycle characteristics								
Seed survival	1	7.95	5.81	**0.019**	2	4.06	2.93	*0.062*

analysis revealed a marginally significant negative relationship between biomass production in 2014 and abundance of omnivorous nematodes (Table 3, Supplementary material Appendix 1 Fig. A2a). Furthermore, biomass slope (without *O. viciifolia* monoculture) was negatively related to plant feeder type diversity as well as marginally significant and positively related to predator–prey ratio and abundance of predatory nematodes (Table 3, Supplementary material Appendix 1 Fig. A3).

Plant feeder type diversity as well as abundance of predators and omnivores differed among monoculture performance categories (Table 2, Fig. 2a–c). PFT diversity and abundance of omnivores were higher in soil of plant species without biomass (zero_BM$_{2014}$) than in soil of plant species with biomass in 2014 (neg_BM$_{slope}$, pos_BM$_{slope}$), while abundance of predators was lower in soil of plant species without biomass (zero_BM$_{2014}$) than in soil of plant species with increasing monoculture performance over time (pos_BM$_{slope}$).

AMF colonization showed marginally significant positive relationship with biomass production in 2014 (Table 3, Supplementary material Appendix 1 Fig. A2b).

Monoculture performance related to plant nutrient economy

SLA in 2014 tended to be negatively related to biomass production in 2014 (Supplementary material Appendix 1 Fig. A2c) and nitrogen slope tended to be positively related to biomass slope (Table 3, Supplementary material Appendix 1 Fig. A4a). Monoculture performance categories differed marginally significant in leaf nitrogen slope (Table 2, Fig. 2d): species without biomass in 2014 (zero_BM$_{2014}$) showed a stronger decline in leaf nitrogen concentrations over time (more negative leaf nitrogen slope) than species with positive biomass slope (pos_BM$_{slope}$). Species with a negative biomass slope (neg_BM$_{slope}$) were characterised by an intermediate decline in leaf nitrogen over time (Table 2, Fig. 2d).

