Plant community stability is associated with a decoupling of prokaryote and fungal soil networks

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Abstract

Soil microbial networks play a crucial role in plant community stability. However, we lack knowledge on the network topologies associated with stability and the pathways that shape these networks. In a 13-year mesocosm experiment, we determined how natural grassland soil and soil abandoned from agricultural practices 60 years before the start of the experiment affected soil microbial network topologies. Abandoned arable soil promoted destabilising properties both above- and belowground. Aboveground, instability was associated with invading plant species reaching dominance. Belowground, instability was associated with soil microbial networks coupled in prokaryote and fungal responses, which were both shaped by a few, dominating plant community parameters. Conversely, in stable, natural grassland communities, soil prokaryote and fungal responses were decoupled. This decoupling was associated with different sets of plant community parameters shaping prokaryote and fungal niches. We conclude that plant community stability is associated with soil microbial networks with a high niche differentiation.

Introduction

Plants live in complex, interactive networks with soil microbial communities. These interactive networks are increasingly found to act as a structuring force in a large array of plant community processes and characteristics such as plant community stability (Bardgett & Caruso, 2020; Bardgett & van der Putten, 2014; Bever et al., 2012; in 't Zandt et al., 2021; van der Putten et al., 2016). Plant community stability describes the ability of communities to resist and recover from biotic and abiotic

perturbations, and has become an increasingly pressing issue with the ongoing change in climate and human interventions (Hernandez et al., 2021; IPCC, 2021). However, understanding the driving forces of community stability is a major challenge due to the complexity of the underlying plant-soil-microbiota interactions. At the same time, the complexity of ecological interactions itself has long been considered to be a key component of community stability (Montoya et al., 2006; Pimm, 1985). To predict, protect and restore plant communities, we need to understand the role of plant-soil-microbiota interactions and their complexity in community stability processes.

Interactive networks between plants and soil microbiota result from both direct and indirect effects that plants and soil microbiota have on each other via, for example, pathogenicity, facilitation, and soil resource cycling (Bezemer et al., 2010; de Vries et al., 2013; Eisenhauer et al., 2010). Although complex, ecological networks have a comprehensible structure with well-defined patterns in relation to network stability (Montoya et al., 2006; Pimm, 1985). Network theory predicts that species connectiveness, negative interactions, few strong and many weak interactions and clustering of species increase the stability of ecological networks to perturbations (Coyte et al., 2015; Hernandez et al., 2021; Saint-Béat et al., 2015; Stouffer & Bascompte, 2011). In essence, these network properties minimise the risk of change when a perturbation occurs by creating dependencies between species, promoting species asymmetry and buffering against the propagation of perturbation effects among subsections of the network (Coyte et al., 2015; de Vries et al., 2018; McCann et al., 1998; Neutel et al., 2002; Saint-Béat et al., 2015). Most of this network theory is derived from ecological food web theory, but has been shown to be applicable to microbial networks (Coyte et al., 2015; de Vries et al., 2018; Hernandez et al., 2021). However, in comparison to food webs, microbial networks lack a strong directional structure and are therefore based on co-occurrences of taxa alone (Coyte et al., 2015). Yet, plants play a critical role in shaping the environmental niches of soil microbial communities via the input of a large variety of chemical compounds into the soil environment via, for example, dead organic material and root exudates as well as the uptake of soil nutrients (Canarini et al., 2019; Sokol et al., 2022). The plant community may therewith play an essential role in shaping soil microbial network stability. We currently lack knowledge on the role of the plant community in shaping soil microbial networks and therewith the importance of these pathways as stabilising mechanisms.

From a plant community perspective, plant diversity has long been considered to result in stability. In diverse plant communities, negative effects of perturbations on certain plant species are considered to be compensated for by positive effects on other species, temporarily taking over their function in the community (Loreau & de Mazancourt, 2013). In other words, plant community diversity increases the probability of finding resistance and/or recovery to a perturbation due to a higher sampling effect. At the same time, plant diversity may not guarantee stability if diversity itself does not create the

pathways that lead to stability (Lepš et al., 2001; Saint-Béat et al., 2015). Instead, plant species identity and therewith plant community composition is expected to act as a stabilising mechanism via reciprocal specialisation of plants and soil microbiota. This reciprocal specialisation creates complex networks and, in particular, negative feedback loops that avoid plant species dominance, species loss and communities tipping into alternative states (Bardgett & Caruso, 2020; Bever et al., 2012; in 't Zandt et al., 2021; van der Putten et al., 2016). As a result, direct effects of plant community composition on microbial networks are likely critical pathways in community stabilising mechanisms. Conversely, inherently more generic effects of plant community diversity and indirect pathways via soil chemical changes may result in microbiota responding in tandem. If such more generic effects are strong or not compensated for, this may lead to plant community instability. To understand the drivers of plant community stability, we need to define the importance of both overall (i.e., plant diversity and productivity) and compositional (where plant identity plays a distinctive role) plant community components in shaping soil microbial networks and to test whether these plant community components create direct or indirect effects via soil chemical changes.

Here, we test to what extent and via which pathways overall and compositional plant community components shape soil microbial biomass and networks after the growing season. We compare these pathways between dry grassland communities established on natural grassland soil and soil abandoned from agricultural practices 60 years before the start of the experiment. Plant communities on natural grassland soils are typically stable communities, while communities on abandoned arable soil are more strongly impacted by plant species invasion (Kulmatiski et al., 2006; Mattingly & Orrock, 2013). We created plant communities by sowing a seed mixture of 44 perennial dry grassland species in outdoor mesocosms filled with natural grassland soil and abandoned arable soil (Münzbergová, 2012). Plant communities were left to establish for 5 years, after which natural invasion by both native and exotic species from outside the sown species pool was allowed and occurred substantially the following 8 years. This long-term plant community development resulted in communities with natural variation in, amongst others, plant diversity and plant community composition. We combined four datasets: plant community aboveground measurements over the 13 years and after the 13th growing season: soil chemistry, total microbial biomass pools (PLFA/NLFA analysis) and soil microbial community composition (16S and ITS amplicon sequencing). First, we test whether plant communities on abandoned arable soil show a decreased long-term stability aboveground and whether this translates to soil microbial communities with destabilising properties in their prokaryote and fungal co-occurrence networks. Second, using structural equation modelling (SEM), we test whether the relative contribution of overall plant community (aboveground and belowground productivity, plant diversity) and plant compositional pathways in shaping soil microbial networks is affected by soil

origin. We distinguish past plant community factors (initial plant invasion impact and developmental trajectories) from factors in the year of sampling as well as direct plant-microbial pathways from indirect pathways occurring via soil chemical changes. Third, we determine the exact plant-soil-microbiota pathways that are consistently changed between stable and instable plant communities, and whether these changes relate to particular putative functions and metabolic characteristics of the microbial communities involved. Taken together, these analyses unfold the pathways via which plant communities shape soil microbial networks and how these are associated with plant community stability.

Results

Plant invasion had a larger impact on plant communities on abandoned arable soil

Plant community diversity gradually declined in time, but was drastically increased 1-2 years after the start of plant invasion on both natural grassland and abandoned arable soil (Fig 1A). Despite this decline in diversity, aboveground productivity of the communities remained relatively constant over time. In addition, aboveground productivity was not affected by the start of invasion and showed little difference between the two soil origins (Fig 1B). The proportion of invaded species biomass, on the other hand, was consistently higher in abandoned arable soil communities than in communities established on natural grassland soil (Fig 1C).

Plant community composition in time was analysed using non-metric multidimensional scaling (NMDS) and was described by three axes representing: residence period of the plant species (early versus late residency in the communities; axis 1), residence time of the plant species (short versus long residency in the communities; axis 2) and compositional dominance of plant species (axis 3) (Fig S1-S2). The axis based on species residence period (NMDS1) showed a gradual turnover in plant composition in time, which was consistently higher in the abandoned arable compared to the natural grassland community (Fig 1D). The axis based on species residence time (NMDS2) showed similar patterns over time as plant diversity. Generally, plant communities consisted increasingly of species with longer residence times as communities developed, but invasion drastically increased the number of individuals with short residence times on both natural grassland and abandoned arable soil (Fig 1E). On natural grassland soil, these newly invaded species were gradually lost in time, while on abandoned arable soil, communities varied in whether the newly invaded species were lost or were able to occur permanently (Fig S3). These patterns did, however, not result in consistently different plant community composition between the two soil origins after plant invasion started (after 2012; Fig 1F). However, on abandoned arable soil, plant community composition showed a larger variation in dominance of plant species compared to natural grassland communities by the 13th growing season:

arable soil plant communities were either dominated by various invaded species or by the sown species *Tanacetum corymbosum* (Fig S1A-B).

