Do soil-borne fungal pathogens mediate plant diversity–productivity relationships? Evidence and future opportunities

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Abstract

1. From the establishment of the first biodiversity experiments in the 1990s, studies have consistently reported positive relationships between plant diversity and productivity in grasslands. However, the predominant hypotheses that may explain this pattern have changed. Initially, there was a strong focus on plant–plant interactions such as facilitation and resource partitioning, but the results from the first experiments that manipulated soil communities have led to a paradigm shift.

2. In the current view on mechanisms that drive plant diversity–productivity relationships, fungal pathogen-induced reductions of plant productivity at low diversity play an important role. This role rests on two assumptions: the effects of pathogens (a) are plant-species specific (i.e. not all plant species are affected equally by a fungal pathogen) and (b) display negative density dependence (i.e. decrease with decreasing host plant density and hence, with increasing plant species richness).

3. Here, we review the empirical evidence for these two assumptions. In the biodiversity literature, this is mainly based on indirect approaches, such as soil sterilization, plant–soil feedback studies and plant biomass patterns. The identification and functional characterization of the fungal pathogens that actually drive the plant diversity–productivity relationship have only recently started.

4. Synthesis. Nevertheless, these studies, together with studies on plant–pathogen interactions in agricultural crops and forests, clearly suggest host-specific, negative density-dependent effects of fungal pathogens are common. Moreover, recent studies suggest that the reduced impact of pathogens at high plant diversity depends not just on host density but also on effects of neighbouring (non-host) plant species on the pathogen. Understanding how neighbouring plants affect the interactions between a pathogen and its host plants and disentangling the role of plant–pathogen interactions from other mechanisms potentially driving diversity–productivity relationships are important future challenges.
1 | INTRODUCTION

Societal concerns about the magnitude of habitat destruction and potential detrimental impacts of biodiversity loss led to renewed scientific interest in the relationship between diversity and the functioning of ecosystems. A conference in 1993 led to the hypothesis that a loss of diversity could lead to reduced primary productivity, less efficient use of limiting resources and decreased ecosystem stability (Schulze & Mooney, 1993). In the emerging field of biodiversity–ecosystem functioning research, field experiments manipulating plant species richness in grassland communities became the main approach to test this hypothesis. The first large experiments, such as the biodiversity experiment at Cedar Creek (Minnesota, USA) and the pan-European BIODEPTH experiment, showed an increasing but decelerating (loglinear) relationship between plant species richness and plant above-ground productivity (Hector et al., 1999; Tilman, Knops, et al., 1997). Meta-analyses of biodiversity experiments, most of which manipulated grassland plant species richness, confirmed species richness had a consistent positive effect on productivity (Barry et al., 2020; Cardinale et al., 2006).

The general interpretation of this pattern by the authors of these studies was that increased species richness led to greater complementarity: based on the assumption that species have at least partly non-overlapping niches, the total niche space covered increases with species richness, leading to a more complete utilization of available resources (Barry et al., 2019 and refs therein). After the publication of the results of the first biodiversity experiments, however, alternative explanations were offered that initiated a long debate (Aarssen, 1997; Grime, 1997; Huston, 1997; Wardle, Bonner, & Nicholson, 1997). Perhaps the most influential of these alternative explanations was what would become known as the selection effect (Aarssen, 1997; Huston, 1997; Loreau & Hector, 2001). This effect is a combination of the sampling effect, which states that the chance of a particular species being present in a mixture increases with increasing species richness, and two assumptions: (a) species differ in productivity and (b) productive species at least partly replace less productive species in mixed communities. This selection effect results in a positive relationship between plant species richness and productivity in which the productivity of the most diverse mixtures does not exceed that of the most productive monoculture, but the lower limit of productivity increases with species richness (Tilman, Isbell, & Cowles, 2014; Tilman, Lehman, & Thomson, 1997). In an attempt to disentangle selection effects from the so-called complementarity effects, Loreau and Hector (2001) developed an additive partitioning approach. This approach separates the net effect of plant species richness (the difference in biomass between the mixture and the average of the monocultures of the component species) into a selection effect (assessed as the covariance across species between productivity in monoculture and shift in performance in mixtures) and the complementarity effect. The latter measures whether on average, the relative performance of species in mixtures compared to that in monoculture deviates from zero. Using this approach, most studies showed complementarity effects were more important than selection effects (Fargione et al., 2007; Loreau & Hector, 2001; Marquard et al., 2009; van Ruijven & Berendse, 2005). A meta-analysis not only confirmed the important role of complementarity effects but also showed these effects increased with experimental duration (Cardinale et al., 2007). In addition, several long-term studies showed that with time, an increasing fraction of diverse communities outperforms even the most productive monoculture, a phenomenon known as transgressive overyielding (de Kroon et al., 2012; Marquard et al., 2009; Reich et al., 2012; Tilman et al., 2014). The question remained, however, which mechanisms were driving these complementarity effects. In the plant biodiversity literature, several potential mechanisms have been identified, including resource partitioning, facilitation and interactions with other organisms, such as nitrogen-fixing bacteria (in symbiosis with legumes), arbuscular mycorrhizal fungi and plant pathogens (see Barry et al., 2019 for a recent review). In this review, we focus on soil-borne fungal pathogens as potential drivers of complementarity effects.