Monoculture performance related to plant life-cycle characteristics

Biomass slope tended to be positively related to the number of viable seeds in topsoil (Supplementary material Appendix 1 Fig. A4b) and was positively related to seedling density (Table 3, Supplementary material Appendix 1 Fig. A4c). The number of viable seeds in topsoil and seedling density in 2014 differed significantly among monoculture performance categories (for hurdle model output see Supplementary material Appendix 1 Table A2). While 12 out of 15 species without biomass in 2014 (zero_BM$_{2014}$) did not have a viable seed bank, the proportion was smaller in categories containing species, which produced biomass in 2014 (neg_BM$_{slope}$: 16 out of 30; pos_BM$_{slope}$: 8 out of 15). In line with these results, 12 out of 15 species in category zero_BM$_{2014}$ had no emerging seedlings in 2014, while only half of the species in category neg_BM$_{slope}$ (15 out of 30) and few species in category
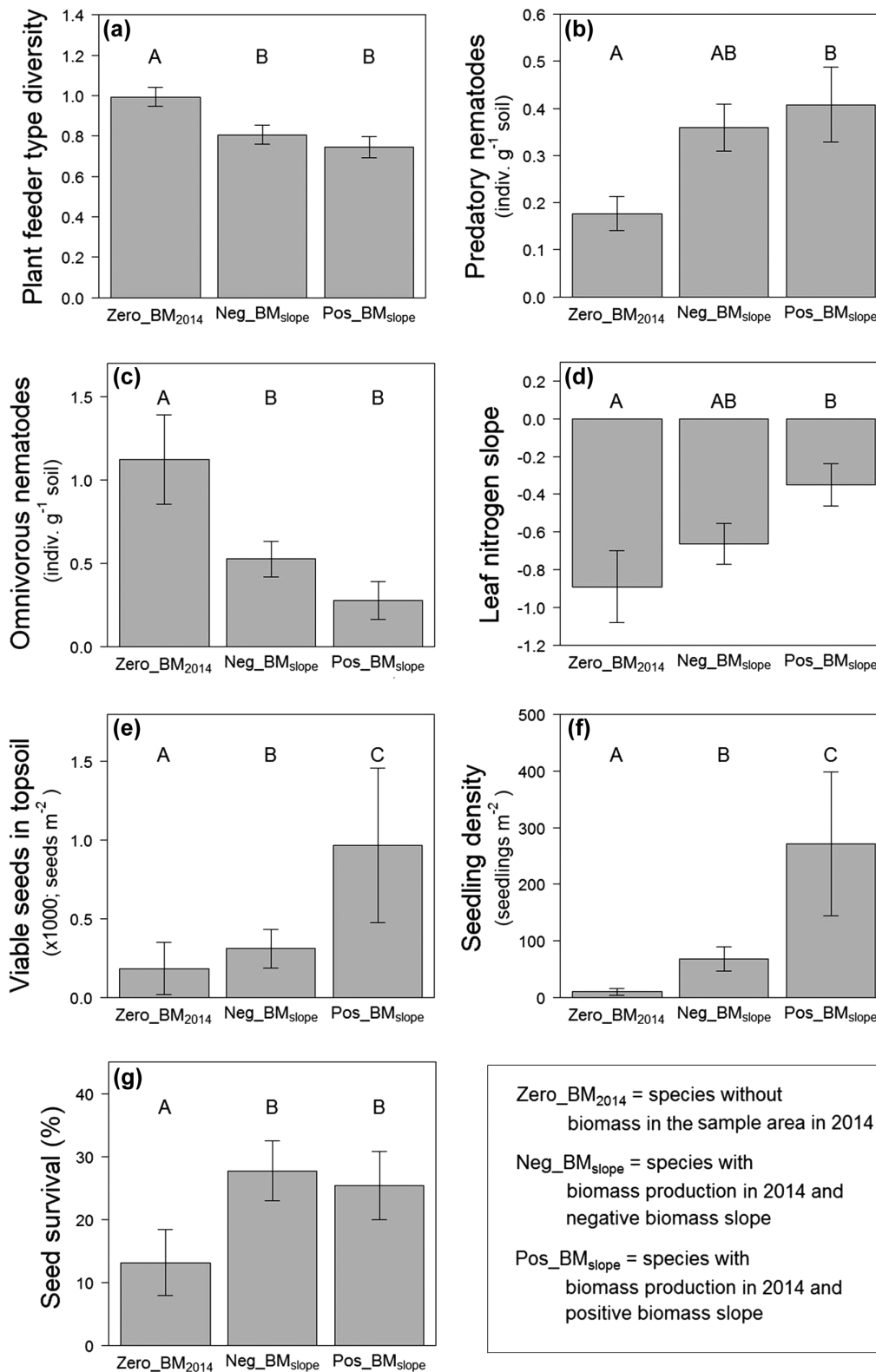
Figure 2. Plant feeder type diversity (a), abundance of predatory nematodes (b), abundance of omnivorous nematodes (c) (a–c = nematode communities), leaf nitrogen slope (d) (nutrient economy), number of viable seeds in topsoil (e), seedling density (f) and seed survival (g) (e–g = life-cycle characteristics) for monoculture performance categories: zero_BM_{2014} (n = 15 species), neg_BM_{slope} (n = 30 species), pos_BM_{slope} (n = 15 species). Bars show mean values (± 1 SE); letters above bars indicate significant (a–f; p < 0.05) or marginally significant (g; p < 0.1) differences among categories (Tukey's HSD test). All variables were measured in 2014, except seed survival, which was recorded in winter 2014/2015. Leaf nitrogen slopes were calculated from leaf nitrogen concentrations measured in different years from 2003 to 2014.