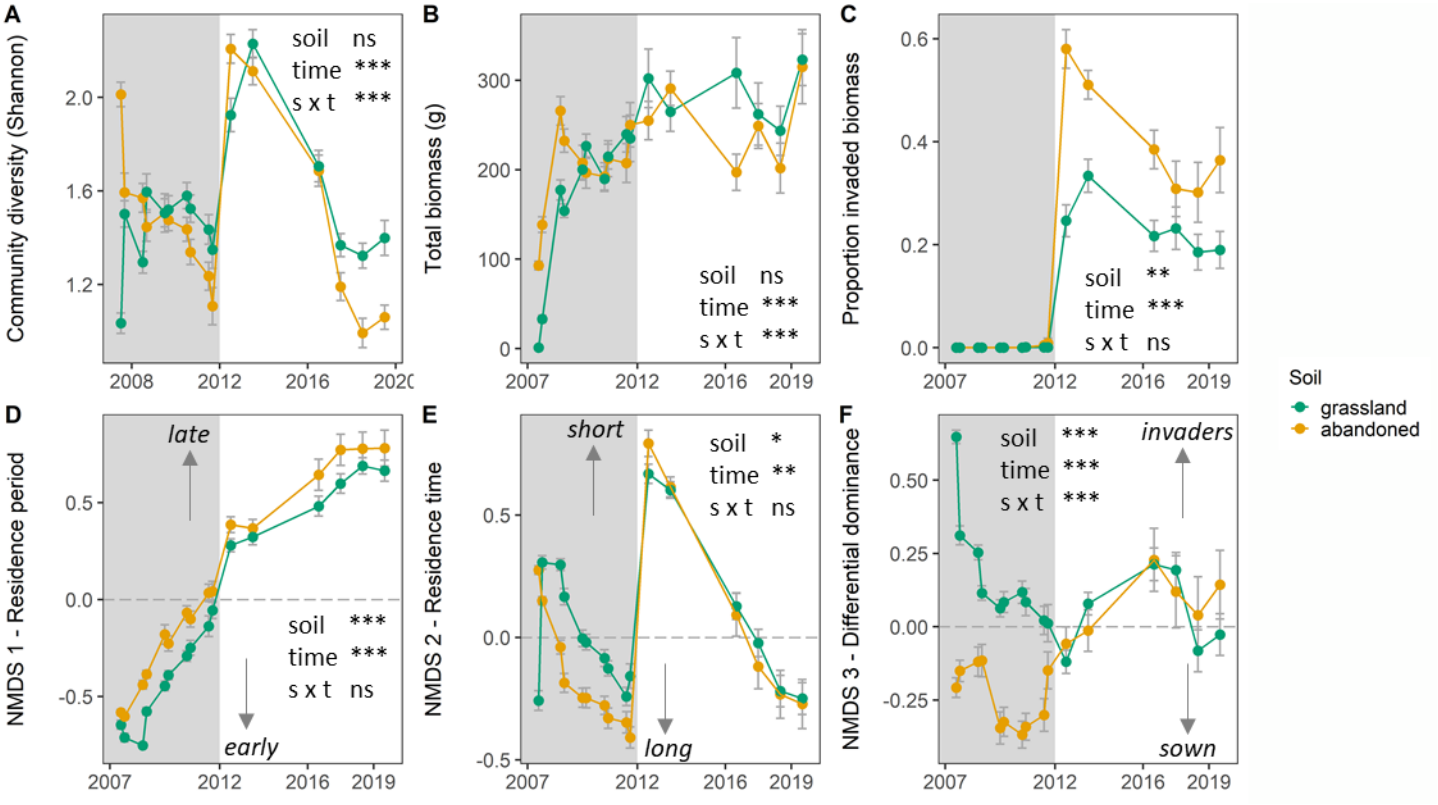

Figure 1 Plant community development over time on natural grassland (green) and abandoned arable soil (yellow). Plant community (A) diversity, (B) total biomass, (C) biomass proportion of invaded plant species, and community compositional NMDS scores related to plant species (D) residence period, (E) residence time and (F) differential dominance. In D-F arrows with text indicate the interpretation of the NMDS scores. Note that in F, this interpretation only concerns the period *after* invasion started; 2012 onwards. Grey shading indicates the time period in which communities established and no natural species invasion took place. From 2012 onwards, natural invasion of species from outside the sown species pool occurred. Averages ± SE are shown (n = 30) and results of linear mixed effect models including sowing density as a random effect are presented. Significance codes: *** = $p < 0.001$; ** = $p < 0.01$; ns = not significant, $p > 0.05$. For figures on species distribution on NMDS axes, see Fig S1-S2.

Soil origin affected soil chemistry and microbial soil communities

After the 13th growing season, natural grassland and abandoned arable soils differed significantly in chemistry, microbial biomass pools and microbial community composition (Fig 2, S4). Abandoned arable soil was significantly higher in total N, plant available NO_3^-, NO_2^-, and NH_4^+, total and organic C and total P. In addition, prokaryote and fungal richness as well as bacterial biomass were significantly higher in abandoned arable than natural grassland soil. Conversely, soil pH, fungal and AMF biomass, and belowground productivity were higher in natural grassland soil (Fig S4). Both prokaryote and fungal community composition were significantly different between natural grassland and abandoned arable soil (Fig 2). Microbial co-occurrence networks indicated highly connected prokaryote and fungal communities in both natural and abandoned soil with few dominating OTUs (Fig S5). Against

150 expectation, microbial network properties commonly associated with stability showed little difference
151 between natural grassland and abandoned arable soil for both prokaryote and fungal networks (Table
152 S1).

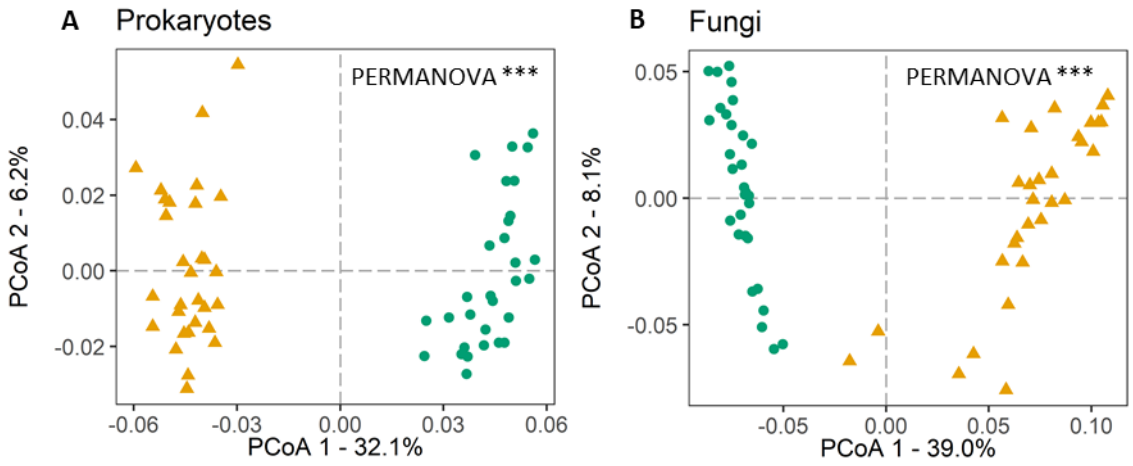

Figure 2 PCoA on Bray-Curtis dissimilarity of (A) prokaryote 16S and (B) fungal ITS rRNA of communities established on natural grassland (green circles) and abandoned arable soil (yellow triangles). Amplicon sequencing was performed at the end of the 13th growing season (*n* = 30 per soil origin).

153 *Microbial networks in natural grassland soil were decoupled in prokaryote and fungal responses*
154 To understand the microbial network topologies in more detail, we clustered similarly responding
155 OTUs across the 30 plant communities of each soil origin. We did this based on both positive and
156 negative co-occurrences (Fig S5). Similarly responding prokaryote OTUs were captured in 9 and 10
157 clusters for natural grassland and abandoned arable soil, respectively. For fungi, 21 and 18 clusters
158 were needed for natural grassland and abandoned arable soil communities, respectively (Fig 3).
159 Importantly, both for prokaryote and fungal networks, taxonomic families largely clustered together,
160 indicating similar responses of closely related taxa and showing the validity of the clustering approach
161 (Fig S6-S7; Table S2-S5).

162 We summed the relative abundance of the different OTUs within each cluster. On average, three
163 dominant prokaryote clusters occurred that held most of the 16S rRNA reads recovered for both soils.
164 In abandoned arable soil, these patterns were similar for the fungal clusters showing 3 large clusters.
165 On natural grassland soil, however, fungal networks showed 5 larger clusters (Fig 3). We then tested
166 for correlations between all clusters in each soil origin. Most strikingly, in natural grassland soil, only
167 a single correlation between the dominant prokaryote and fungal clusters occurred, while various
168 strong positive and negative correlations occurred between the most dominant clusters in abandoned
169 arable soils (Fig 3). In other words, responses of dominant prokaryote and fungal clusters in networks
170 of natural grassland soils were decoupled, while in abandoned arable soil, dominant prokaryote and
171 fungal clusters responded in tandem.
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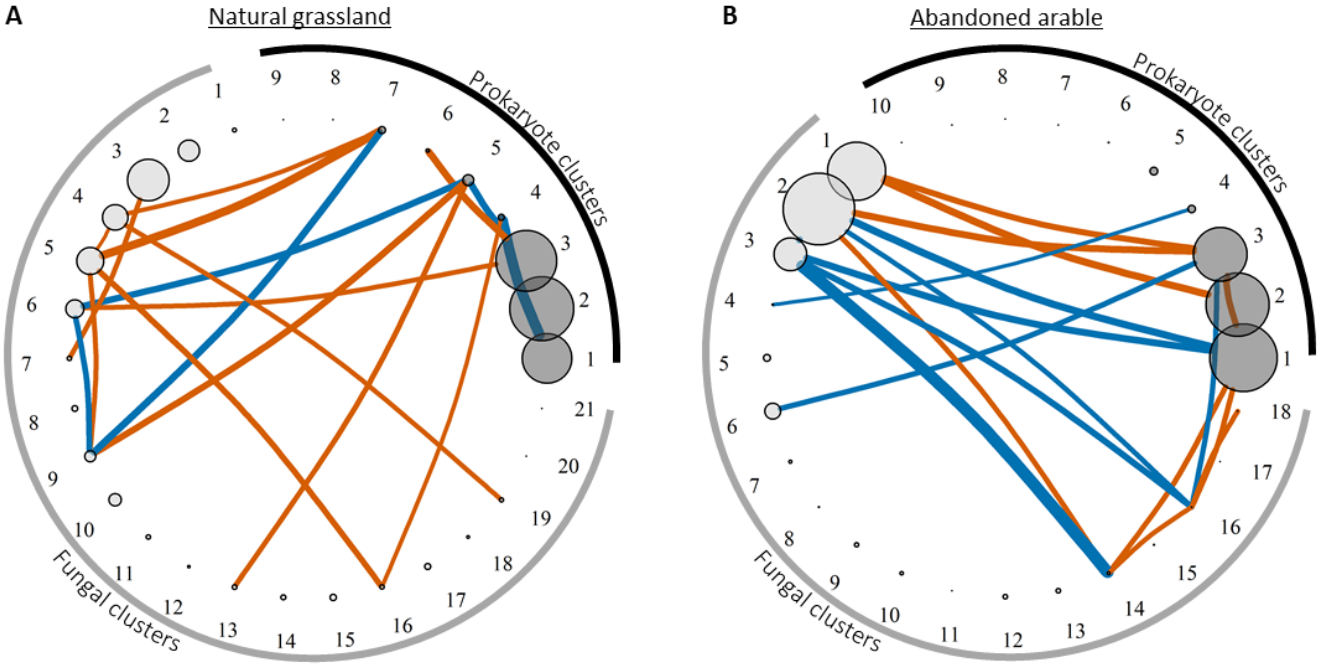

Figure 3 Correlation network of prokaryotes (dark grey) and fungal network clusters (light grey) in (A) natural grassland and (B) abandoned arable soil. Only significant correlations after Bonferroni correction for multiple testing are presented ($p < 0.0017$ and $p < 0.0018$ for natural grassland and abandoned arable correlations, respectively). Negative correlations are indicated in vermillion, positive in blue. Width of the lines indicate the strength of the correlation and size of the vertices indicate the average size of the microbial network cluster based on relative reads within prokaryote and fungal clusters each. Note that this means that cluster sizes can be compared between the two soils *within* the prokaryotes and fungal groups each, but that prokaryote and fungal clusters are not scaled to each other and are therefore not directly comparable (but see Fig S4 for bacterial and fungal biomass comparisons). Correlations result from linear mixed effect models including sowing density as a random factor. For taxonomic and putative soil functions of each microbial network cluster, see Fig S6-S7 and Table S2-S5.

Soil origin affected the pathways via which plant communities shaped microbial networks

We hypothesised that the plant community plays an important role in shaping soil microbial networks and biomass pools, and, importantly, that these pathways are affected by soil origin. Because the plant community was sampled before the microbial soil communities, we were able to test this directional hypothesis using structural equation modelling (SEM). For each soil origin, we tested how plant community parameters from the year of sampling and from the past (plant invasion impact and plant community development trajectories) affected microbial biomass pools and clusters at the end of the 13th growing season (Fig 4A). Moreover, we tested whether these effects resulted from overall plant community factors (belowground productivity, aboveground productivity and plant diversity) or plant community composition (NMDS scores). In addition, we determined whether these pathways occurred via direct plant-microbiota interactions or indirectly via soil chemical changes (Fig 4A). The obtained SEM models explained, on average, 55% of the variation in microbial biomass pools and 50% of the variation in the dominant microbial clusters over both soil origins. For the smaller microbial clusters, the explained variation varied more strongly (between 15 and 71%; Table S6).