2 | THE ROLE OF SOIL-BORNE FUNGI IN BIODIVERSITY EXPERIMENTS

The strengthening of the positive effect of plant species richness on productivity with time has been attributed to a positive feedback of productivity to nitrogen cycling, in which increased plant productivity (at high diversity) leads to enhanced soil organic matter and nitrogen accumulation, resulting in higher nitrogen mineralization and, consequently, increased plant productivity (Cong et al., 2014; Eisenhauer, Reich, & Isbell, 2012; Formara & Tilman, 2008; Mueller, Tilman, Formara, & Hobbie, 2013). However, a temporal increase in plant species richness effects can also be caused by a decrease in functioning in low-diversity communities (Guerrero-Ramirez et al., 2017; Marquard et al., 2013). In the Wageningen biodiversity experiment, for example, biomass production in diverse mixtures did not increase over time, but decreased in monocultures (Guerrero-Ramirez et al., 2017).

In agriculture, it is well established that the productivity of a single plant species is likely to decrease over time due to accumulation of natural enemies if grown at the same location for multiple years (Boudreau, 2013). Ecological studies also provide ample evidence
that plant species can change their growth conditions, and soil conditions in particular (Ehrenfeld, Ravit, & Elgersma, 2005). For example, plants can change the soil by depleting resources (Lambers, Raven, Shaver, & Smith, 2008), releasing toxic compounds (Hierro & Callaway, 2003) or accumulating soil biota that affect plant growth (Bever, 1994; van der Putten, van Dijk, & Peters, 1993). These interactions, known as plant–soil feedbacks, are predominantly negative (Kulmatiski, Beard, Stevens, & Cobbold, 2008). Several studies suggest that soil-borne pathogens play an important role in these negative feedbacks. Important groups of soil pathogens that can, individually or collectively (Raaijmakers, Paulitz, Steinberg, Alabouvette, & Moënne-Locoaz, 2009), cause negative plant–soil feedback include plant-parasitic nematodes (van der Putten & van der Stoel, 1997), soil-borne pathogenic fungi (Termorshuizen, 2014), protozoa and bacteria (Latz et al., 2012). However, there are strong indications that soil-borne fungi are the key determinants (Gilbert, 2002; Maron, Marler, Kilonomos, & Cleveland, 2011; Mommer et al., 2018; Schnitzer et al., 2011). Moreover, the negative effects of species-specific soil fungal pathogens on plant biomass may be more host density-dependent because of their low mobility (Termorshuizen, 2017) compared to foliar pathogens and above-ground herbivores.

Elton (1958) already proposed that plant diseases can be reduced in diverse ecosystems if high diversity is associated with low host density. Although increased diversity can sometimes lead to amplification of disease, for example by increasing the number of vectors for the disease, recent reviews suggest that dilution of disease risk at high diversity is more common. These so-called dilution effects are often associated with a reduction in host density with increasing diversity (Keesing et al., 2010; Keesing, Holt, & Ostfeld, 2006). At low diversity, where the density of a particular plant species is often high and conspecific plants grow in close proximity, soil-borne pathogens can strongly reduce plant growth. In contrast, at high diversity the density of a host plant will be lower on average and distance between conspecifics will be larger, potentially reducing the negative impact of soil fungal pathogens on plant biomass. This negative density dependence is particularly important because it can allow plant mixtures to outperform monocultures, potentially explaining the positive complementarity effects observed in grassland biodiversity experiments (see Box 1).