Table 3. Summary of regression analyses of biomass production in 2014 (without species in category Zero_BM$_{2014}$) and biomass slope (with and without the outlier *Onobrychis viciifolia*) as functions of soil biota, traits related to nutrient economy and life-cycle characteristics. Listed are the direction of the relationship (+/−; according to positive or negative correlation), coefficients of determination (R^2) and p-values (p). Significant relationships ($p < 0.05$) are given in bold, and marginally significant relationships ($p < 0.1$) are given in italics. For list of variables, abbreviations and units see Table 1.

	Biomass production in 2014 (BM $\neq$ 0 g m^{-2})			Biomass slope (*O. vic.* deleted)			Biomass slope		
	+/−	R^2	p	+/−	R^2	p	+/−	R^2	p
Soil biota									
Nematode communities									
Total number of nematodes	+	0.06	0.119	−	<0.01	0.549	−	0.02	0.269
Genus richness	−	<0.01	0.654	+	<0.01	0.636	+	<0.01	0.511
Genus diversity	−	0.02	0.348	−	0.03	0.207	+	0.02	0.330
Plant-feeding nematodes	+	0.04	0.212	+	<0.01	0.970	−	<0.01	0.478
Plant feeder type diversity	−	0.03	0.251	−	0.09	**0.020**	−	0.04	0.144
Fungal-feeding nematodes	+	0.01	0.561	−	<0.01	0.886	−	0.02	0.251
Bacterial-feeding nematodes	+	0.06	0.105	−	<0.01	0.792	−	<0.01	0.851
Predatory nematodes	+	<0.01	0.558	+	0.05	*0.088*	+	0.05	*0.074*
Predator–prey ratio	+	<0.01	0.797	+	0.06	*0.057*	+	0.08	**0.028**
Omnivorous nematodes	−	0.09	*0.052*	−	0.04	0.107		0.03	0.178
AMF colonization	+	0.14	*0.053*	+	0.06	0.237	+	0.06	0.237
Nutrient economy									
Leaf nitrogen conc. 2014	−	<0.01	0.894	−	0.02	0.322	−	0.02	0.322
Leaf nitrogen slope	+	0.02	0.337	+	0.05	*0.096*	+	0.02	0.250
Specific leaf area 2014	−	0.08	*0.056*	−	<0.01	0.675	−	<0.01	0.675
Specific leaf area slope	−	0.01	0.445	+	0.02	0.286	+	0.01	0.361
Life-cycle characteristics									
Viable seeds in topsoil	+	<0.01	0.582	+	0.05	*0.076*	+	0.06	*0.063*
Seedling density	+	0.01	0.429	+	0.12	**0.007**	+	0.11	**0.008**
Seed survival	+	0.02	0.383	+	0.03	0.220	+	0.02	0.302

pos_BM$_{slope}$ (3 out of 15) did not show seedling emergence in 2014. Furthermore, if a viable seed bank or seedlings were found, their quantities differed significantly among the monoculture performance categories. Species with positive biomass slope (pos_BM$_{slope}$) had the highest number of viable seeds in topsoil and seedling density, while species with negative biomass slope (neg_BM$_{slope}$) had intermediate and species without biomass (zero_BM$_{2014}$) had the lowest numbers of viable seeds and seedlings (Supplementary material Appendix 1 Table A2, Fig. 2e–f). Moreover, the number of surviving buried seeds (seed survival) was significantly lower in species that did not produce any biomass in 2014 than in species with biomass (Table 2, Fig. 2g).