We calculated the relative contribution of each group of plant community parameters in shaping soil microbial properties using the effect sizes of the SEM pathways. Overall, we found that both plant community parameters in the year of sampling and from the past contributed significantly to shaping soil microbial communities. Furthermore, the relative contribution of plant community parameters differed between plant communities established on natural grassland and abandoned arable soil (Fig 4B). Three consistent differences occurred. (i) In natural grassland soil, microbial biomass was shaped by a multitude of pathways resulting from both the year of sampling as from the past. In abandoned arable soil, on the other hand, the year of sampling was more important in shaping soil microbial biomass. This effect resulted from microbial biomass being predominantly shaped by direct pathways of the overall plant community (Fig 4B). (ii) In the year of sampling, direct effects of plant community composition contributed with 15% to fungal network cluster formation in natural grassland soil. In contrast, in abandoned arable soil, plant community composition effects in the year of sampling occurred via indirect pathways and shaped both prokaryote and fungal networks clusters (Fig 4B). (iii) Past plant community composition effects in natural grassland soil were generally more important than in abandoned arable soil. Moreover, in natural grassland soil, past plant community effects resulted from a multitude of plant community factors via both direct and indirect pathways with microbial biomass, prokaryote clusters and fungal clusters each shaped by a unique set of pathways. In contrast, in abandoned arable soil, past plant community effects on microbial communities largely resulted from direct, plant compositional effects (Fig 4B).

(i) Year of sampling: plant diversity effects overruled in abandoned arable soil

In abandoned arable soil, the year of sampling had the strongest effects on shaping microbial biomass due to strong effects of plant diversity. In abandoned arable soil, a high plant diversity in the year of sampling was associated with a low bacterial, fungal and AMF biomass (Fig 5B; Table S7-S8). Plant diversity therewith also had dominant effects on soil microbial networks and, amongst others, increased the relative abundance of a large cluster of putative slow growing prokaryotes, while decreasing a large cluster of putative fungal soil saprotrophs (Table 1, S8).

In contrast, in natural grassland soil, belowground productivity was most important in shaping soil bacterial biomass and networks in the year of sampling (Fig 5A, C). Belowground productivity increased putative fast cycling microbiota likely profiting from plant rhizodeposits, while decreasing putative slow cycling microbiota (Table S7). Moreover, in natural grassland soil, plant diversity effects were almost solely indirect and related to a decrease in soil nutrient availability: total N and P (Table 1; Fig 5, S7-S8; Table S7). Via these soil chemical pathways, a high plant diversity decreased bacterial biomass and putative fast-growing microbiota in favour of slower-growing taxa (Table 1, S7).

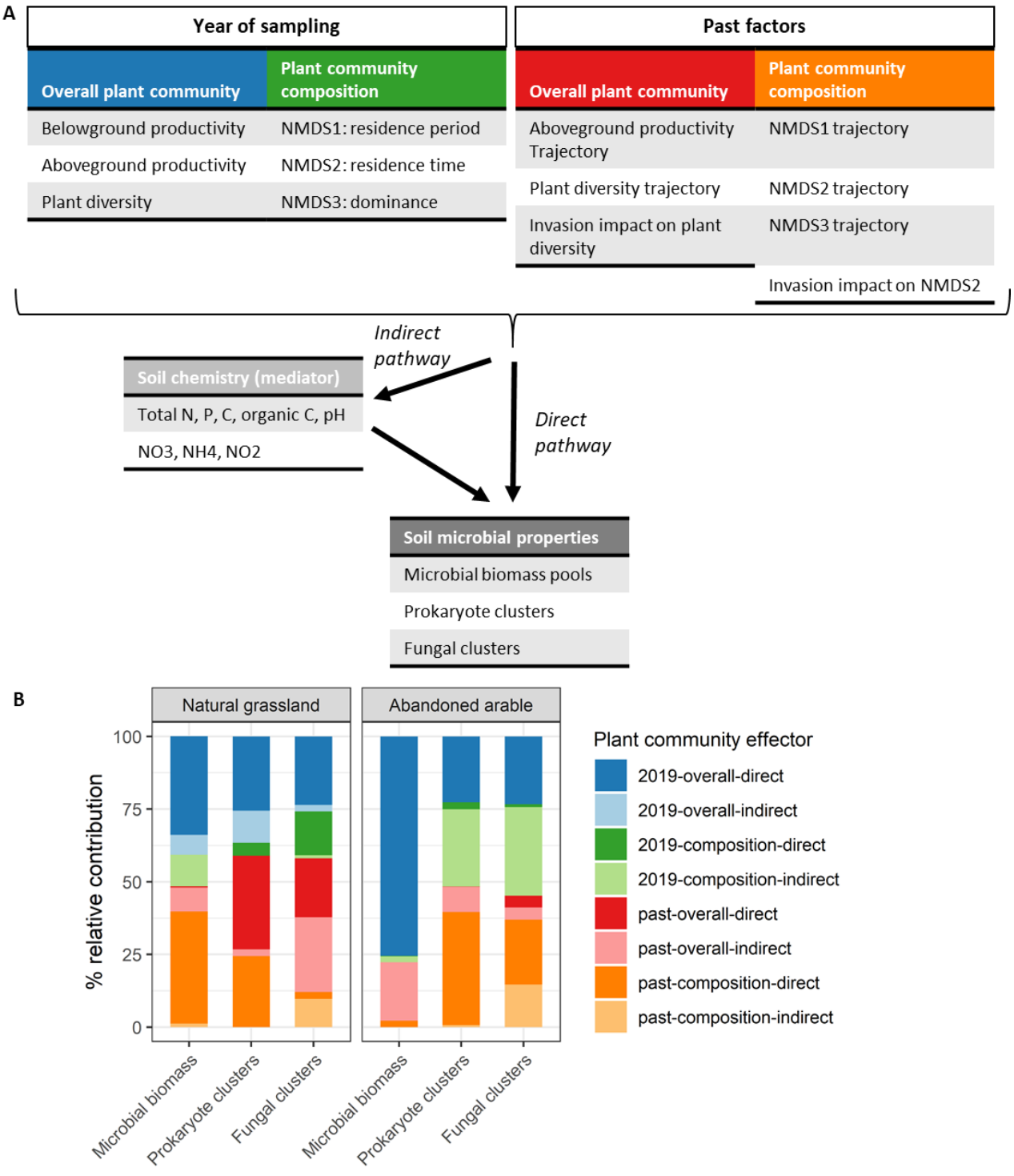

Figure 4 Structural equation modelling (SEM) coupling natural variation in plant communities, soil chemistry and microbial biomass pools and co-occurrence networks in plant communities established on natural grassland and abandoned arable soil. (A) Plant community factors implemented in the SEM approach from the year of sampling (2019) and the past (2007-2019), the considered soil chemical factors as mediators in indirect pathways and the soil microbial properties that were affected either via direct or indirect pathways (n = 30). (B) Combined, relative contribution of each group of plant community factors in shaping soil microbial properties in natural grassland and abandoned arable soil. The relative contribution is based on the effect sizes of the SEM pathways scaled to the size of the microbial parameters affected and corrected for the number of potential pathways in the SEM to allow for direct comparison of the various microbial properties. Colours between A and B match the four main groups of plant factors. In B, dark colours indicate direct pathway contributions and faded colours indirect pathways.

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(ii) Year of sampling: plant community composition via soil chemical changes shaped both prokaryote and fungal networks in abandoned arable soil

In the year of sampling, the difference in contribution of plant community composition to microbial networks of the two soil origins was related to both the involved compositional axes as the pathways (direct versus *in*direct). In natural grassland soil, plant compositional effects in the year of sampling were largely related to plant species residence time (NMDS2) and were mostly important in affecting fungal networks via direct pathways (Fig 5E, S8): plant communities with a high proportion of species with long residence times associated with more putative soil and litter saprotrophs and fewer putative root endophytes and fast-growing soil saprotrophs (Table 1, S7-S8).

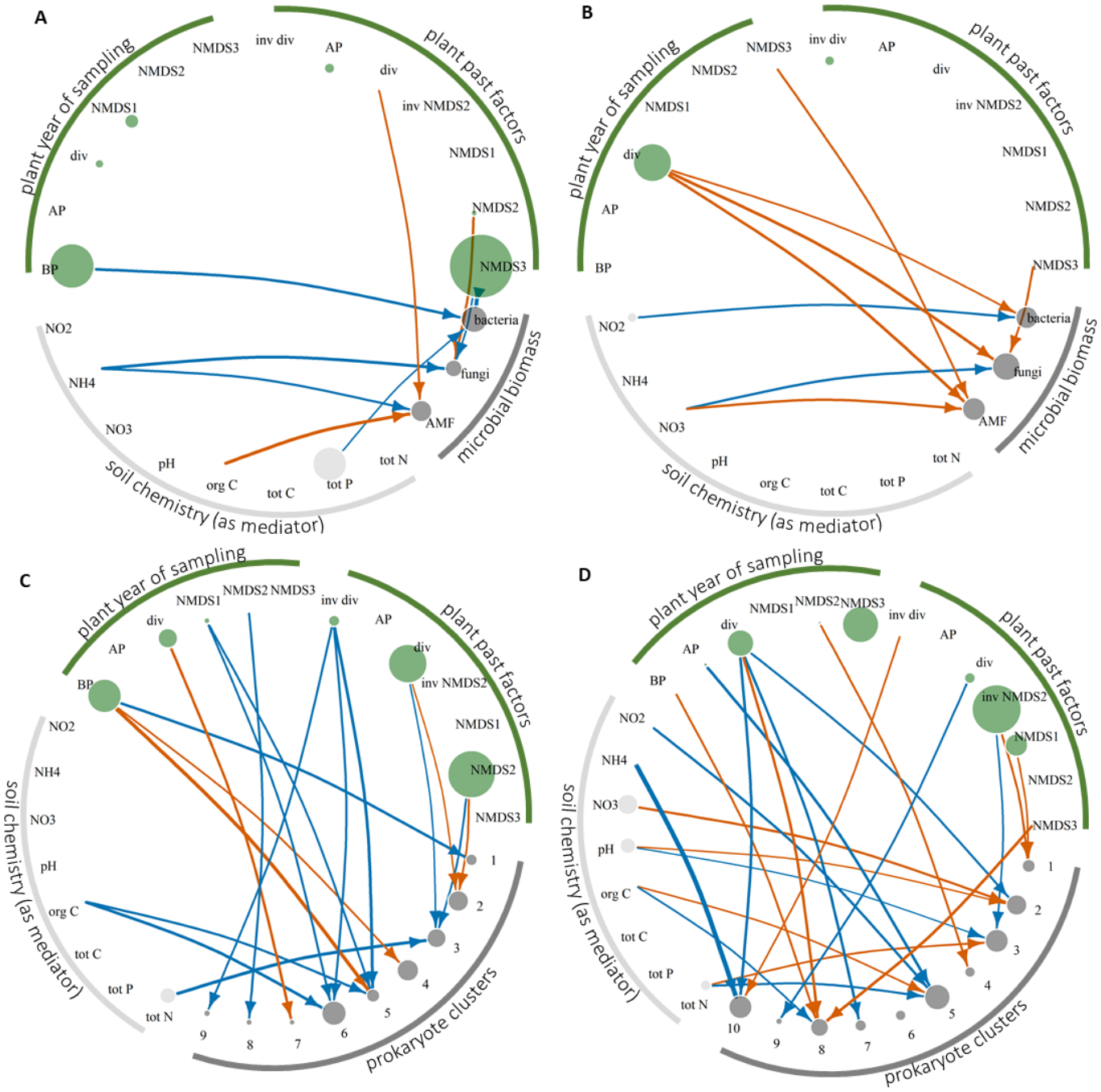
In abandoned arable soil, on the other hand, plant compositional dominance (NMDS3) played a more prominent role (Fig 5, S8). These pathways occurred via soil chemical changes and shaped both prokaryote and fungal networks (Table 1, S7-S8). Plant communities dominated by invaded species had higher soil NO_3^- and pH. These effects were associated with an increase in microbial communities typical for fast soil nutrient cycling: amongst others, ammonia oxidising archaea (AOA), putative plant pathogens and mycoparasites were increased, while fungal and AMF biomass, putative slow growing chemoheterotrophic bacteria and soil saprotrophs were decreased (Table 1; S7-8).