In 2011, two studies shifted the focus of the biodiversity-ecosystem functioning field from plant–plant interactions to the role of soil biota. The first experiment was performed by Schnitzer et al. (2011) and tested the hypothesis that negative density-dependent effects of host-specific soil fungal pathogens drive the positive plant diversity–productivity relationship. They performed a plant diversity experiment on four soils: (a) intact field soil, (b) sterilized field soil (eliminating soil biota), (c) sterilized field soil inoculated with pathogenic and saprophytic microbes isolated from the field soil and (d) sterilized field soil inoculated with spores of arbuscular mycorrhizal fungi (AMF). Their results showed a strong effect of soil treatment on the plant diversity–productivity relationship. First, the classical loglinear increase in plant biomass with plant species richness observed on field soil changed to a weak linear relationship on sterilized soil, due to a strong positive effect of sterilization on plant biomass at low diversity. More importantly, their results also showed a ‘recovery’ of the classical loglinear relationship on sterilized soil when inoculated with (pathogenic and saprophytic) microbes from the field soil. Adding AMF spores did not change the plant diversity–productivity relationship observed on sterilized soil (Schnitzer et al., 2011). The second study showed that a positive relationship between plant species richness and biomass became insignificant when treating the soil with fungicides (Maron et al., 2011).

These experiments strongly suggest that soil-borne fungal pathogens play a role in the positive plant diversity–productivity relationship. However, because soil sterilization and fungicide use eliminate all potential plant-fungal interactions, they provide little support for host specificity. More importantly, a positive relationship between plant diversity and productivity in the presence of soil fungal pathogens does not necessarily mean that the effects of the pathogens are negatively density-dependent. This is illustrated in Box 1, which shows four hypothetical scenarios for biomass dynamics in species mixtures (I–IV) when one species is accumulating a host-specific fungal pathogen, reducing its biomass over time. In monoculture, this leads to a gradual reduction in biomass over time. In mixture, however, the temporal biomass patterns can be different, depending on the occurrence of negative density dependence (NDD) and compensation, in which the decline in biomass of the infected species is compensated by increased biomass production of the other species (Gilbert, 2002; Gonzalez & Loreau, 2009). Without these mechanisms (scenario I in Box 1), the temporal biomass patterns of both species will be similar in monoculture and mixture and as a consequence, no effect of plant diversity will occur in mixture. Yet, compensation and NDD, alone or in combination, lead to a positive effect of diversity on biomass production (scenarios II–IV). In the absence of NDD, but with compensation, this positive diversity effect is mainly driven by a positive selection effect. With NDD (scenarios III and IV) however, complementarity effects drive the positive effect of diversity.

These hypothetical scenarios suggest that more insight into the occurrence of negative density-dependent effects of soil fungal pathogens may be obtained by analysing the temporal patterns in species biomass across the diversity gradient in biodiversity experiments. Negative density-dependent effects of soil fungal pathogens should result in a pattern in which species biomass decline over time is most pronounced in monoculture. Marquard et al. (2013) tested this prediction using data of 60 species over 8 years in the Jena Experiment (Weisser et al., 2017) in monoculture and mixtures that differed in plant species composition and found limited support. Over time, species biomass on average decreased in both monocultures and mixtures, with a marginally significant stronger decrease in monocultures. However, large variation in biomass dynamics over time was observed, and several species performed worse in mixtures than in monocultures. Here, we analysed the temporal biomass dynamics of species in replicated monocultures and eight-species mixtures in the Wageningen Biodiversity Experiment.
Hypothetical biomass dynamics of two plant species over time when one of the species (in orange) is affected by a host-specific root pathogen, reducing its biomass by 10% each time step. As the biomass of this species decreases over time, the average monoculture biomass (grey line) also decreases over time. The temporal biomass patterns in mixture may be very different, depending on the occurrence of negative density dependence (NDD) and the interactions between the plant species. Based on the assumption that the density of a particular species decreases with increasing species richness, NDD will lead to a reduction of the negative effect of the fungal pathogen in mixture. Here, we assume the negative effect of the pathogen is reduced by 50% in mixture. For plant interactions, we only include compensation: the decline in biomass of the infected species is compensated by increased biomass production of the other species (Gilbert, 2002; Gonzalez & Loreau, 2009). The occurrence of these two effects has direct implications for the plant diversity effects (Loreau & Hector, 2001). The net effect (NE) is the difference in biomass between the average monoculture and the sum of the species in mixture (grey lines in panels I–IV). This effect can be divided into the complementarity effect (CE), which measures whether on average, the relative performance of species in mixture exceeds that expected based on the monocultures, and the selection effect (SE), which calculates the contribution of the covariance between species biomass in monoculture and deviations from expected biomass in mixture.