Relationships between multiple biotic drivers

The two leading axes of the PCA including 57 species (Fig. 3) explained about 37.1% of the total variation in predictor variables. The first principle component (PC) accounted for 20.3% of the variance and had high negative loadings for plant feeders, bacterial feeders, fungal feeders and total number of nematodes (Supplementary material Appendix 1 Table A3). The first PC did not separate monoculture performance categories. The second PC explained 16.8% of variance and had high negative loadings for the three studied life-cycle characteristics and high positive loadings for nematode genus diversity, plant feeder type diversity and abundance of omnivores (Supplementary material Appendix 1 Table A3). Species that did not produce any biomass in 2014 (zero_BM$_{2014}$) were clearly separated from species, which produced biomass in 2014 (pos_BM$_{slope}$, neg_BM$_{slope}$) on the second PC ($F_{2,54} = 8.96$, $p < 0.001$; Tukey's HSD: $p = 0.002$ for neg_BM$_{slope}$ versus zero_BM$_{2014}$; $p < 0.001$ for pos_BM$_{slope}$ versus zero_BM$_{2014}$; $p = 0.130$ for pos_BM$_{slope}$ versus neg_BM$_{slope}$; Fig. 3), indicating that species without biomass in 2014 were characterized by higher genus diversity, plant feeder type diversity and number of omnivorous nematodes and a lower likelihood of successful generative reproduction (smaller viable seed bank, lower seed survival, lower seedling densities). Furthermore, PCA and correlation matrix results (Supplementary material Appendix 1 Table A4) showed significant relationships among variables of all three biotic drivers.

Discussion

Our study shows that the performance of species growing in long-term monoculture can be highly variable, from decreasing to increasing performance over time, which is in line with previous results obtained with a time series of nine years (Marquard et al. 2013). Our univariate and multivariate analyses of different biotic drivers documented that there is no single variable explaining the varying performance of monocultures across all study species. Variables from all categories of the investigated drivers (nematode communities, nutrient
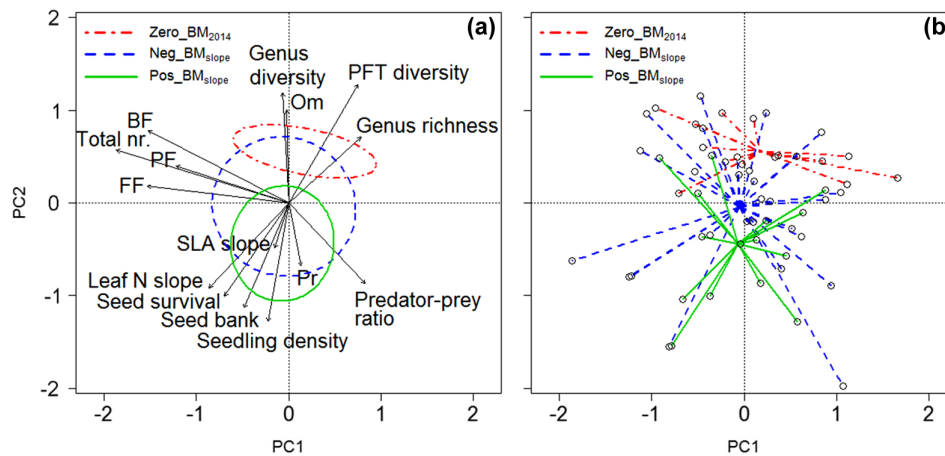
Figure 3. Standardized principal components analysis (PCA; first versus second axes) of 57 species growing in 12 year-old monocultures characterized by 10 nematode community variables (total number of nematodes ('Total nr.'), genus richness and diversity, plant feeders ('PF'), plant feeder type diversity ('PFT diversity'), bacterial feeders ('BF'), fungal feeders ('FF'), predators ('Pr'), predator–prey ratio, omnivores ('OM')); two nutrient economy variables (specific leaf area slope ('SLA slope'), leaf nitrogen slope ('Leaf N slope')) and three life-cycle characteristics (seedling density, number of viable seeds in topsoil ('Seed bank'), seed survival). Shown are monoculture performance categories in (a, b) as ellipses indicating the standard deviation of point scores for each category (a), and as circles for the position of different monocultures in ordination space with spider-diagrams for group centroids (b). For list of variables, transformation and units see Table 1.

economy, life-cycle characteristics) were related to the long-term monoculture performance, but the weak effects of single drivers suggested that the relative importance of these drivers varied to some degree among species.