(iii) *Past: a multitude of plant community pathways shaped microbial networks in natural grassland soil*

The differences in the contribution of past effects to microbial communities in natural grassland and abandoned arable soil were related to the diversity in involved pathways. In natural grassland soil, microbial biomass was mostly determined by the trajectory of plant compositional dominance in time (NMDS3) (Fig 5). Communities with increasing dominance of invaded species had higher bacterial and fungal biomass and putative fungal plant pathogens and nematode parasites. Conversely, a gradual increase in dominance of the sown species *Tanacetum corymbosum* was associated with an increase in, amongst others, a different set of putative fungal plant pathogens (Table S7). Past effects on prokaryote clusters in natural grassland soil occurred mainly via the trajectories of plant diversity and plant compositional residence time (NMDS2) (Fig 5). Both trajectories largely regulated the balance between two dominant prokaryote clusters carrying chemoheterotrophic bacteria (Table S7). Past effects on fungal clusters, on the other hand, largely resulted from the initial impact of invasion on plant diversity (the increase in diversity between 2011 and 2013) (Fig 5, S7). Communities in which plant diversity was drastically increased by this initial invasion event had an increased AMF biomass, various putative saprotrophs and plant pathogens, while various other putative saprotrophs, plant pathogens and nematode parasites were decreased (Table 1, S7). These changes occurred both via

direct pathways and indirect pathways: a decrease in organic C and an increase in pH (Table S7; Fig S9).

In contrast, in abandoned arable soil, one pathway dominated in particular, namely the initial impact of invasion on plant community composition between 2011 and 2012 (Fig 5, S8). Plant communities that had many new plants establish in this first year of invasion had more prokaryotes associated with fast soil nutrient cycling and fewer putative fungal plant pathogens. Moreover, the putative fungal saprotroph community was strongly modified (Table S8). These microbial community changes were in part associated with a decreased total C and organic C (Table S8; Fig S9).



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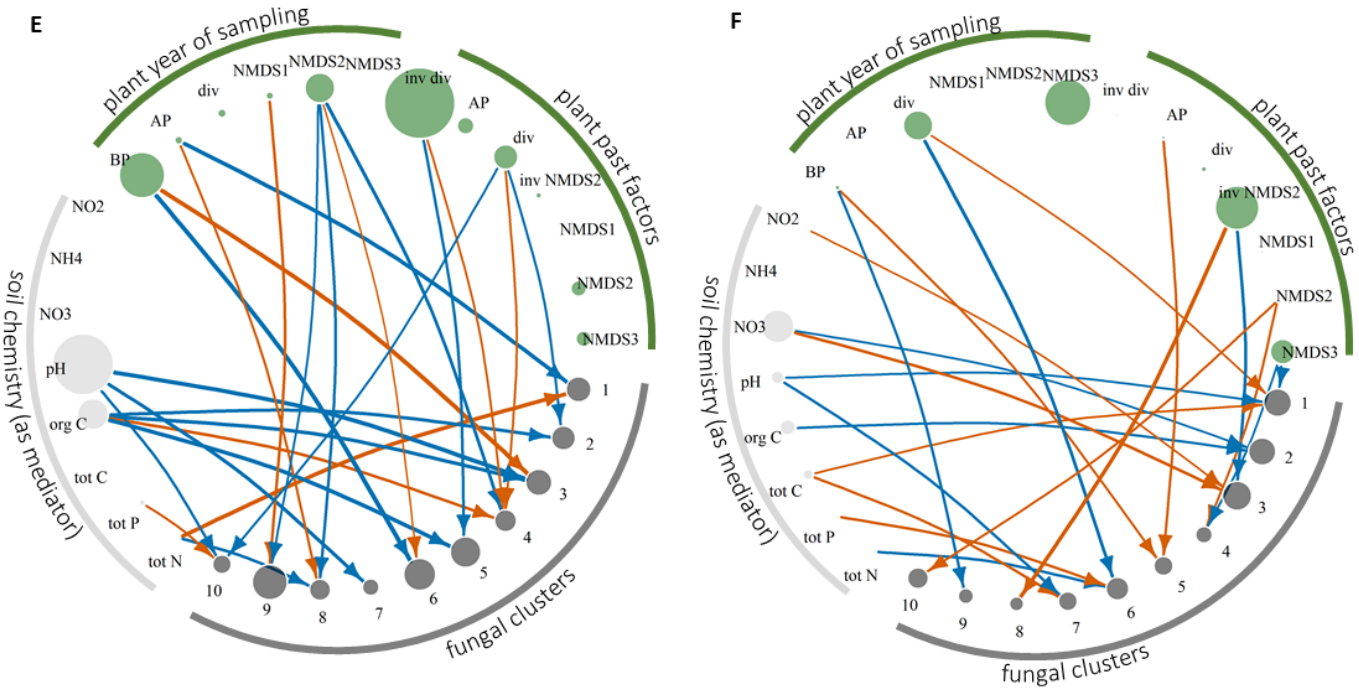

Figure 5 continued. Significant pathways obtained from structural equation models testing effects of plant community parameters in the year of sampling (2019) and the past (2007-2019) on soil microbial communities. Direct effects were separated from *in*direct effects that occurred via changes in soil chemical properties. Microbial community changes were measured in terms of (A, B) microbial biomass, (C, D) prokaryote and (E, F) fungal network clusters in communities established on (left; A, C, E) natural grassland and (right; B, D, F) abandoned arable soil. Plant vertices are indicated in green, soil chemical vertices in light grey and microbial vertices in dark grey. Plant vertice sizes *in*dicate the summed direct and indirect pathway effect sizes onto microbial parameters. Soil chemical vertices indicate only the summed *in*direct pathway effect sizes. Microbial vertices indicate the summed direct and *in*direct pathway effect sizes that these microbial parameters were affected by. All summed pathway effect sizes were scaled to the size of the microbial parameters involved. Negative pathways are represented in vermillion, positive in blue. Arrows indicate the direction of the pathways and the width of the arrows its effect size. Soil chemical pathways are only included when the plant community affected the soil chemical variable. For plant-soil chemical pathways, see Fig S8.
Plant year of sampling factors: BP – belowground productivity, AP – aboveground productivity, div – plant diversity, NMDS1 – plant composition related to species residence period, NMDS2 – plant composition related to species residence time, NMDS3 – plant composition related to species differential dominance. Plant past factors: inv div – initial invasion effect size on plant diversity (increase in diversity between 2011 and 2013), AP – aboveground productivity trajectory, div – plant diversity trajectory, inv NMDS2 – initial invasion effect size on plant composition NMDS2 (increase in new individuals between 2011 and 2012), NMDS1-3 – plant compositional trajectories (see Fig 4A, S1-S2 for more details). Only significant pathways are included ($p < 0.05$; $n = 30$). To increase figure readability, only fungal clusters 1-10 are presented in E and F, see Fig S8 for the other fungal clusters (plant and soil chemical vertices do indicate relative effect sizes based on all fungal clusters).

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Table 1 Summarised effects of the most consistent differences in plant community parameters in the year of sampling on microbial soil networks between natural grassland and abandoned arable soil communities.

		Pathway	Natural grassland soil	Abandoned arable soil
Year of sampling	**Plant diversity**	Direct	↑ soil and litter saprotrophs, animal parasites (1 small) ↓ unknown (1 small)	↑ dominant slow chemoheterotrophs, slow N-cycling taxa, fast soil saprotrophs, fast plant pathogens (1 large, 3 small) ↓ bacterial, fungal and AMF biomass, slow chemoheterotrophs, dominant soil saprotrophs (1 large, 1 small)
		*In*direct	↑ nitrifying taxa, soil and litter saprotrophs (4 small) ↓ bacterial biomass, dominant fast chemoheterotrophs, fast plant pathogens (1 large, 4 small)	
	Composition - residence time (NMDS2)	Direct	↑ root endophytes, dominant soil saprotrophs (1 large, 3 small) ↓ dominant fast and other soil and litter saprotrophs (1 large, 1 small)	↓ chemoheterotrophs (1 small)
	Composition – differential dominance (NMDS3)	Direct	↑ soil saprotrophs, plant pathogens (2 small)	↓ AMF biomass, dung and litter saprotrophs, plant pathogens (1 small)
		*In*direct		↑ dominant AOA and N-fixing taxa, dominant fast and other soil saprotrophs, large diversity plant pathogens, mycoparasites (3 large, 2 small) ↓ fungal and AMF biomass, dominant slow chemoheterotrophs, soil and litter saprotrophs (1 large, 2 small)

A high NMDS2 score indicates communities consisting of typically short-residence plant species. Vice versa, a low NMDS2 score indicates communities consisting of typically long-residence plant species. A high NMDS3 score indicates communities dominated by invaded plant species, while a low NMDS3 score indicates communities dominated by the sown *Tanacetum corymbosum*. Note that displayed functions and metabolic characteristics are all putative. See Table S7-S8 for more details and past plant community pathways, and Table S2-S5 for details on each cluster.

Discussion

Plant community stability is associated with a decoupling in prokaryote and fungal responses

We tested to what extent and via which pathways plant communities shaped end of season soil microbial networks. We compared these pathways between dry grassland communities established on natural grassland soil and soil abandoned from agricultural practices 60 years before the start of the 13-year long mesocosm experiment. Overall, abandoned arable soil created destabilising properties in plant-soil-microbial networks compared to natural grassland soil. In line with various studies, plant communities on abandoned arable soil were less resistant to plant species invasion than communities on natural grassland soil (Kulmatiski et al., 2006; Mattingly & Orrock, 2013). This aboveground instability was mirrored in the soil microbial networks occurring after the 13th growing season. In abandoned arable soil, strong positive and negative co-occurrences between, in particular, the dominant prokaryote and fungal network clusters indicated strong, in tandem responses of these

dominant groups. Since each microbial network cluster was related to a unique set of plant community and soil chemical properties, the observed in tandem patterns indicate that prokaryotes and fungal responses were coupled because these groups occupied the same soil niches (Davison et al., 2021; Dumbrell et al., 2010). Indeed we found that prokaryote and fungal networks were both shaped by the same, dominating pathways associated with the plant community. These observed patterns are typical for instable networks, since disturbance effects can easily propagate in networks that have strongly connected clusters (Fig 6) (Guimerà et al., 2010; Stouffer & Bascompte, 2011). As a result, perturbation effects are able to reshape microbial networks as a whole rather than affecting a small proportion. Aboveground plant community instability on abandoned arable soil was thus mirrored in soil microbial network topology. The latter networks indicated a low microbial niche differentiation, which was for an important part related to the plant community.