In the first scenario, without compensation and NDD (scenario I), temporal biomass patterns will be similar in mixture and monoculture, and biomass of both species in the two-species mixture will be equal to half of that in monoculture. As a consequence, no effects of plant species richness will occur (note that we assume no other potentially important mechanisms such as resource partitioning or facilitation take place). In the other scenarios, however, positive effects of diversity on biomass emerge over time. With compensation only (scenario II), mixture biomass will increasingly exceed the average monoculture biomass over time, leading to a positive net diversity effect. This effect is largely due to a selection effect, as the most productive species in monoculture (not affected by the pathogen) increases its performance in mixture by partly replacing the less productive infected species. Nevertheless, the complementarity effect will also be positive because one species performs better than expected from monoculture, whereas the relative performance in mixture of the other species (affected by the pathogen) is equal to its expected performance based on its monoculture. With NDD (scenarios III and IV), complementarity effects become more prominent and selection effects decrease. Note that with NDD only (III), SE gets negative because the least productive species in monoculture (the one affected by the pathogen) shows increased performance in mixture due to NDD. When both compensation and NDD operate (IV), both species will perform better in mixture than in monoculture over time, leading to a positive complementarity effect, while SE will be close to zero.
LONG-TERM BIOMASS DYNAMICS IN THE WAGENINGEN BIODIVERSITY EXPERIMENT

The Wageningen Biodiversity experiment was initiated in Spring 2000 and terminated in 2011. It consisted of 102 plots of 1 m$^2$, planted with monocultures (six replicates per species) and mixtures of two (24 plots), four (24) and eight species (6). The species pool of the experiment consisted of four grasses (Agrostis capillaris, Anthoxanthum odoratum, Festuca rubra and Holcus lanatus) and four forbs (Centaurea jacea, Leucanthemum vulgare, Plantago lanceolata and Rumex acetosa). Nitrogen-fixing legumes were excluded to avoid potentially confounding effects on the relationship between plant species richness and productivity. Above-ground biomass was harvested annually by clipping in August. See van Ruijven and Berendse (2003) for more information on the design of the study. A positive relationship between plant species richness and above-ground biomass of the community emerged in the second year of the study and strengthened over time. Complementarity effects were positive and also increased over time, whereas selection remained close to zero throughout the experimental period (de Kroon et al., 2012). Here, the above-ground biomass of the eight plant species in monoculture and eight-species mixtures over 11 years was analysed using mixed models with plot as a random factor and species richness as a fixed factor. Linear and quadratic terms of year were treated as repeated observations within plot and included as covariates in the model. Biomass data were log transformed. In monoculture, biomass of seven out of eight species declined over time (one species showed no significant temporal patterns), in line with the expectation that plants would accumulate pathogens that negatively affect plant biomass production. In contrast, temporal biomass dynamics in mixture showed a large variation among species (Figure 1). Half of the species showed less negative temporal patterns in mixture, consistent with negative density-dependent effects of pathogens (see scenarios III and IV in Box 1). Two species (A. odoratum and C. jacea) actually increased over time in mixtures (Figure 1), consistent with a combination of compensation and NDD (scenario IV in Box 1).