Monoculture performance related to soil biota

Although we detected large differences in the abundance of plant-feeding nematodes in soil of the monocultures, they did not significantly influence biomass performance, which is in line with other long-term studies (de Deyn et al. 2004, Viketoft et al. 2005). However, other nematode variables were related to monoculture performance. First, the observed negative relationship between plant feeder type diversity and abundances of omnivores, respectively, and monoculture performance may indicate that these nematode variables negatively influence productivity of poorly-performing species. However, it is equally possible that these relationships were the consequence and not the cause of plant performance. Monoculture plots with low target biomass had a 'hidden' higher plant species diversity due to invading weed species, at least between two weeding events, which can explain the higher values of these nematode variables in soil of poorly-performing species (especially in soil of species without biomass). Second, the abundance of predatory nematodes as well as the predator–prey ratio were positively related to plant monoculture performance, supporting the hypothesis that predators enhance top–down control (Khan and Kim 2007) and contribute to a positive effect of soil biota on plant biomass production (Eisenhauer et al. 2011). Furthermore, by feeding on prey, predators release nutrients for plants, which may increase the resistance against plant-feeding nematodes (Khan and Kim 2007) and thus increase productivity over time. Therefore, higher abundance of predatory nematodes may contribute to

a positive PSF. Such an effect, which may slow down negative net effects, can be strengthened over time (Zuppinger-Dingley et al. 2016) due to co-selection between plant species and beneficial soil biota (van Moorsel et al. 2018), which may explain the increasing productivity over time in 15 (pos_BM$_{slope}$) out of 60 species tested in the present study.

Contrary to our expectations that AMF colonization positively influences plant monoculture performance, we only found a marginally significant positive relationship between AMF colonization and biomass production in 2014. Unfortunately, the study of AMF colonization was restricted to 27 mostly well-performing plant species out of the pool of 60 species due the lack of root samples that could be obtained from species with no or very few individuals in the plot. Therefore, we cannot exclude the possibility that we underestimated the role of AMF as a biotic driver of monoculture performance. Additionally, it is important to note that AMF colonization does not necessarily reflect the efficiency in the relationship for the plant (Wagg et al. 2011). Also, AMF can benefit plants in other ways then biomass production, such as pathogen defence or recruitment success (van der Heijden 2004, de la Peña et al. 2006).

Monoculture performance related to plant nutrient economy

Positive relationships between leaf nitrogen, specific leaf area and net photosynthesis rate (Evans 1983, 1989, Reich et al. 1998) as well as between leaf nitrogen and soil nitrogen (Sinclair et al. 2000) are well established from several studies suggesting that higher nutrient availability in the soil may lead to higher photosynthesis rates and thus higher plant productivity. Furthermore, SLA and leaf nitrogen concentration are known as important components of the 'leaf economics

spectrum' (Wright et al. 2004) differentiating between species with rapid resource acquisition and use ('fast' species with high SLA and leaf nitrogen concentrations) and species with a more conservative use of resources ('slow' species with low SLA and leaf nitrogen concentrations) (Reich 2014). In our study, we found a marginal significant negative relationship between SLA and biomass production in 2014 ($p < 0.06$), suggesting that plant species with a low biomass production in 2014 pursued a 'fast' strategy (high SLA), while species with a high biomass production in 2014 were 'slow' species (low SLA). Furthermore, 'fast' species tended to loose more leaf nitrogen over time, i.e. plant species without biomass in 2014 (zero_BM$_{2014}$) showed stronger declines in leaf nitrogen concentrations over time (as long they were available) than species with a positive biomass slope (pos_BM$_{slope}$; $p < 0.06$), but had highest leaf nitrogen concentrations in the early years of the Monoculture Experiment (2003; 2004) compared to 'slow' species (Supplementary material Appendix 1 Table A5). This suggests that 'fast' species consumed more nitrogen at the beginning than 'slow' species, but possibly suffered from resource depletion over time because most nutrients incorporated in plant tissues were removed with mown biomass in our experiment. In contrast, 'slow' species may have benefitted from more balanced use of nitrogen over the years causing a more stable monoculture performance.