In natural grassland soil, on the other hand, prokaryote and fungal responses were largely decoupled. Given that each microbial cluster was related to a unique set of plant community and soil chemical properties, this decoupling indicates that prokaryote and fungal communities largely occupied separate soil niches (Davison et al., 2021; Dumbrell et al., 2010). In line, we found that prokaryote and fungal networks were for an important part shaped by different plant community pathways, creating separate soil niches for the two groups. In addition, the fungal networks itself showed a higher niche differentiation in natural grassland soil than abandoned arable soil. Interestingly, fungal alpha-diversity (Shannon diversity and the number of unique taxa) was lower in natural grassland than abandoned arable soil, indicating that the structure of, in particular, fungal networks is more important to stability than fungal diversity per se. The observed microbial network topologies are associated with the capacity to buffer against the propagation of perturbation effects: a perturbation affecting one or a few of the microbial clusters will not cascade into affecting unconnected clusters (Fig 6; compartmentalization in Guimerà et al., 2010; Stouffer & Bascompte, 2011). Plant community stability is thus associated with a decoupling of prokaryote and fungal responses, which likely plays a critical role in buffering the propagation of disturbance effects in plant communities.

Plant diversity driven soil resource depletion plays a key role in microbial network stability

One of the key differences between stable and instable plant communities resulted from the effect of plant diversity on soil microbial communities. Plant diversity in the year of sampling was one of the most important pathways shaping soil microbial communities in abandoned arable soil, likely overriding other plant community effects. In abandoned arable soil, a low plant diversity was associated with a high bacterial, fungal and AMF biomass, and a loss of slower-growing prokaryotes

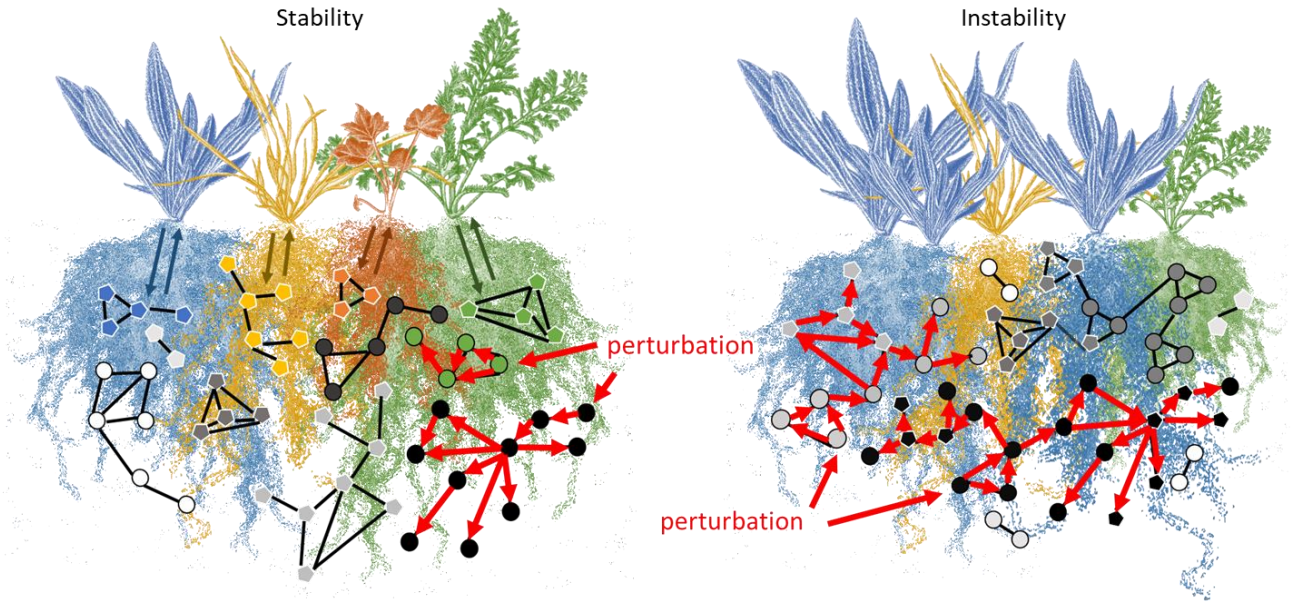

Figure 6 Conceptual framework reconciling the importance of the decoupling of prokaryote and fungal responses in buffering the propagation of local perturbation effects. In (A) stable settings, prokaryotes (circles, black border) and fungi (pentagons, white border) occur in separate clusters. This separation results from different factors shaping prokaryote and fungal niches. For example, fungal clusters are for an important part associated with different plant species (colours of fungal pentagons match colour of the plant species). The resulting microbial networks are likely to buffer local perturbation effects as effects on a subset of the occurring clusters are not likely to propagate to unconnected clusters (red arrows showing the spread among connected taxa). In (B) unstable settings, prokaryotes and fungi respond in tandem and create three dominating clusters. This coupling results from a few dominant factors shaping both prokaryote and fungal soil niches. The resulting microbial networks are likely to propagate disturbance effects throughout large parts of the network given the high connectiveness of microbial taxa into dominant clusters (red arrows).

and small microbial clusters. To understand these effects, it is important to realise that plant diversity differences in our communities resulted from local plant species extinction and invasion processes over time. Because species loss and invasion are typically non-random processes, a low plant diversity represented a situation in which certain plant species were successful and had excluded less successful species (Smith & Knapp, 2003). It is therefore likely that plant species with a certain successful strategy took over in low diversity communities and created plant communities low in plant functional diversity and effects on soil multifunctionality (Zavaleta et al., 2010). In other words, plant species in low diverse assemblies affected microbial communities in relative similar ways, resulting in the coupling of prokaryote and fungal responses, and therewith instable microbial networks (Fig 6).

In contrast, in natural grassland soil, plant diversity effects were almost solely related to soil N and P depletion. Via these soil chemical pathways, plant diversity decreased bacterial biomass, and other possibly more opportunistic prokaryotes and fungi. Indeed, resource depletion by plant communities is often found to increase with plant diversity (Oelmann et al., 2021; Walde et al., 2021). Soil resource limitation typically limits growth of nutrient acquisitive plant species and therefore plays an essential role in avoiding exclusion of nutrient conservative plant species to maintain diversity at high levels (Grime, 1973). Similar mechanisms are likely to have promoted microbial niche differentiation in

natural grassland soil in the year of sampling (Fig 6). Moreover, such resource limitation mechanisms also occurred from past effects in which the increase of plant diversity with initial plant species invasion in 2012 created long-lasting reductions in organic C. Soil organic C was a particular important parameter associated with fungal networks and therewith decoupled fungal responses from prokaryote responses. While plant diversity is regarded to result in stability by increasing the chance that a plant species is present which can take over lost functions in a community during disturbances (Lepš et al., 2001; Loreau & de Mazancourt, 2013; Saint-Béat et al., 2015), plant diversity also promotes stability via soil resource depletion, driving niche differentiation of soil microbial communities (Fig 6).

It has to be noted that the observed decreases in microbial biomass with increasing plant diversity in both soil origins are opposite to patterns found in many plant diversity experiments (Chen et al., 2019). These opposing effects likely result from the difference in establishment of the plant diversity gradients. Here, local extinction and invasion of plant species resulted in natural variation of plant diversity over time and, therefore, likely resulted in dominant effects of successful plant species strategies at low diversity. In contrast, diversity experiments commonly use planted gradients and initial diversity instead of realised diversity measurements (Hagan et al., 2021). In line with our observations, various studies indicate that diversity gradients affected by extinction and invasion processes can result in negative relationships between realised diversity and ecosystem functioning (Hagan et al., 2021; Mouquet & Loreau, 2003). Such opposing patterns may especially occur if low diversity treatments in planted gradients do not represent groups of plant species which would be most successful in the particular environmental conditions and would naturally come to dominate at low diversity. To properly understand the importance of plant diversity in ecosystem dynamics, plant diversity effects will therefore have to be considered within the processes that created variation in plant diversity in the first place.

Direct, plant compositional effects play a key role in microbial network stability

The second key difference between stable and instable plant communities resulted from the effects of plant community composition on soil microbial communities. In stable, natural grassland soil, plant community composition affected microbial communities via direct pathways. For fungi, these effects occurred mainly in the year of sampling, while for prokaryotes these mainly occurred via developmental trajectories in time. These direct, plant community compositional effects therewith played an important role in decoupling prokaryote and fungal responses in natural grassland soil.

In natural grassland soil, in the year of sampling, plant communities consisting of a large proportion of long-resident plant species were associated with a putative saprotroph community likely specialised

to less available C sources. Plant compositional effects in the year of sampling therefore likely related to a slowing of organic matter turnover in communities consisting largely of species with long residence times (Mayer et al., 2021). Plant compositional trajectories in time mainly regulated dominant chemoheterotrophs. In addition, compositional trajectories suggested putative fungal plant pathogen accumulation associated with a gradual increase in dominance of various invading plant species or the sown species *Tanacetum corymbosum*. Importantly, different sets of putative plant pathogens were involved with the gradual increase of invading species and *T. corymbosum*. An increased plant pathogen accumulation with plant species dominance is a well-known ecological mechanism that is key to plant stabilising processes in, amongst others, grasslands ecosystems (Bever et al., 2012). Indeed, this so-called self-limitation mechanism was also found to increase with plant species time since establishment in a new range (Aldorfová et al., 2020; Diez et al., 2010) and similar temporal processes have been suggested to occur within plant communities itself (in 't Zandt et al., 2022). We conclude that direct effects of plant community composition play a key role in microbial niche differentiation and are essential components to stability in plant and microbial networks (Fig 6).

In contrast, in abandoned arable soil, direct plant compositional effects in the year of sampling were almost completely absent. Instead, plant compositional effects in the year of sampling were driven by indirect effects resulting from plant species compositional dominance: dominance of various invading plant species was associated with increased nutrient cycling after the 13th growing season. Moreover, past compositional effects were dominated by the initial impact of invasion in 2012. The latter also suggested that soil nutrient cycling had been increased when many new species established between 2011 and 2012 (the initial invasion event). Indeed, increased nutrient cycling is commonly observed when ruderal plant species invade communities (Kulmatiski et al., 2006; Zhang et al., 2019). Given the involvement of many N-cycling prokaryotes and ammonia oxidising archaea (AOA), highly plant-controlled processes such as rhizosphere priming (the acceleration of organic matter turnover via root exudation) may play an important role in these self-promotional pathways (Kuzyakov & Xu, 2013; Thion et al., 2016). The plant community pathways via soil resource cycling affected large parts of the microbial community in tandem, coupling prokaryote and fungal responses. We therefore conclude that soil resource cycling is critical in mediating community stability.