**Figure 1** Above-ground biomass (g/m$^2$) of eight plant species over time in monoculture (blue) and eight-species mixtures (red) in the Wageningen Biodiversity experiment. Half of the eight species show significantly different temporal patterns in monoculture and mixture, as indicated by significant interactions between time and species richness (T × S), which are consistent with negative density-dependent effects of specific fungal pathogens. Two species (Anthoxanthum odoratum and Centaurea jacea) show positive temporal patterns in mixture, but negative or neutral patterns in monocultures. Leucanthemum vulgare initially showed similar declines in monoculture and mixture, but increased again in mixture (not in monoculture) in later years. Plantago lanceolata initially showed more pronounced decline in biomass over time in monoculture than in mixture, consistent with negative density dependence. However, this pattern disappeared in the second half of the experiment. The other half of the species showed similar declines over time in monoculture and mixture (Agrostis capillaris and Festuca rubra), a stronger decline in mixture (Holcus lanatus) or no significant temporal patterns (Rumex acetosa). Note that if biomass production per individual plant is similar in monoculture and mixture, the expected biomass in the eight-species mixture would be 1/8 of that in monoculture. Circles show $M ± SE (N = 6)$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns = not significant [Colour figure can be viewed at wileyonlinelibrary.com]
L. vulgare initially showed similarly strong declines in mixture and in monoculture, but its biomass increased again in mixtures (not in monocultures) in the last years (Figure 1). This suggests negative density dependence mainly played a role in the establishment of the next generation for this species. P. lanceolata initially showed a stronger decrease in monoculture than in mixture (consistent with NDD), but in later years decreased in mixture but not in monoculture. Other species showed temporal patterns that are difficult to explain by NDD or compensation. Two species (A. capillaris and F. rubra) showed similar declines in monoculture and mixture (consistent with no compensation and NDD, see scenario I in Box 1), and one species (H. lanatus) showed a more pronounced decline over time in mixture than in monoculture (Figure 1). Perhaps the accumulation of pathogens reduced the interspecific competitive strength of this species, which led to its competitive replacement in mixtures. This is line with plant-soil feedback experiments that include a competition treatment. These studies tend to show more pronounced negative soil feedback effects under interspecific competition (Hendriks et al., 2013; Petermann, Fergus, Turnbull, & Schmid, 2008).

In conclusion, analysis of biomass dynamics of individual species can provide more insights into the mechanisms operating in diverse communities. Indeed, several plant species showed temporal patterns consistent with negative density-dependent effects of pathogens, which can explain the positive complementarity effects found in this study (de Kroon et al., 2012). However, it cannot be ruled out that these patterns in mixtures were driven by other organisms and/or mechanisms. For example, the increased biomass production over time in mixture, as shown by two species, and the increase in complementarity effects can also be a response to the increased accumulation of organic matter and nutrient mineralization we found at higher diversity (Cong et al., 2014). Information about the pathogens in monocultures and mixtures is needed to identify plant-soil pathogen interactions as the key driver of these temporal patterns, and of positive effects of plant diversity on biomass as a whole.

4 | Generality of Plant–Soil Feedback as Drivers of Biodiversity Effects

One approach that has often been used in ecology to study the impact of soil organisms is to assess plant-soil feedback (PSF). Initially, these studies compared plant performance on soil conditioned by individuals of the same plant species to that on sterilized soil. Due to the limitations associated with soil sterilization, such as enhanced nitrogen availability (see Brinkman, Van der Putten, Bakker, & Verhoeven, 2010 for a review), later studies compared performance on ‘own’ soil to that on soil conditioned by other plant species (also referred to as indirect feedback, see van der Putten et al. (2013). For example, Schnitzer et al. (2011) used a long-term plant diversity experiment to regrow plant species on soils conditioned by different plant monocultures for 5 years. They found increased root disease incidence in plants grown on their own monoculture soil compared to plants grown on monoculture soil of other plant species. In their study, this difference in disease incidence did not lead to significant differences in plant biomass. However, other studies have shown reduced biomass on soil conditioned by the same species (Harrison & Bardgett, 2010; Hendriks et al., 2013; Meisner et al., 2014) or plant functional group (Cortois, Schröder-Georgi, Weigelt, van der Putten, & Deyn, 2016; Petermann et al., 2008).

Together, these studies show a clear degree of specificity in plant-soil feedback: PSF effects of particular species are often more pronounced on the same species or group of species. However, what has often been lacking in PSF studies is the identification of the organisms that potentially drive PSF: the soil-borne fungal pathogens. Treatments such as soil sterilization and fungicide application potentially affect a range of soil organisms, including symbionts such as arbuscular mycorrhizal fungi and nitrogen-fixing bacteria. Similarly, soil conditioning by particular plant species will also affect multiple components of the soil community (Geisen, Wall, & van der Putten, 2019). As a consequence, PSF effects reflect the net result of a range of positive and negative plant-soil interactions (Brinkman et al., 2010; van der Putten et al., 2013). We argue that identification of the fungal pathogens in the soil is a necessary next step to enhance our understanding of the specific role of these pathogens in plant diversity-productivity relationships and plant community dynamics in general.