Monoculture performance related to plant life-cycle characteristics

In biodiversity experiments such as the Jena Experiment plant communities are established by a single sowing event. As the life of plant individuals is not infinite (average age of perennial herb species in the Jena Experiment ranges from 1 to 5 years, but some individuals are as old as the biodiversity experiment, see Roeder et al. 2017), the long-term persistence of plant populations depends on re-establishment from seeds or clonal growth. In our study, species with decreasing productivity over time (zero_BM$_{2014}$ and neg_BM$_{slope}$) had no or a low number of viable seeds in the topsoil (seed bank) and seedling density (recruitment), while better-performing species (pos_BM$_{slope}$) had a high number of seeds and seedlings. The observed productivity loss in species without biomass in 2014 (zero_BM$_{2014}$) was associated with low seed survival, which could be induced either by interaction with soil biota (deep burial by animals or attack by seed predators) (Leishman et al. 2000) or being an inherent characteristic of the species themselves. Some grassland species, however, mainly reproduce by clonal growth (Coffin and Lauenroth 1989) and are less dependent on the successful establishment from seeds. According to the literature (Klotz et al. 2003), only a few species with biomass in 2014 predominantly reproduced via seeds rather than by clonal growth (neg_BM$_{slope}$: 10 out of 30; pos_BM$_{slope}$: 4 out of 15), while the percentage was higher in species that did not produce any biomass in 2014 (7 out of 15). We suggest that the inability to form a permanent seed bank as well as the lack of clonal growth had negative impacts on biomass performance.

Specificity of extinct plant species

To further assess the specificity of species, which did not produce any biomass in the study year (zero_BM$_{2014}$), we inspected, in which experimental year plant species in the category zero_BM$_{2014}$ produced no biomass in the sampling area for the first time and did not appear again in the subsequent years. In the following, this event is called 'extinction'; however, it does not mean that the species was necessarily lost on the whole plot (1×1 m). Overall, 11 out of 15 species went extinct after more than ten years: two species in 2013 and nine species in 2014. Especially, for the latter species, which disappeared for the first time in our sampling year, we cannot prove if they came back in the subsequent year. From the remaining species, only one species was never well established in monoculture and already disappeared in 2004 (*Cardamine pratensis*; for more information about extinction years see Supplementary material Appendix 1 Table A1). Because of the late extinction of most species, we think that the differences in the measured variables among the monoculture performance categories found in this study are plausible and not the result of vegetation-free plots. Nevertheless, we are not able to exclude other factors, which were, at least in part, responsible for the relationships found in the study, e.g. that poorly-performing species were more sensitive for replacement by weed species influencing, inter alia, the nematode community.

One possible reason for the high loss of species from 2013 to 2014 is a flood event in summer 2013 (Wright et al. 2015). The nine species, which went extinct in 2014 showed a strong loss of productivity over time (biomass slope: -0.041 ± 0.008; except *Luzula campestris*, which was always poorly established), and likely the small remaining populations were particularly vulnerable to the flood. The lack of recovery in 2014 can be explained by the fact that these species did not form a permanent seed bank.