Conclusion

We found remarkably different topologies in soil microbial networks in plant communities established on natural grassland soil and plant communities established on soil abandoned from extensive agricultural practices 60 years before the start of the experiment. Microbial networks in stable, natural

grassland soil were largely decoupled in prokaryote and fungal responses, while prokaryote and fungal networks in instable, abandoned arable soil largely responded in tandem. We were able to link this coupling/decoupling of prokaryote and fungal soil networks to the way that, amongst others, plant diversity and plant community composition shaped soil microbial niches. Both aboveground parameters therefore likely provide important, easy to measure predictors of belowground microbial network stability of grassland globally (Bardgett & Caruso, 2020). Similarly, both these plant community factors are promising aspects to consider in designing plant communities that are both diverse in plant species and diverse in plant species' effects on soil microbial networks to increase stability of agricultural systems (Isbell et al., 2017). At the same time, we showed that soil chemical pathways played an important role in both stable and instable microbial networks. Future challenges therefore lie in connecting plant and microbial community stability to its driving forces across a multitude of ecosystems, soil resource conditions, land management and perturbation types (Bardgett & Caruso, 2020; Ingrisch & Bahn, 2018).

Materials and methods

Experimental design

Experimental plant communities consisting of 44 perennial dry grassland species were sown in May 2007 (Table S9) (Münzbergová, 2012). Plant species were sown in equal proportions with three sowing densities on two soil types: a dry natural grassland soil (excavated near Encovany, Czech Republic; 50°31'44.6"N, 14°15'12.6"E) and a soil on which dry natural grasslands was turned into agricultural land, extensively managed and abandoned 60 years before soil was collected (excavated near Institute of Botany, Czech Academy of Sciences; 50°0'7.11"N, 14°33'20.66"E) (n = 10, 60 plant communities in total; Fig S10A). The two soils mainly differed in soil nutrient availability with the natural grassland soil being significantly lower in total N, organic C and plant available P and K than the abandoned arable soil (Münzbergová, 2012). The three sowing densities represent 25%, 100% and 400% of the seed density per m^{-2} as estimated at the natural dry grassland location (see Supplementary methods and (Münzbergová, 2012)). Despite significant effects of sowing density in the first three years of the experiment (Münzbergová, 2012), sowing density did not significantly affect above- and belowground plant, microbial and chemical properties in the current study (data not shown). Sowing density was therefore incorporated as a random effect rather than a fixed factor in all analyses.

Plant communities were grown in 90 L mesocosms (diameter 65 cm, height 36 cm) in the experimental garden of the Institute of Botany, Czech Academy of Sciences (Münzbergová, 2012). This location provided similar environmental conditions to the natural grassland location. Plant

communities did not receive any watering or fertiliser. Only in rare periods of extreme drought when plants showed signs of wilting, communities were watered with rain water.

Importantly, mesocosms were regularly inspected and all species from outside the sown species pool were weeded until September 2011. After this, plant species from the experimental surroundings of the mesocosms were allowed to invade into the established plant communities (Fig S10B; Table S9).

Plant species aboveground proportions

Plant community aboveground biomass was harvested every July and September from 2007 until 2011. From 2012 onward, aboveground biomass was harvested only once a year in July. These time points are similar to management practises at the natural grassland site. Aboveground biomass was cut off 3 cm above the soil and from 2007 until 2011, biomass was sorted per plant species, dried at 60 ℃ for at least 48 h, after which dry weight was determined. From 2012 onward, plant species biomass was estimated by determining the percentile abundance of each plant species per mesocosm and multiplying this by the total aboveground biomass cut at 3 cm height and dried at 60℃ for at least 48 h. In 2014 and 2015, aboveground biomass was cut, but no species proportions were determined.

Soil sampling

After the growing season in December 2019, soil cores of 6 cm in diameter and 36 cm length were taken at six random positions in each plant community (Fig S10B). Aboveground plant parts were removed and soil of the six cores was thoroughly mixed by passing it through a 2 mm mesh. Roots that did not pass the mesh were collected, dried at 60 ℃ for at least 48 h after which dry weight was determined. Subsamples from the mixed soils were taken for soil chemical determination of total nitrogen (N), total and organic C, plant available NO_3^-, NH_4^+ and NO_2^-, K, P and pH (see Supplementary methods). Furthermore, subsamples for analyses of total bacterial, fungal and arbuscular mycorrhizal fungi (AMF) biomass using PLFA and NLFA analyses were taken as well as microbiome community composition using 16S and ITS amplicon sequencing (see Supplementary methods).

Plant community diversity and composition

All analyses were performed in R version 3.6.1 (R Core Team, 2019). Plant community diversity was calculated based on the Shannon index using the function *diversity* of the vegan package (Oksanen et al., 2018). Plant community compositional changes were assessed using non-metric multidimensional scaling (NMDS) based on square root transformed plant community dissimilarity (Bray-Curtis). For this, aboveground biomass of species with in total > 10 observations over all replicates and all years was analysed using *metaMDS* from the vegan package (Oksanen et al., 2018). Community composition

effects were distributed over three axes, which separated communities based on plant species residence period and residence time for axis 1 and 2, respectively, and differential dominance of plant species for axis 3 (Fig S1-2).

Plant community developmental trajectories in time

Plant community development in time was calculated to describe variation in developmental trajectories between the communities and used in the SEM models (see below). In all cases, parameters were calculated based on only the July aboveground measurements to avoid the earlier years to have a relative stronger effect on the trajectories. The overall increase/decrease trend in aboveground productivity and NMDS axis score 3 over time were estimated by a linear model of which the beta slope was taken using a linear model and *lm.beta* (R Core Team, 2019). Shannon diversity and NMDS axis score 2 showed gradual increases/decreases before and after invasion in 2012 with a drastic increase when invasion started. Trajectories before and after the start of invasion in 2012 were estimated by a linear model of which the beta slope was taken. For NMDS2, scores in the first year 2007 were excluded as scores were often very different from the following years, yet had a large influence on the obtained slopes. The drastic increase in diversity due to invasion took place over 2 years. The effect size of this increase was calculated as the Shannon diversity index before invasion in 2011 minus the diversity index in 2013. The drastic change in community composition with invasion largely occurred in one year. The effect size of invasion on community composition was calculated as the NMDS2 score before invasion in 2011 minus the NMDS2 scores in 2012. NMDS axis 1 scores over time showed a gradual increase that levelled off over time. These temporal patterns were described by a Michaelis Menten saturation curve of the form: $y = a \cdot t / (1 + b \cdot t) + c$, where t is time in years, using nls2 (Grothendieck, 2013). Parameter b from this formula indicates the extent to which the line levels off with a low value indicating a fast levelling off and a high value a slow levelling off. In other words, parameter b indicates compositional turnover speed.

Microbial community analyses

Demultiplexed raw FASTQ files were analysed using the SEED2.0 pipeline (Větrovský et al., 2018) for prokaryote 16S and fungal ITS rRNA sequences. In short, sequences were quality trimmed, clustered into OTUs at ≥ 97% sequence similarity and chimeric sequences were removed. Taxonomic information was obtained using the RDP (v16) and UNITE (v8.3) databases for 16S and ITS, respectively (Cole et al., 2014; Nilsson et al., 2019). Beta-diversity (Bray-Curtis) was calculated based on center log ratio (clr) transformed read counts and visualised using PCoA with the phyloseq package (McMurdie

& Holmes, 2013). Significant separation between soil origins was tested with PERMANOVA using *adonis* from the vegan package (Oksanen et al., 2018).

We constructed co-occurrence networks across the 30 plant communities on each soil type for both prokaryote (bacteria and archaea) and fungal communities. We first filtered each dataset to exclude rare OTUs with total < 100 reads and OTUs that were present in < 5 samples per soil origin. Co-occurrence networks were then calculated using the SpiecEasi package (Kurtz et al., 2015). In brief, networks were inferred based on clr transformed read counts, neighbourhood selection (MB method) and we selected optimal stability parameters using the StARS selection approach (threshold 0.05, nlambda 20 with 999 replications) (Liu et al., 2010). We clustered similarly responding OTUs in each network using the Spin-glass algorithm of the igraph package (Newman & Girvan, 2004; Reichardt & Bornholdt, 2006; The igraph Core Team, 2020; Traag & Bruggeman, 2009). This approach clusters OTUs based on both positive and negative edges as well as their weight. Present and absent edges as well as positive and negative edges were given a similar importance, and unlimited spins (clusters) were provided. Relative read counts were summed per cluster per sample and used in further correlation and structural equation models (SEM).

We inferred putative dominant metabolic and functional characteristics of each microbial network cluster. This was done based on the significant relations between the relative abundance of the cluster to soil chemical and plant community parameters from SEM (see section Structural Equation Modelling) in combination with knowledge on the dominant microbial families and genera present in each cluster. The latter information was obtained from literature, and, for fungal communities, from the FungalTraits database (Põlme et al., 2020). It has to be noted that such an inference on putative microbial traits has to be treated with caution, however, our approach of including relations with soil chemistry and plant community parameters is more rigorous than solely relying on microbial family and genera knowledge. Moreover, the use of network clusters avoids the common issue of excluding unidentified taxa or taxa with unknown putative functions and, at the same time, informs on the co-occurrence of taxa with different putative traits.

Overall statistics

Analysis of variances were performed using linear mixed-effects models in R version 3.6.1 (R Core Team, 2019). All models in which ‘time’ (continuous, scaled) and ‘soil origin’ were included as a dependent variable, included ‘mesocosm’ and ‘sowing density’ as random effects to take repeated measures and initial sowing densities into account. Models were fitted using *lmer* of the lme4 package (Bates et al., 2015). Mesocosm position in the garden had negligible effects, in many cases resulted in overfitting of the models and was therefore dropped as a random effect.

Correlations between plant, soil chemistry and microbial parameters including network clusters were performed using *lme* of the nlme package (Pinheiro et al., 2019) and included sowing density as a random factor. For these multiple correlations, p-values were corrected for multiple testing using the Bonferroni correction (Bonferroni, 1936). In all models, data was ln- or sqrt-transformed when model residuals did not follow a normal distribution. In case of heterogeneity of variances, data weighting per soil type using *varIdent* from the nlme package was incorporated (Zuur et al., 2009). Following Tukey HSD post-hoc tests were performed using emmeans (Lenth, 2018).

Structural equation modelling

We hypothesised that plant community parameters in the year of sampling and developmental trajectories in time affected plant available and total resources in the soil with potential cascading effects onto microbial communities. Alternatively, these plant parameters could have had direct effects on microbial communities. We used structural equation models (SEM) to test this hypothesis, which specifically allowed us to separate direct effects of plant communities on soil microbial communities from indirect effects via soil chemical changes. All SEMs were fit using piecewiseSEM (Lefcheck, 2016) and *lme* of the nlme package (Pinheiro et al., 2019) with initial sowing density as a random effect (n = 30). Overall model fit was assessed using direction separation tests (d-sep) based on Fisher's C statistics with models being accepted if $p > 0.1$. We simplified our models using a backward stepwise elimination procedure for which we consecutively removed pathways with the highest p-value (in 't Zandt et al., 2020). Endogenous variables were allowed to drop from the model in case effects were not significant ($p > 0.05$). The model with the lowest Akaike information criterion (AIC) was then selected as the best fit base model (see Supplementary Methods).