5 | Identifying Fungal Pathogens

Next-generation sequencing (NGS) techniques have increased our understanding of soil microbial communities, including the root-associated fungal communities. Plant roots are colonized by a wide variety of fungi (Jumpponen, Jones, & Blair, 2010; Philippot, Raaijmakers, Lemanceau, & van der Putten, 2013; Vandenkooymhuyse, Baldauf, Leyval, Stracek, & Young, 2002) and several studies have shown that fungal communities differ between plant species (Hannula et al., 2017; Leff et al., 2018; Mommer et al., 2018; Wehner et al., 2014). Identifying the ecological function of the different actors in these diverse fungal communities is still a challenge, however. First, the fungal kingdom is very diverse, estimated to include two to four million species (Blackwell, 2011; Hawksworth & Lücking, 2017). Consequently, the fungal community in a soil or root sample is often also diverse and high-throughput sequencing studies commonly yield hundreds to thousands of operational taxonomic units (OTUs). For example, 966 OTUs were found in the roots of 25 herbaceous plant species in the family Asteraceae in a grassland reserve in Germany (Wehner et al., 2014). Many OTUs lack a formal taxonomic description: often 10%-30% of the OTUs remain unidentified and often only 10%-40% of the OTUs match well-described species (Tedersoo et al., 2014). As a consequence, few studies have identified the root fungal pathogen communities of plant species in grasslands. Yet, novel bioinformatic approaches have been developed to unravel...
diverse fungal communities and characterize the ecological role of the fungal taxa detected using NGS techniques. For instance, the open annotation tool FUNGuild (Nguyen et al., 2016) makes it possible to separate fungal OTUs into broad ecological guilds (e.g. saprotrophs, pathogens or mycorrhizal fungi), independent of sequencing platform or analysis pipeline. This tool associates functional guilds at relatively high taxonomic levels, for example, at the genus or family level. This may introduce a bias, as these taxonomic levels can include fungal taxa with divergent ecological strategies (Nilsson et al., 2019). Therefore, a two-step approach, which combines tools like FUNGuild to identify potential fungal pathogens with a literature survey to check the pathogen status for the fungi identified to species level, is recommended to increase the reliability of the functional assignment (Nilsson et al., 2018). Using this approach in a recent study of the fungal communities in roots from monocultures and mixtures of the Wageningen Biodiversity experiment, 988 OTUs were detected, of which 261 could be assigned to distinct functional categories and 31 OTUs were identified as root pathogens. Importantly, these root pathogen communities differed between the eight plant species in monoculture (Mommer et al., 2018), supporting host specificity of root fungal pathogens. More studies using NGS techniques to identify root fungal pathogens in grasslands will allow us to identify phylogenetic patterns across plant species in, and assess the role of abiotic conditions on, plant species’ root fungal communities.

At the same time, more information on the biology of these fungi is needed. The presence of a well-described pathogenic fungal species does not necessarily imply pathogenicity. Even the identification of a well-described active pathogenic fungal species may not be sufficient to infer pathogenic effects on plants. Recent studies show that pathogenic fungi can also live as endophytes within plants without causing disease (Brader et al., 2017; Lofgren et al., 2018). Moreover, several fungal pathogens well known to cause disease in crops, such as Fusarium oxysporum and Rhizoctonia solani, previously considered to be generalist pathogens, show large genotypic variation across strains, leading to more specialist host ranges (Gordon & Martyn, 1997; Termorshuizen, 2017). This shows that isolation of fungal pathogens and subsequent pathogenicity tests are needed to confirm host specificity. In the Wageningen Biodiversity experiment, two root pathogens isolated from the roots of the forb L. vulgare (Paraphoma chrysanthemicola) and the grass A. odoratum (Gaumannomyces incarnatus) were used in bioassays with both plant species. Each fungal species significantly reduced the growth of the plant species from which it was isolated, but not that of the other species (Mommer et al., 2018), demonstrating host specificity. Currently, such studies have only been done for a very small subset of pathogen and plant species. These studies show that reduction of seedling biomass is a common effect of root pathogenic fungi (see Ampt, van Ruijven, Raaijmakers, Termorshuizen, & Mommer, 2019 for an overview), which supports the role of these pathogens in the biodiversity–productivity relationships. However, more studies determining the pathogenic effects of these fungi on plant species, also in relation to other important factors such as plant age and soil conditions, are needed to get a more complete picture of host specificity in plant–fungal pathogen interactions in grasslands.