Linking the biotic drivers to the different life strategies of plant species

Based on the 'fast' – 'slow' continuum by Reich (2014), we assume that plant species pursue either an exhaustive ('fast') or a conservative ('slow') life strategy when growing in monoculture. 'Fast' species likely had a rapid and exhaustive use of resources and lacked a buffering of population growth through a permanent seed bank or clonal growth resulting in loss of productivity over time. In contrast, 'slow' species were characterised by positive nematode effects (top–down control by predatory nematodes), a conservative use of resources and still a high reproductive success after 12 experimental years resulting in a positive performance over time (Fig. 4). Our findings are in line with a study by Cortois et al. (2016) using the same species and soil of the Jena Experiment in a short-term greenhouse PSF study. The results showed that conservative species (slow growth, resource conservative, well defended) benefit more from positive PSF effects than exploitative ones (fast growth, rapid resource use, poorly
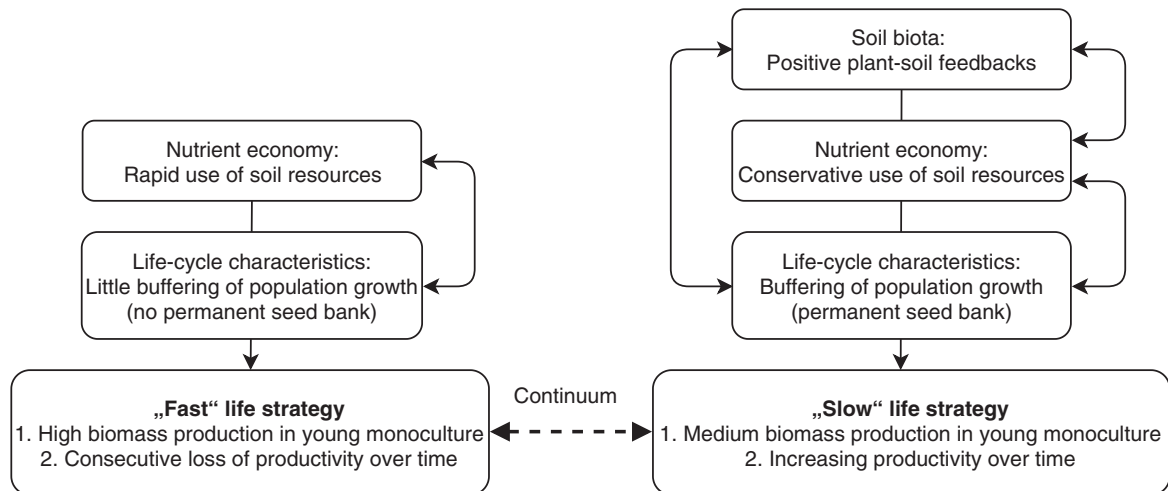
Figure 4. Conceptual framework showing the potential influence of three biotic drivers (soil biota, nutrient economy, life-cycle characteristics) on plant monoculture performance resulting in a 'fast'–'slow' continuum (Reich 2014) among grassland species.

defended). Our findings provide further evidence that these strategies also involve different life-cycle characteristics which are important for the long-term persistence of plant populations (Salguero-Gómez et al. 2016).

During the last decade, it has been generally accepted that monocultures lose productivity over time due to increasing negative PSF effects (Kulmatiski et al. 2008, Petermann et al. 2008, van der Putten et al. 2013). This assumption has mostly been tested by short-term greenhouse or mesocosm experiments (Kulmatiski et al. 2008, Petermann et al. 2008), while long-term field studies over several years are missing. Our study using 12-years old monocultures of the Jena Experiment (Monoculture Experiment) under field conditions showed that indeed many, but not all species decreased in monoculture productivity over time. Although our analyses were mainly correlative, we conclude that the interplay of different biotic drivers determines the long-term performance of grassland species in monocultures, reflecting a 'fast' versus 'slow' continuum (Fig. 4). This framework could provide the basis for further experimental studies, e.g. with phytometers, to prove the existence and direction of PSF effects and their interaction with other biotic drivers as determinants of the long-term monoculture performance of grassland species.

Data availability statement

Data are available from the Pangea Digital Repository: <https://doi.org/10.1594/PANGAEA.866358> (Dietrich et al. 2019).

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