We created 64 unique SEM models (3 microbial pools natural soil + 3 microbial pools abandoned soil + 9 16S clusters natural soil + 10 16S clusters abandoned soil + 21 ITS clusters natural soil + 18 ITS clusters abandoned soil). We extracted the direction and effect size of each significant pathway of each model ($p < 0.05$). We then calculated the contribution of each plant parameter to changes in microbial biomass pools and microbial co-occurrence clusters both via direct and indirect pathways. For each plant community parameter, its importance in affecting microbial biomass pools, prokaryote clusters and fungal clusters was calculated including both direct and indirect pathways. For each significant, direct plant-microbial pathway, we multiplied the path effect size with the relative size of the microbial pool or cluster it was affecting. This multiplication sized the pathway effect to its relative importance to the microbial community as a whole. For indirect pathways, we multiplied the effect size of the plant parameter onto the soil chemical variable with the effect size of the soil chemical variable onto the microbial variable. The obtained effect sizes were again scaled to the relative size of

563 the microbial variable they were affecting. We then created an overview of the importance of plant
564 community parameters in the year of sampling and from the past, in which we separated overall from
565 compositional plant community effects and direct from indirect pathways (Fig 4A). For this we
566 summed the above effect sizes of each plant community parameter belonging to each group, scaled
567 these to the number of potential pathways within each group to be able to compare the various
568 groups directly and calculated each groups relative contribution in affecting microbial biomass,
569 prokaryote and fungal clusters in each soil origin (Fig 4B).
570

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577

578 **Author contributions**

579 ZM designed and set up the experiment; ZM maintained the experiment and led the sampling
580 campaigns; TC analysed soil samples for PLFA/NLFAs; ZK performed the downstream microbial
581 bioinformatics; DitZ performed all further microbial, soil and plant data analyses and statistics; DitZ
582 wrote the publication, all others co-commented. All authors contributed critically to the manuscript
583 and gave final approval for publication.
584

585 **Data availability**

586 Upon acceptance of the manuscript, data and R code will be made publicly available.
587

588 **References**

589 Aldorfová, A., Knobová, P., & Münzbergová, Z. (2020). Plant–soil feedback contributes to predicting
590 plant invasiveness of 68 alien plant species differing in invasive status. *Oikos*, *129*(8), 1257–
591 1270. https://doi.org/10.1111/oik.07186

592 Bardgett, R. D., & Caruso, T. (2020). Soil microbial community responses to climate extremes:
593 Resistance, resilience and transitions to alternative states. In *Philosophical Transactions of the*
594 *Royal Society B: Biological Sciences* (Vol. 375, Issue 1794). Royal Society Publishing.
595 https://doi.org/10.1098/rstb.2019.0112

596 Bardgett, R. D., & van der Putten, W. H. (2014). Belowground biodiversity and ecosystem
597 functioning. *Nature*, *515*(7528), 505–511. https://doi.org/10.1038/nature13855

598 Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using
599 lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

600 Bever, J. D., Platt, T. G., & Morton, E. R. (2012). Microbial population and community dynamics on
601 plant roots and their feedbacks on plant communities. *Annual Review of Microbiology*, *66*(1),
602 265–283. https://doi.org/10.1146/annurev-micro-092611-150107

603 Bezemer, T. M., Fountain, M. T., Barea, J. M., Christensen, S., Dekker, S. C., Duyts, H., Van Hal, R.,
604 Harvey, J. A., Hedlund, K., Maraun, M., Mikola, J., Mladenov, A. G., Robin, C., De Ruiter, P. C.,
605 Scheu, S., Setälä, H., Šmilauer, P., & van der Putten, W. H. (2010). Divergent composition but
606 similar function of soil food webs of individual plants: plant species and community effects.
607 *Ecology*, *91*(10), 3027–3036. https://doi.org/10.1890/09-2198.1

608 Bonferroni, C. E. (1936). Teoria statistica delle classi e calcolo delle probabilità. In *Pubblicazioni del R*
609 *Istituto Superiore di Scienze Economiche e Commerciali di Firenze*.

610 Canarini, A., Kaiser, C., Merchant, A., Richter, A., & Wanek, W. (2019). Root exudation of primary
611 metabolites: Mechanisms and their roles in plant responses to environmental stimuli. In
612 *Frontiers in Plant Science* (Vol. 10). Frontiers Media S.A.
613 https://doi.org/10.3389/fpls.2019.00157

614 Chen, C., Chen, H. Y. H., Chen, X., & Huang, Z. (2019). Meta-analysis shows positive effects of plant
615 diversity on microbial biomass and respiration. *Nature Communications*, *10*(1), 1–10.
616 https://doi.org/10.1038/s41467-019-09258-y

617 Cole, J. R., Wang, Q., Fish, J. A., Chai, B., McGarrell, D. M., Sun, Y., Brown, C. T., Porras-Alfaro, A.,
618 Kuske, C. R., & Tiedje, J. M. (2014). Ribosomal Database Project: Data and tools for high
619 throughput rRNA analysis. *Nucleic Acids Research*, *42*(D1).
620 https://doi.org/10.1093/nar/gkt1244

621 Coyte, K. Z., Schluter, J., & Foster, K. R. (2015). The ecology of the microbiome: Networks,
622 competition, and stability. *Science*, *350*(6261), 663–666.
623 https://doi.org/10.1126/science.aad2602

624 Davison, J., Moora, M., Semchenko, M., Adenan, S. B., Ahmed, T., Akhmetzhanova, A. A., Alatalo, J.
625 M., Al-Quraishy, S., Andriyanova, E., Anslan, S., Bahram, M., Batbaatar, A., Brown, C., Bueno, C.
626 G., Cahill, J., Cantero, J. J., Casper, B. B., Cherosov, M., Chideh, S., … Öpik, M. (2021).
627 Temperature and pH define the realised niche space of arbuscular mycorrhizal fungi. *New*
628 *Phytologist*, *231*(2), 763–776. https://doi.org/10.1111/nph.17240

629 de Vries, F. T., Griffiths, R. I., Bailey, M., Craig, H., Girlanda, M., Gweon, H. S., Hallin, S., Kaisermann,
630 A., Keith, A. M., Kretzschmar, M., Lemanceau, P., Lumini, E., Mason, K. E., Oliver, A., Ostle, N.,
631 Prosser, J. I., Thion, C., Thomson, B., & Bardgett, R. D. (2018). Soil bacterial networks are less
632 stable under drought than fungal networks. *Nature Communications*, *9*(1), 3033.
633 https://doi.org/10.1038/s41467-018-05516-7

634 de Vries, F. T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M. A., Bjørnlund, L., Jørgensen, H. B.,
635 Brady, M. V., Christensen, S., de Ruiter, P. C., D'Hertefeldt, T., Frouz, J., Hedlund, K., Hemerik,
636 L., Gera Hol, W. H., Hotes, S., Mortimer, S. R., Setälä, H., Sgardelis, S. P., … Bardgett, R. D.
637 (2013). Soil food web properties explain ecosystem services across European land use systems.
638 *Proceedings of the National Academy of Sciences of the United States of America*, *110*(35),
639 14296–14301. https://doi.org/10.1073/pnas.1305198110

640 Diez, J. M., Dickie, I., Edwards, G., Hulme, P. E., Sullivan, J. J., & Duncan, R. P. (2010). Negative soil
641 feedbacks accumulate over time for non-native plant species. *Ecology Letters*, *13*(7), 803–809.
642 https://doi.org/10.1111/j.1461-0248.2010.01474.x

643 Dumbrell, A. J., Nelson, M., Helgason, T., Dytham, C., & Fitter, A. H. (2010). Relative roles of niche
644 and neutral processes in structuring a soil microbial community. *ISME Journal*, *4*(3), 337–345.
645 https://doi.org/10.1038/ismej.2009.122

646 Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., Partsch, S., Sabais, A. C.
647 W., Scherber, C., Steinbeiss, S., Weigelt, A., Weisser, W. W., & Scheu, S. (2010). Plant diversity
648 effects on soil microorganisms support the singular hypothesis. *Ecology*, *91*(2), 485–496.
649 https://doi.org/10.1890/08-2338.1

650 Grime, J. P. (1973). Competitive exclusion in herbaceous vegetation. *Nature*, *242*(5396), 344–347.
651 https://doi.org/10.1038/242344a0

652 Grothendieck, G. (2013). *nls2: non-linear regression with brute force* (0.2).

653 Guimerà, R., Stouffer, D. B., Sales-Pardo, M., Leicht, E. A., Newman, M. E. J., & Amaral, L. A. N.
654 (2010). Origin of compartmentalization in food webs. *Ecology*, *91*(10), 2941–2951.
655 https://doi.org/10.1890/09-1175.1

656 Hagan, J. G., Vanschoenwinkel, B., & Gamfeldt, L. (2021). We should not necessarily expect positive
657 relationships between biodiversity and ecosystem functioning in observational field data.
658 *Ecology Letters*, *24*(12), 2537–2548. https://doi.org/10.1111/ele.13874

659 Hernandez, D. J., David, A. S., Menges, E. S., Searcy, C. A., & Afkhami, M. E. (2021). Environmental
660 stress destabilizes microbial networks. *ISME Journal*, *15*(6), 1722–1734.
661 https://doi.org/10.1038/s41396-020-00882-x

662 Ingrisch, J., & Bahn, M. (2018). Towards a comparable quantification of resilience. *Trends in Ecology*
663 *and Evolution*, *33*(4), 251–259. https://doi.org/10.1016/j.tree.2018.01.013

664 in 't Zandt, D., Herben, T., Brink, A., Visser, E. J. W., & Kroon, H. (2021). Species abundance
665 fluctuations over 31 years are associated with plant–soil feedback in a species-rich mountain
666 meadow. *Journal of Ecology*, *109*(3), 1511–1523. https://doi.org/10.1111/1365-2745.13574

667 in 't Zandt, D., Hoekstra, N. J., Caluwe, H., Cruijsen, P. M. J. M., Visser, E. J. W., & Kroon, H. (2022).
668 Plant life-history traits rather than soil legacies determine colonisation of soil patches in a
669 multi-species grassland. *Journal of Ecology*, *110*(4), 889–901. https://doi.org/10.1111/1365-
670 2745.13850

671 in 't Zandt, D., Hoekstra, N. J., Wagemaker, C. A. M., Caluwe, H., Smit-Tiekstra, A. E., Visser, E. J. W.,
672 & Kroon, H. (2020). Local soil legacy effects in a multispecies grassland community are
673 underlain by root foraging and soil nutrient availability. *Journal of Ecology*, *108*(6), 2243–2255.
674 https://doi.org/10.1111/1365-2745.13449

675 IPCC. (2021). *Climate change 2021: the physical science basis. contribution of working group I to the*
676 *sixth assessment report of the intergovernmental panel on climate change* (V. Masson-
677 Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I.
678 Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O.
679 Yelekçi, R. Yu, & B. Zhou, Eds.). Cambridge University Press.