6 | NEGATIVE DENSITY DEPENDENCE IN ROOT PATHOGENIC FUNGI

Although incomplete, the studies performed to date in which root pathogenic fungi were identified suggest that host-specific effects of soil-borne pathogenic fungi on plants are common. The most important step, however, is to determine whether the negative effects of these host-specific root pathogens become less pronounced with increasing plant diversity. Negative density dependence is a prerequisite for an important role of pathogens in driving the positive relationship between plant species richness and productivity (see Box 1), but what is the evidence for negative density dependence in plant–root fungal pathogen interactions? Some studies have investigated the above-ground fungal pathogen communities in plant diversity experiments in grasslands. These studies have shown decreased abundance and/or disease severity of fungal pathogens with increasing plant species richness (Mitchell, Tilman, & Groth, 2002; Rottstock, Joshi, Kummer, & Fischer, 2014). Below-ground, Schnitzer et al. (2011) found similar patterns when regrowing plant species on soils conditioned by 1, 4 or 16 plant species in a long-term biodiversity experiment: the number of lesions in the roots was reduced on soils from mixed communities. However, the fungal species present in the soil and those that colonized the roots were not identified. In the Wageningen Biodiversity experiment, we did not quantify disease incidence, but we showed that 50% of the root-pathogenic fungi identified in roots from plant monocultures were not found in the eight-species mixtures (Mommer et al., 2018). Together, these studies provide indications that plant diversity can negatively affect root fungal pathogens. Providing direct evidence for negative density-dependent effects of specific root fungal pathogens on host plants in grasslands remains a challenge for future research.

As far as we know, only studies from other ecosystems have provided support for negative density dependence in root fungal pathogen effects. In (tropical) forests, negative density dependence is considered an important mechanism contributing to local tree diversity. The main idea is that host-specific natural enemies accumulating on adult trees limit the success of conspecific seedlings close to the adult tree, favouring the establishment of heterospecific seedlings and promoting tree diversity. In this effect, commonly referred to as the Janzen–Connell effect (Janzen, 1970), distance to the adult tree is often used as a proxy for host density. Several studies have demonstrated increased seedling mortality or decreased seedling biomass due to root pathogenic fungi such as Fusarium oxysporum and Pythium spp. as a function of decreasing distance to adult trees in the field (Johnson, Beaulieu, Bever, & Clay, 2012; Liu, Etienne, Liang, Wang, & Yu, 2015; Mangan et al., 2010; Packer & Clay, 2000).

Studies that experimentally manipulate host density to study density-dependent effects of root pathogenic fungi are limited to
agricultural systems. For example, the dispersal of damping-off disease (caused by Pythium irregularare) in small-scale stands of garden cress (Lepidium sativum) under controlled conditions increased with seedling density (Burdon & Chilvers, 1975). In the field, the incidence and severity of pea root rot (caused by Aphanomyces euteiches) increased with increasing plant density (Willocquet, Jumel, & Lemarchand, 2007). A similar effect has been observed for southern blight disease (caused by Sclerotium rolfsii) in carrots (Smith, Campbell, Jenkins, & Benson, 1988). Such experiments have yet to be performed for grasslands plants and pathogens, and would strongly enhance our understanding of the role of soil pathogenic fungi in plant diversity–productivity relationships.