680 Isbell, F., Adler, P. R., Eisenhauer, N., Fornara, D., Kimmel, K., Kremen, C., Letourneau, D. K., Liebman,
681 M., Polley, H. W., Quijas, S., & Scherer-Lorenzen, M. (2017). Benefits of increasing plant
682 diversity in sustainable agroecosystems. *Journal of Ecology*, *105*(4), 871–879.
683 https://doi.org/10.1111/1365-2745.12789

684 Kulmatiski, A., Beard, K. H., & Stark, J. M. (2006). Soil history as a primary control on plant invasion in
685 abandoned agricultural fields. *Journal of Applied Ecology*, *43*(5), 868–876.
686 https://doi.org/10.1111/j.1365-2664.2006.01192.x

687 Kurtz, Z. D., Müller, C. L., Miraldi, E. R., Littman, D. R., Blaser, M. J., & Bonneau, R. A. (2015). Sparse
688 and compositionally robust inference of microbial ecological networks. *PLoS Computational*
689 *Biology*, *11*(5), 1–25. https://doi.org/10.1371/journal.pcbi.1004226

690 Kuzyakov, Y., & Xu, X. (2013). Competition between roots and microorganisms for nitrogen:
691 Mechanisms and ecological relevance. *New Phytologist*, *198*(3), 656–669.
692 https://doi.org/10.1111/nph.12235

693 Lefcheck, J. S. (2016). piecewiseSEM: piecewise structural equation modelling in R for ecology,
694 evolution, and systematics. *Methods in Ecology and Evolution*, *7*(5), 573–579.
695 https://doi.org/10.1111/2041-210X.12512

696 Lenth, R. (2018). *emmeans: estimated marginal means, aka least-squares means* (1.2.1).
697 https://cran.r-project.org/package=emmeans

698 Lepš, J., Brown, V. K., Diaz Len, T. A., Gormsen, D., Hedlund, K., Kailová, J., Korthals, G. W., Mortimer,
699 S. R., Rodriguez-Barrueco, C., Roy, J., Santa Regina, I., van Dijk, C., & van der Putten, W. H.
700 (2001). Separating the chance effect from other diversity effects in the functioning of plant
701 communities. *Oikos*, *92*(1), 123–134. https://doi.org/10.1034/j.1600-0706.2001.920115.x

702 Liu, H., Roeder, K., & Wasserman, L. (2010). Stability Approach to Regularization Selection (StARS) for
703 high dimensional graphical models. *Advances in Neural Information Processing Systems*, *24*(2),
704 1432–1440.

705 Loreau, M., & de Mazancourt, C. (2013). Biodiversity and ecosystem stability: a synthesis of
706 underlying mechanisms. *Ecology Letters*, *16*(SUPPL.1), 106–115.
707 https://doi.org/10.1111/ele.12073

708 Mattingly, W. B., & Orrock, J. L. (2013). Historic land use influences contemporary establishment of
709 invasive plant species. *Oecologia*, *172*(4), 1147–1157. https://doi.org/10.1007/s00442-012-
710 2568-5

711 Mayer, M., Rewald, B., Matthews, B., Sandén, H., Rosinger, C., Katzensteiner, K., Gorfer, M., Berger,
712 H., Tallian, C., Berger, T. W., & Godbold, D. L. (2021). Soil fertility relates to fungal-mediated
713 decomposition and organic matter turnover in a temperate mountain forest. *New Phytologist*,
714 *231*(2), 777–790. https://doi.org/10.1111/nph.17421

715 McCann, K., Hastings, A., & Huxel, G. R. (1998). Weak trophic interactions and the balance of nature.
716 *Nature*, *395*(6704), 794–798. https://doi.org/10.1038/27427

717 McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R package for reproducible interactive analysis
718 and graphics of microbiome census data. *PLoS ONE*, *8*(4).
719 https://doi.org/10.1371/journal.pone.0061217

720 Montoya, J. M., Pimm, S. L., & Solé, R. V. (2006). Ecological networks and their fragility. *Nature*,
721 *442*(7100), 259–264. https://doi.org/10.1038/nature04927

722 Mouquet, N., & Loreau, M. (2003). Community patterns in source-sink metacommunities. *American*
723 *Naturalist*, *162*(5), 544–557. https://doi.org/10.1086/378857

724 Münzbergová, Z. (2012). Seed density significantly affects species richness and composition in
725 experimental plant communities. *PLoS ONE*, *7*(10).
726 https://doi.org/10.1371/journal.pone.0046704

727 Neutel, A. M., Heesterbeek, J. A. P., & de Ruiter, P. C. (2002). Stability in real food webs: Weak links
728 in long loops. *Science*, *296*(5570), 1120–1123. https://doi.org/10.1126/science.1068326

729 Newman, M. E. J., & Girvan, M. (2004). Finding and evaluating community structure in networks.
730 *Physical Review E - Statistical, Nonlinear, and Soft Matter Physics*, *69*(2 2), 1–15.
731 https://doi.org/10.1103/PhysRevE.69.026113

732 Nilsson, R. H., Larsson, K. H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D.,
733 Kennedy, P., Picard, K., Glöckner, F. O., Tedersoo, L., Saar, I., Kõljalg, U., & Abarenkov, K. (2019).
734 The UNITE database for molecular identification of fungi: Handling dark taxa and parallel
735 taxonomic classifications. *Nucleic Acids Research*, *47*(D1), D259–D264.
736 https://doi.org/10.1093/nar/gky1022

737 Oelmann, Y., Lange, M., Leimer, S., Roscher, C., Aburto, F., Alt, F., Bange, N., Berner, D., Boch, S.,
738 Boeddinghaus, R. S., Buscot, F., Dassen, S., De Deyn, G., Eisenhauer, N., Gleixner, G., Goldmann,
739 K., Hölzel, N., Jochum, M., Kandeler, E., … Wilcke, W. (2021). Above- and belowground
740 biodiversity jointly tighten the P cycle in agricultural grasslands. *Nature Communications*, *12*(1),
741 4431. https://doi.org/10.1038/s41467-021-24714-4

742 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara,
743 R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2018). *vegan:*
744 *community ecology package* (2.4-6). https://cran.r-project.org/package=vegan

745 Pimm, S. L. (1985). The complexity and stability of ecosystems. *Nature*, *315*(6021), 635–636.

746 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2019). *nlme: linear and nonlinear*
747 *mixed effects models* (3.1-140).

748 Põlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B. D., Clemmensen, K. E., Kauserud, H., Nguyen,
749 N., Kjøller, R., Bates, S. T., Baldrian, P., Frøslev, T. G., Adojaan, K., Vizzini, A., Suija, A., Pfister, D.,
750 Baral, H. O., Järv, H., Madrid, H., Nordén, J., … Tedersoo, L. (2020). FungalTraits: a user-friendly
751 traits database of fungi and fungus-like stramenopiles. *Fungal Diversity*, *105*(1).
752 https://doi.org/10.1007/s13225-020-00466-2

753 R Core Team. (2019). *R: a Language and Environment for Statistical Computing* (3.6.0). R Foundation
754 for Statistical Computing.

755 Reichardt, J., & Bornholdt, S. (2006). Statistical mechanics of community detection. *Physical Review E*
756 *- Statistical, Nonlinear, and Soft Matter Physics*, *74*(1), 1–16.
757 https://doi.org/10.1103/PhysRevE.74.016110

758 Saint-Béat, B., Baird, D., Asmus, H., Asmus, R., Bacher, C., Pacella, S. R., Johnson, G. A., David, V.,
759 Vézina, A. F., & Niquil, N. (2015). Trophic networks: How do theories link ecosystem structure

760 and functioning to stability properties? A review. *Ecological Indicators*, *52*, 458–471.
761 https://doi.org/10.1016/j.ecolind.2014.12.017

762 Smith, M. D., & Knapp, A. K. (2003). Dominant species maintain ecosystem function with non-
763 random species loss. *Ecology Letters*, *6*(6), 509–517. https://doi.org/10.1046/j.1461-
764 0248.2003.00454.x

765 Sokol, N. W., Slessarev, E., Marschmann, G. L., Nicolas, A., Blazewicz, S. J., Brodie, E. L., Firestone, M.
766 K., Foley, M. M., Hestrin, R., Hungate, B. A., Koch, B. J., Stone, B. W., Sullivan, M. B., Zablocki,
767 O., Trubl, G., McFarlane, K., Stuart, R., Nuccio, E., Weber, P., … Pett-Ridge, J. (2022). Life and
768 death in the soil microbiome: how ecological processes influence biogeochemistry. *Nature*
769 *Reviews Microbiology*. https://doi.org/10.1038/s41579-022-00695-z

770 Stouffer, D. B., & Bascompte, J. (2011). Compartmentalization increases food-web persistence.
771 *Proceedings of the National Academy of Sciences of the United States of America*, *108*(9), 3648–
772 3652. https://doi.org/10.1073/pnas.1014353108

773 The igraph Core Team. (2020). *igraph - The network analysis package*.

774 Thion, C. E., Poirel, J. D., Cornulier, T., de Vries, F. T., Bardgett, R. D., & Prosser, J. I. (2016). Plant
775 nitrogen-use strategy as a driver of rhizosphere archaeal and bacterial ammonia oxidiser
776 abundance. *FEMS Microbiology Ecology*, *92*(7), 1–11. https://doi.org/10.1093/femsec/fiw091

777 Traag, V. A., & Bruggeman, J. (2009). Community detection in networks with positive and negative
778 links. *Physical Review E - Statistical, Nonlinear, and Soft Matter Physics*, *80*(3), 1–6.
779 https://doi.org/10.1103/PhysRevE.80.036115

780 van der Putten, W. H., Bradford, M. A., Pernilla Brinkman, E., van de Voorde, T. F. J., & Veen, G. F.
781 (2016). Where, when and how plant-soil feedback matters in a changing world. *Functional*
782 *Ecology*, *30*(7), 1109–1121. https://doi.org/10.1111/1365-2435.12657

783 Větrovský, T., Baldrian, P., & Morais, D. (2018). SEED 2: A user-friendly platform for amplicon high-
784 throughput sequencing data analyses. *Bioinformatics*, *34*(13), 2292–2294.
785 https://doi.org/10.1093/bioinformatics/bty071

786 Walde, M., Allan, E., Cappelli, S. L., Didion-Gency, M., Gessler, A., Lehmann, M. M., Pichon, N. A., &
787 Grossiord, C. (2021). Both diversity and functional composition affect productivity and water
788 use efficiency in experimental temperate grasslands. *Journal of Ecology*, *August*, 1–15.
789 https://doi.org/10.1111/1365-2745.13765

790 Zavaleta, E. S., Pasari, J. R., Hulvey, K. B., & Tilman, G. D. (2010). Sustaining multiple ecosystem
791 functions in grassland communities requires higher biodiversity. *Proceedings of the National*
792 *Academy of Sciences of the United States of America*, *107*(4), 1443–1446.
793 https://doi.org/10.1073/pnas.0906829107

794 Zhang, P., Li, B., Wu, J., & Hu, S. (2019). Invasive plants differentially affect soil biota through litter
795 and rhizosphere pathways: a meta-analysis. *Ecology Letters*, *22*(1), 200–210.
796 https://doi.org/10.1111/ele.13181

797 Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and*
798 *extensions in ecology with R*. Springer-Verlag.

799