7 | JUST HOST DENSITY? THE ROLE OF NEIGHBOURING PLANT SPECIES

So far, plant diversity has mainly been treated as a proxy for host density. However, the effect of plant diversity may not be limited to a decrease in host density. For example, some studies have shown that the chance of fungal infection decreases with increasing phylogenetic distance between neighbouring plant species (Gilbert & Webb, 2007; Parker et al., 2015). In general, neighbouring species can have either reducing, neutral or even amplifying effects on disease transmission (Keesing et al., 2006). Obviously, if neighbouring plant species are also hosts for the pathogen, the effects of increasing plant density (and reducing host density) on pathogenic effects will be small (Otten, Filipe, & Gilligan, 2005) and may even be negative if neighbouring species amplify pathogen transmission. In addition, some soil-borne fungal pathogens can persist in a plant species as an endophyte without causing disease symptoms. A host plant with such ‘asymptomatic hosts’ as neighbours is more likely to become infected than a plant surrounded by non-hosts that are not colonized (Malcolm, Kulda, Gugino, & Jiménez-Gasco, 2013). Yet, even the effects of non-host neighbours on fungal pathogen transmission may differ considerably (Keesing et al., 2006). So far, the underlying mechanisms of neighbours on root fungal pathogens are poorly understood (Ostfeld & Keesing, 2012) and have rarely been investigated explicitly in diverse grassland communities. We propose three potential ways by which neighbouring plant species can affect soil-borne fungal pathogens, and consequently the observed strength of negative density dependence in diverse plant communities. The first potential mechanism is the formation of a root barrier. In grasslands, root densities are high and roots of different plant species co-occur on spatial scales of a few millimetres (Frank, Pontes, Maine, & Fridley, 2015; Kesavanurty et al., 2011) and this can obstruct soil-borne fungi navigating to their hosts. For example, the spread of the soil-borne disease Phytophthora blight in pepper was reduced when intercropped with maize. The treatment with the highest degree of root intermingling of the two crops blocked pathogen transmission completely (Yang et al., 2015). Currently, it is unclear whether the roots themselves obscure the signals for the fungal pathogen to germinate and navigate to its host or if it operates via the stimulation of the diversity and activity of the soil microbial communities (Dassen et al., 2017; Eisenhauer et al., 2017; Steinauer, Chatzinotas, & Eisenhauer, 2016), leaving less space for soil-borne pathogen invasion (i.e. the niche exclusion hypothesis; Mallon, Elsas, & Salles, 2015).

The second mechanism is the active suppression of soil-borne fungal pathogens via the presence of antifungal compounds in root exudates (Baetz & Martinoia, 2014; Bednarek & Osbourn, 2009). For example, the medicinal herb Atractylodes lancea released compounds that inhibited the hyphal growth of the soil-borne fungal pathogen Fusarium oxysporum in a peanut intercropping system (Li et al., 2018).

The third mechanism is biological control: the ability of plant species to stimulate specific antagonists of the soil-borne fungal pathogen (van Lenteren, Bolckmans, Köhl, Ravensberg, & Urbaneja, 2018). The microbes associating with plant roots are known to affect the below-ground interactions between a plant and pathogen (Berendsen, Pieterse, & Bakker, 2012; Philipppot et al., 2013). Gilbert, Handelsman, and Parke (1994) hypothesized that plant root-colonizing bacteria may ‘camouflage’ the roots against pathogens. There are clear indications that host plants assemble distinct rhizosphere communities, a process that is plant species and genotype-specific, but is also influenced by environmental factors such as soil type and temperature (Bulgarelli et al., 2012; Philipppot et al., 2013; Thomson et al., 2015). It is likely that these rhizosphere communities are also influenced by neighbouring plant species. For example, Bezem et al. (2010) showed that the identity of neighbouring plants affected the composition of the nematode community in local soil food webs, but the role of neighbour plants on the bacterial and fungal rhizosphere communities of a plant has not been elucidated. Future research aimed at elucidating the role of neighbour identity and host density in plant–root fungal pathogen interactions will not only enhance our understanding of the role of root fungal pathogens in plant diversity–productivity relationships, but may also shed new light on plant coexistence mechanisms in diverse grasslands.

8 | CONCLUSIONS

In search for the mechanisms underlying the positive effects of plant diversity on productivity, ecologists initially focused on plant–plant interactions such as competition and resource partitioning. The publication of the first studies that not only manipulated plant diversity but also soil biota, resulted in a paradigm shift in the field and shifted the attention to negative density-dependent effects of host-specific root fungal pathogens as an important driver of positive diversity effects. The research performed to date strongly suggests that host specificity of these pathogens is the rule rather than the exception, although the identity and biology of many fungi in diverse communities remain unknown. Direct empirical evidence for negative density dependence is still limited for root fungal pathogens in grasslands. However, research into pathogens and effects of host density in
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